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FADS gene variants, diet and atopic phenotypes and lipids in children.

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Abbreviations

AA	arachidonic acid
ALA	α-linolenic acid
DHA	docosahexaenoic acid
EPA	eisosapentaenoic acid
FADS	fatty acid desaturase
FFQ	food frequency questionnaire
GWAS	genome-wide association study
HDL	high-density lipoprotein
IgE	Immunoglobulin E
LA	linoleic acid
LDL	low-density lipoprotein
PUFA	polyunsaturated fatty acid
SNP	single-nucleotide polymorphism

Summary

The prevalence of allergic diseases has increased over the past decades. Dietary changes, especially the altered fatty acid consumption, is suggested to be partly responsible. There is a growing body of evidence that polyunsaturated fatty acids (PUFAs) play a major role in the development of atopic diseases in children, although the results have been inconsistent. Also the development of cardiovascular diseases seems to be dependent on PUFA composition as PUFA levels are believed to lower high blood lipid concentrations. Elevated cholesterol levels during childhood may be an important predictor for later disease in life.

The conversion of essential fatty acids to longer chain, biological active metabolites is regulated by the enzymes Δ^5 and Δ^6 desaturase, which are encoded by the genes fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*). Therefore, it is hypothesized that inter-individual genetic differences may modify the association of dietary fatty acid intake and allergic diseases or lipids.

This thesis comprises five publications, which are based on data from the GINIplus and LISAplus studies.

The first publication investigated the influence of variants in the *FADS1 FADS2* gene cluster on the association of dietary fatty acid and margarine intake and atopic diseases, as well as allergic sensitization. In the stratified analysis, a higher margarine intake was positively associated with a higher risk for asthma in homozygous major allele carriers.

In the second publication, the modulating effect of variants in the *FADS* gene cluster on the association of breastfeeding and asthma up to 10 years of age was evaluated. A strong interaction effect between the duration of exclusive breastfeeding and the six tested SNPs was observed. Individuals carrying at least one minor allele who were exclusively breastfed for more than three months, showed a reduced risk for asthma, whereas there was no genetic effect in homozygous major allele carriers.

The third publication included data from the GINIplus and LISAplus studies to a worldwide meta-analysis of genome-wide association studies on atopic dermatitis. Three new risk loci were identified. Two of these loci are near genes, which are related to epidermal proliferation and differentiation. The third locus is located near immune-related genes. These results underline the hypothesis, that atopic dermatitis is caused by epidermal barrier abnormalities and immunological features. However, further research is needed to identify causal variants at these loci and to understand the mechanisms through which they affect atopic dermatitis.

Regional and socio-economic differences in dietary intake of school-aged children were investigated in the fourth publication. A higher level of parental education was associated with a higher intake of more healthy food, such as bread, butter, eggs, pasta, vegetables/salad and fruit, whereas intakes of margarine, meat products, pizza, desserts and soft drinks were inversely associated with parental education. Additionally, substantial differences in food intake between eastern and western Germany were observed.

In the fifth publication, the association between blood lipid concentrations and variants of the *FADS* gene cluster was assessed. Individuals carrying the homozygous minor

allele were found to have lower levels of total cholesterol and low-density lipoprotein (LDL) compared to homozygous major allele carriers. Carriers of the heterozygous allele had lower levels of high-density lipoprotein (HDL) and higher levels of triglycerides compared to homozygous major allele carriers. Additionally, the influence of dietary n-3 PUFA intake was tested. A higher intake of n-3 fatty acids was related to higher levels of total cholesterol, LDL, HDL and lower triglyceride levels.

In summary, these results support the hypothesis, that there is a causal association between dietary fatty acid intake and atopic diseases or lipids in children and may help to better understand the complex association between allergic inflammation and PUFAs.

Zusammenfassung

Die Prävalenz allergischer Erkrankungen stieg in den vergangenen Jahrzehnten stark an. Es wird vermutet, dass unter anderem veränderte Ernährungsgewohnheiten, insbesondere die Zusammensetzung der Fettsäuren in der Nahrung, teilweise dafür verantwortlich sein könnten. Obwohl nicht alle Untersuchungsergebnisse konsistent sind, gibt es doch deutliche Hinweise darauf, dass mehrfach ungesättigte Fettsäuren (polyunsaturated fatty acids, PUFAs) eine große Rolle bei der Entstehung allergischer Erkrankungen bei Kindern spielen.

Ferner ist bekannt, dass die Aufnahme mehrfach ungesättigter Fettsäuren, neben anderen Faktoren, erhöhten Blutfettwerten entgegenwirkt. Erhöhte Cholesterinwerte in der Kindheit und Jugend können ein Risikofaktor für die Entstehung kardiovaskulärer Erkrankungen im Erwachsenenalter sein.

Die Umwandlung von essentiellen Fettsäuren zu längerkettigen, biologisch aktiven Metaboliten wird durch die Enzyme Δ^5 und Δ^6 Desaturase gesteuert, die durch die Gene *FADS1* und *FADS2* (*fatty acid desaturase 1* und *2*) kodiert werden. Daraus lässt sich die Hypothese ableiten, dass inter-individuelle genetische Unterschiede den Zusammenhang zwischen Fettsäurenaufnahme aus der Ernährung und allergischen Erkrankungen und Lipidwerten beeinflussen können.

Diese Dissertation umfasst fünf Publikationen, basierend auf den Daten der GINIplus und LISAplus Studien.

In der ersten Publikation wird der Einfluss von Varianten aus dem *FADS1 FADS2* Gencluster auf den Zusammenhang zwischen Fettsäurenaufnahme und Margarinekonsum und atopischen Erkrankungen bzw. allergischer Sensibilisierung untersucht. Dabei war eine höhere Margarineaufnahme mit einem höheren Asthmarisiko bei Kindern, die homozygot die häufige Variante tragen, assoziiert.

Die zweite Publikation befasst sich mit dem Zusammenhang zwischen Varianten aus dem *FADS* Gencluster, Stillen und Asthma bis zum Alter von 10 Jahren. Hier wurde ein starker Interaktioneffekt zwischen der Dauer, die ausschließlich gestillt wurde und den sechs getesteten SNPs beobachtet. Kinder, die mindestens ein seltenes Allel tragen und für mindestens drei Monate ausschließlich gestillt wurden, hatten ein deutlich niedrigeres Risiko an Asthma zu erkranken. Für Kinder, die homozygot die häufige Variante tragen, wurde kein genetischer Effekt beobachtet.

Die dritte Publikation ist eine Meta-Analyse von genomweiten Assoziationsstudien, in die auch Daten der GINIplus und LISAplus Studien miteingegangen sind. Es konnten drei neue Risikovarianten für atopischer Dermatitis identifiziert werden. Zwei dieser Loci liegen in der Nähe von Genen, die mit epidermaler Zellproliferation und Zelldifferentierung in Verbindung gebracht werden, während der dritte Locus in der Nähe von Genen liegt, die die Immunantwort regulieren. Diese Ergebnisse stützen die Hypothese, dass atopische Dermatitis durch Defekte in der natürlichen Hautbarriere und der Immunregulation verursacht wird. Allerdings sind weitere Untersuchungen erforderlich um die ursächlichen Varianten an diesen Loci zu identifizieren und den Mechanismus zu verstehen, durch den atopische Dermatitis hervorgerufen wird.

Regionale und sozio-ökonomische Unterschiede in der Ernährung von Kindern im Schulalter wurden in der vierten Publikation untersucht. Ein höherer Grad elterlicher Bildung war mit einem höheren Konsum gesunder Nahrungsmittel, wie Brot, Butter, Eier, Nudeln, Gemüse/Salat und Obst und einer niedrigeren Aufnahme von Margarine, Fleischprodukten, Pizza, Nachspeise und Limonaden assoziiert. Außerdem wurden grundsätzliche Unterschiede in der Ernährung zwischen Ost- und Westdeutschland beobachtet.

In der fünften Publikation wird der Zusammenhang zwischen Lipiden und Varianten des *FADS* Genclusters untersucht. Kinder, die homozygot das seltene Allel tragen, hatten im Vergleich zu Kindern, die das häufige Allel homozygot tragen, niedriger Cholesterinwerte und LDL-Konzentration. Träger des homozygoten Allels zeigten, im Vergleich zu Kindern, die das häufige Allel homozygot tragen, niedrigere HDL-Werte und höhere Triglyzeridwerte. Zusätzlich wurde der Einfluss der n-3 Fettsäurenaufnahme geprüft. Eine höhere n-3 Fettsäurenaufnahme war assoziiert mit einer höheren Cholesterin-, LDL- und HDL-Konzentration und niedrigeren Triglyzeridwerten. Es wurde allerdings keine Interaktion mit dem *FADS* Genotyp beobachtet.

Diese Ergebnisse lassen auf einen Zusammenhang zwischen *FADS* Gencluster, Fettsäurenaufnahme und allergischen Erkrankungen oder Lipiden bei Kindern schließen. Dadurch wird die Hypothese gestützt, dass ein kausaler Zusammenhang zwischen Fettsäurenaufnahme aus der Nahrung und atopischen Erkrankungen bei Kindern besteht. Dies könnte helfen, den komplexen Zusammenhang zwischen allergischer Inflammation und Fettsäuren zu verstehen.

1 Introduction

The prevalence of allergic diseases was increasing over the past decades with about 30-40% of the world population now being affected[1]. Changes in dietary intake, especially the altered fatty acid consumption, were suggested to be partly responsible for the increasing prevalence of allergies[2], although the results are inconsistent[3].

Polyunsaturated fatty acids (PUFAs) have a major impact on health and development and have been associated with cardiovascular diseases[4,5], mental health[6,7] and cognitive development[8,9], immunologial and inflammatory responses as well as related diseases such as allergic diseases[10,11].

The development and programming of the immune system is related to PUFA intake during pregnancy, lactation, infancy and early childhood and the development and manifestation of atopic diseases and allergies can be prevented thereby[12,13].

Among other factors, dietary PUFA intake can attenuate high blood lipid concentrations[14–16]. Elevated cholesterol levels during childhood and adolescence increase the risk for cardiovascular diseases later in life. Treatments which effectively lower cholesterol levels early in life have been shown to prevent disease manifestation in adulthood[17–19].

1.1 Allergy, Allergic Sensitization and Atopy

Allergy is defined as a "hypersensitivity reaction initiated by specific immunologic mechanisms", based on the nomenclature developed by the European Academy of Allergology and Clinical Immunology (EAACI) and updated by the World Allergy Organization (WAO), and can be antibody- or cell-mediated[20]. The presence of specific immunoglobulin E (IgE) antibodies is called sensitization. In most patients allergy is initiated by increased levels of IgE antibodies to specific antigens. Allergic symptoms may be referred to as atopic, if IgE sensitization has been documented by IgE antibodies in serum or by a positive skin prick test[20]. Therefore, atopy is defined as "a personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins. As a consequence, these persons can develop typical symptoms of asthma, rhinoconjunctivitis, or eczema."[20].

Asthma and allergies are complex diseases with high hereditary. The susceptibility to asthma and allergies is influenced by environmental factors, but with a strong genetic background[21]. Recently, a large genome-wide association study on asthma was published[22]. The results show that asthma is genetically heterogeneous with different characteristics in children and adults. Several genes could be identified, initiating as well as down-regulating airway inflammation processes. Additionally, little overlap between asthma and total serum IgE levels was reported. Therefore, the authors suggest that elevated total serum IgE concentration do not play a major role in the development of asthma, but rather, are a secondary effect of asthma[22].

1.2 Polyunsaturated Fatty Acid Metabolism and the *FADS* Gene Cluster

1.2.1 The Polyunsaturated Fatty Acid Metabolism

There is strong evidence that fatty acids play a major role in programming of the immune system and the development of atopic diseases in children[12,13], although the underlying biological mechanism is still not entirely clear and the results have been inconsistent[3,23].

In general, fatty acids containing more than one double bond in the chain are called polyunsaturated fatty acids. The main n-6 PUFA is linoleic acid (LA, 18:2n-6) and the main essential n-3 PUFA is α-linolenic acid (ALA, 18:3n-3)[24]. The essential fatty acids LA and ALA cannot be synthesized by mammalian cells, but they can be metabolized by desaturation (introduction of double bonds) and elongation (lengthening the hydrocarbon chain). The fatty acid metabolism is presented in figure 1. The n-6 PUFA LA is converted into γ-linolenic acid (18:3n-6), dihomo-γ-linolenic acid (20:3n-6) and arachidonic acid (AA, 20:4n-6). ALA can be converted to the long-chain fatty acids eisosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), using the same enzymatic pathway as n-6 PUFAs[24].

The key link between PUFAs and inflammation is the synthesis of eicosanoids, which are generated from PUFAs[25]. AA is the major substrate for eicosanoid synthesis, which are mediators and regulators of inflammation. In contrast, n-3 PUFAs are suggested to have anti-inflammatory effects by decreasing the production of inflammatory eicosanoids and cytokines[25]. Since n-3 and n-6 PUFAs use the same enzymatic pathway, a higher proportion of n-3 PUFAs leads to less substrate which is available for eicosanoid synthesis from AA. Additionally, there is evidence that n-3 PUFAs alter the expression of inflammatory genes and increase the production of anti-inflammatory mediators[24,25].
This led to the hypothesis that n-6 PUFA intake may enhance the development of allergic diseases in susceptible individuals. In contrast, n-3 PUFA are suggested to have protective effects with regard to allergic diseases[3].

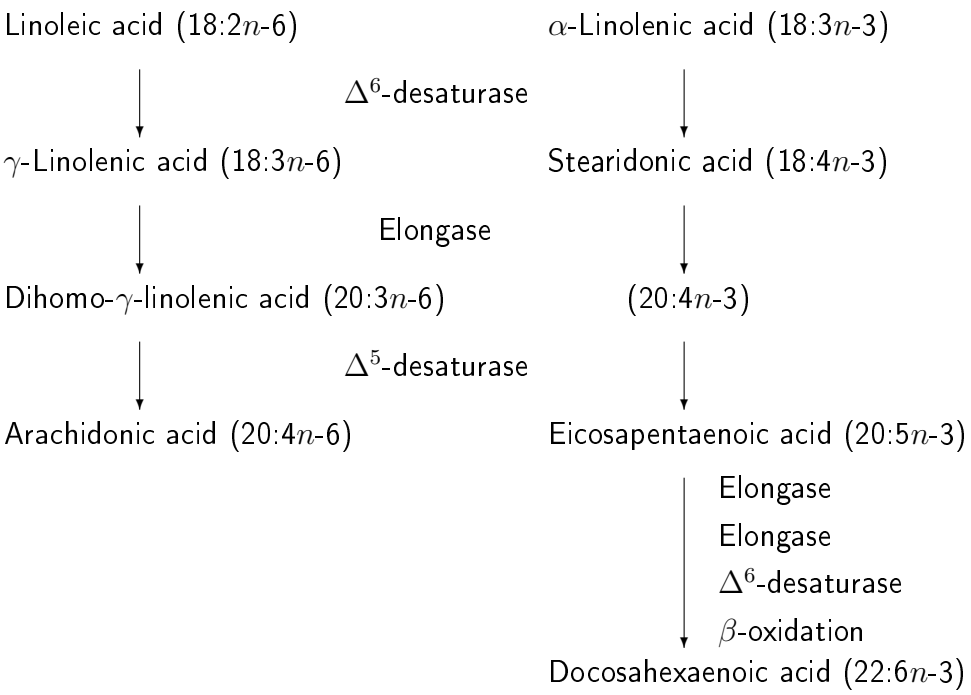
1.2.2 The FADS Gene Cluster

The conversion of the essential fatty acids LA and ALA to longer chain, biological active metabolites is regulated by the enzymes Δ^5 and Δ^6 desaturase, which are encoded by the genes fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*)[26].

The *FADS* gene cluster is located at chromosome 11q12-13.1[27] and comprises the genes *FADS1*, *FADS2* and *FADS3*. Whereas the function of *FADS1* and *FADS2* is well studied, the function of *FADS3* is not entirely clear[28].
Several genetic association studies showed that carriers of the minor alleles of single-nucleotide polymorphisms (SNPs) in the *FADS* gene cluster exhibit increased levels of desaturase substrates and decreased levels of desaturase products. This might arise from lowered transcriptional levels or diminished conversion rates of the enzymes in individuals carrying the minor alleles[29].

Variants of the *FADS* gene cluster can explain up to almost 30% of the variations in the fatty acid levels[29]. Several studies have shown strong associations between

Figure 1: Metabolism of n-6 and n-3 PUFAs (adapted from Calder (2005)[24])



the *FADS* gene cluster and fatty acid levels in serum phospholipids[29,30], plasma and adipose tissue samples[31], erythrocyte cell membranes[30,32], breast milk[30,33,34] and red blood cells[35].

Additionally, recent genome-wide association studies on blood lipid levels in adults have identified several genetic loci including the *FADS* gene cluster[36–40]. Lower total cholesterol[36], low-density lipoprotein (LDL)[36–38], high-density lipoprotein (HDL)[38,39] and higher triglyceride levels[39,40] are all associated with the minor alleles of the tested *FADS* polymorphisms.

Therefore, it is hypothesized that inter-individual genetic differences may influence the association of dietary fatty acid intake and fatty acid related phenotypes[26,41–43]. As the conversion rate of essential fatty acids to longer chain metabolites is hypothesized to be lower in individuals carrying the minor allele, it would be expected that the pro-inflammatory effects of n-6 fatty acids and the protective effects of n-3 fatty acids would be stronger in major allele carriers, because a higher percentage of the essential fatty acids is converted to longer chain, biological active metabolites.
Beside diet and nutrition, the *FADS* gene cluster plays an important role in the regulation of the PUFAs. A modulating effect of variants in the *FADS* gene cluster on intelligence development, risk of myocardial infarction and metabolic syndrome was reported[26]. Further, there is some evidence, that variants in the *FADS* gene cluster may also influence the association of PUFAs on allergies[26,41,43]. In summary, these results make these genes good candidates and underline the importance of further research on the modifying effect of variants in the *FADS* gene cluster on the association of PUFAs and fatty acid related phenotypes.

2 Specific Aims and Results

2.1 Specific Aims

The aim of this thesis was to investigate the association between dietary intake, *FADS* variants and atopic phenotypes or lipids in children. The main objectives were

- to assess the influence of *FADS1 FADS2* gene cluster polymorphisms on the association between dietary fatty acid intake and atopic diseases and allergic sensitization.
- to evaluate the impact of *FADS1 FADS2* gene cluster polymorphisms on the association between breastfeeding and asthma.
- to identify new risk loci for atopic dermatitis in a meta-analysis of genome-wide association studies.
- to describe regional differences between eastern and western Germany with regard to food, nutrient and supplement intake in children, and analyze the association with parental education and equivalent income.
- to investigate whether lipid levels are influenced by the *FADS* genotype already in children and whether this association interacts with dietary intake of n-3 fatty acids.

This thesis is based on five manuscripts, which are published in Clinical & Experimental Allergy, Allergy – the European Journal of Allergy and Clinical Immunology, Nature Genetics, Public Health Nutrition and PLoS ONE.
For all publications, I am the first, or shared first author. I was significantly involved in developing the research question, study design, performed the statistical analyses and interpreted the results. All comments and suggestions from the co-authors were included in the finalized version.

2.2 Study Population and Methods

2.2.1 Study Population

All manuscripts are based on data from the GINIplus[44] and LISAplus[45] studies. Briefly, a total of 5991 mothers and their newborns were recruited into the German Infant study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development (GINIplus) between September 1995 and June 1998 in Munich and Wesel. Infants with at least one allergic parent and/or sibling were allocated to the interventional study arm investigating the effect of different hydrolysed formulas for allergy prevention in the first year of life[44]. All children without a family history of allergic diseases and children whose parents did not give consent for the intervention were allocated to the non-interventional arm. The influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany PLUS the influence of traffic emissions and genetics (LISAplus) Study is a population based birth cohort study. A total of 3097 healthy, fullterm neonates were recruited between

1997 and 1999 in Munich, Leipzig, Wesel and Bad Honnef. The participants were not pre-selected based on family history of allergic diseases[45].
Detailed descriptions of the LISAplus and GINIplus studies have been published elsewhere ([44] and[45], respectively).

2.2.2 The Food Frequency Questionnaire

A food frequency questionnaire (FFQ) was developed to measure children's usual food and nutrient intake over the past 12 months, and more specifically to estimate energy, fatty acid and antioxidant intake at 10 years of age. The FFQ comprised a list of 82 food items accompanied by several questions about the preferred fat and energy content of products, preparation methods, diets and food preferences, buying habits and dietary supplement use. The consumption frequencies and portion size estimates were converted to average consumption in grams per day and linked to the German Nutrient Data Base (BLS) version II.3.1[46]. The design of the FFQ, including the selection of the food item list, validation, and the calculation of food and nutrient intake is described further by Stiegler et al.[47]. Information obtained by FFQ was used for the first, second, fourth and fifth manuscript.

2.2.3 Tested Variants of the *FADS* Gene Cluster

Six SNPs of the *FADS1 FADS2* gene cluster (rs174545, rs174546, rs174556, rs174561, rs174575 and rs3834458) were typed. Five of these variants (rs174545, rs174546, rs174556, rs174561 and rs3834458) were selected based on the previous publications in adult populations[29,48]. Moreover, the SNP rs174575 was included in order to obtain a better coverage of the *FADS* gene cluster. This SNP was selected based on a previous publication in children[49].

2.2.4 Methods

Up to now, the association between dietary fatty acid intake and the development of atopic diseases has been inconsistent[3]. The first publication (chapter 4) investigated whether the association between dietary fatty acid intake and atopic diseases and allergic sensitization is modified by variants of the *FADS* gene cluster.
Margarine and fatty acid intake was assessed by FFQ. Information on atopic diseases for each year of life up to 10 years of age was collected using a self-administered questionnaire completed by the parents and specific IgE antibodies against common food and inhalant allergens were measured. Six variants of the *FADS1 FADS2* gene cluster were tested. In total, complete information was available for 2000 children. In this analysis, the impact of inter-individual genetic differences on the association between dietary fatty acid intake and atopy was investigated.

The second manuscript (chapter 5) evaluated the effect of variants of the *FADS* gene cluster on the association between exclusive breastfeeding and the development of asthma. The analysis was based on 2245 children. Information on ever having a doctor's diagnosis of asthma up to 10 years of age and breastfeeding during the first 6 months was collected using parental questionnaires. Six SNPs of the *FADS1 FADS2*

gene cluster were tested. Within this study, the inconsistencies in the association between breastfeeding and asthma may be partly explained by inter-individual genetic differences.

The scope of the third publication (chapter 6) was to identify new genetic risk variants for atopic dermatitis in a meta-analysis of genome-wide association studies. Within the EArly Genetics & Lifecourse Epidemiology (EAGLE) Consortium a well-powered, two-stage genome-wide association meta-analysis was performed. The discovery analysis included 5606 affected individuals and 20565 controls from 16 population-based cohorts and the ten most strongly associated new susceptibility loci were examined in additional 5419 affected individuals and 19833 controls from 14 studies.

The aim of the fourth publication (chapter 7) was to analyze the effect of socio-economic factors and regional differences in food consumption and supplement intake. Information on food, nutrient and supplement intake of 3435 children derived by FFQ was analyzed in respect to regional differences between eastern and western Germany and the association with parental education and equivalent income was assessed.

In adults, it had been shown that the blood lipid concentrations are strongly influenced by variants of the *FADS* gene cluster. Therefore, the fifth publication (chapter 8) investigated whether blood lipid levels are influenced by polymorphisms in the *FADS* gene cluster already in children and whether this association interacts with dietary fatty acid intake. During the 10 year follow-up of the GINIplus and LISAplus studies total cholesterol, HDL, LDL and triglycerides were measured. Six SNPs of the *FADS* gene cluster were genotyped and dietary fatty acid intake was assessed by FFQ. In this analysis, the influence of *FADS* variants on lipid concentrations in children was evaluated.

2.3 Results

In the first publication (chapter 4) the influence of polymorphisms in the *FADS1 FADS2* gene cluster on the association between dietary fatty acid intake and atopic diseases and allergic sensitization was evaluated. Margarine intake was associated with an increased risk for asthma in individuals carrying the homozygous major allele. The ratio of n-3 to n-6 fatty acids was related to higher hay fever risk, although this association was not significant after adjustment for multiple testing. These results suggest that the effect of dietary fatty acid intake on atopic diseases might be modulated by the *FADS1 FADS2* genes in children.

In the second publication (chapter 5) the impact of variants of the *FADS* gene cluster on the association of breastfeeding and asthma was assessed. A strong, highly significant interaction effect was identified. Exclusive breastfeeding for at least 3 months showed a protective effect in heterozygous and homozygous carriers of the minor allele, whereas no effect of exclusive breastfeeding was observed for individuals carrying the homozygous major allele. These results suggest a modulating effect of the *FADS1 FADS2* gene cluster on the association of the duration of exclusive breastfeeding and the development of asthma in later life.

A meta-analysis of genome-wide association studies on atopic dermatitis identified three new risk loci. Three polymorphisms reached genome-wide significance in the combined analysis of discovery and replication cohorts. Two of these loci are near genes, which are related to epidermal proliferation and differentiation (rs479844 upstream of *OVOL1* and rs2164983 near *ACTL9*). The third locus, rs2897442 in *IL4–KIF3A* located at 5q31.1, seems to be composed of two independent signals (*IL4–KIF3A* and *IL13–RAD50*), which contain cytokine and immune-related genes. Additionally, the association with the well-studied *FLG* locus and two signals which were recently identified by two GWA studies could be replicated. These results strengthen the hypothesis, that atopic dermatitis is caused by epidermal barrier abnormalities and immunological features. However, further research is needed to identify causal variants at these loci and to understand the mechanisms through which they affect atopic dermatitis (chapter 6).

The fourth publication (chapter 7) aimed to describe regional differences between eastern and western Germany and the association between dietary intake and the level of parental education and equivalent income. A higher level of parental education was associated with a higher intake of healthy food, like bread, butter, eggs, pasta, vegetables/salad and fruit, whereas intakes of margarine, meat products, pizza, desserts and soft drinks were inversely associated with parental education. The association between dietary intake and equivalent income was weaker. Additionally, substantial differences in food intake between eastern and western Germany were observed. Therefore, nutritional education programs and dietary recommendations for school-aged children should take these regional and socio-economic differences into account.

The association between blood lipid concentrations and variants of the *FADS* gene cluster was investigated in the fifth publication (chapter 8). Individuals carrying the homozygous minor allele were found to have lower levels of total cholesterol and LDL compared to homozygous major allele carriers. Carriers of the heterozygous allele had lower levels of HDL and higher levels of triglycerides compared to homozygous major allele carriers. Additionally, the influence of dietary n-3 PUFA intake was tested. A higher intake of n-3 fatty acids was related to higher levels of total cholesterol, LDL, HDL and lower triglyceride levels. However, these associations did not interact with the *FADS1 FADS2* genotype. These results show, that total cholesterol, HDL, LDL, and triglyceride levels are influenced by the *FADS* gene cluster already in 10 year old children. This might differentially predispose individuals to the development of cardiovascular diseases later in life.

2.4 Strengths and Limitations

A detailed discussion of strengths and limitations can be found in the five publications. Here, a few major aspects will be briefly summarized.

Dietary intake was assessed by a FFQ, which is generally believed to overestimate dietary intakes[50]. As an objective measurement, this FFQ was developed and validated for the specific aim to assess intake of n-3 and n-6 fatty acids. Thus, this developed FFQ for children goes beyond common used other FFQs. The FFQ measured dietary

intakes over the past 12 months and was validated for dietary fatty acid intake[47]. Fatty acid concentrations in blood at 10 years of age were not available so far.

Further, it should be considered, that five of the six SNPs from the *FADS* gene cluster, which were analyzed, were selected based on previous publications in adult populations[29,48]. The SNPs are in high linkage disequilibrium with each other and do not cover the complete region of the *FADS* gene cluster.

A longitudinal analysis to identify age dependent differences is not possible due to a low prevalence especially of asthma in children up to 10 years of age. The definition of asthma and allergies was based on questionnaire information on having a doctor's diagnosis for each year of live. A clinical ascertainment of these information in each year of life was beyond the scope of this study. However, the presence of specific IgE antibodies was measured at 6 and 10 years of age and blood lipid levels were measured during the 10 year follow-up.

Further, the results of the meta-analysis of genome-wide association studies on atopic dermatitis underline the importance of careful phenotyping. In this analysis, several studies with differences in case definition and age of onset were included and some loci showed evidence for heterogeneity. Additional sensitivity analyses on age of onset and a more stringent definition based on reported physician's diagnosis could partly explain the heterogeneity.

3 Conclusion and Outlook

This thesis aimed to investigate the association between dietary intake, the *FADS1 FADS2* gene cluster and atopic phenotypes as well as lipids in children. An association between dietary fatty acid intake, the *FADS1 FADS2* gene cluster and allergies was identified. Margarine intake was related with an increased risk for asthma in children carrying the homozygous major allele. Additionally, a protective effect of breastfeeding for at least three months on the development of asthma later in life was observed in children carrying at least one minor allele, whereas there was no association for individuals carrying the homozygous major allele. Further, it could be shown that total cholesterol, HDL, LDL and triglyceride concentrations are associated with dietary n-3 PUFA intake and variants in the *FADS* gene cluster already in 10-year-old children, although no interaction with dietary fatty acid intake was observed.

So far, the results from several studies, which have investigated the association between dietary PUFA intake and atopic diseases, were not conclusive[3,23]. Although the mechanism seems biological plausible, the evidence remains scarce. One reason for this might be inter-individual genetic differences. In fact, the results presented in this thesis indicate that individual differences in the fatty acid metabolism, influenced by the *FADS* genes, may be partly responsible for these inconsistencies.

These individual differences in the fatty acid metabolism, presumably induced by the *FADS* genotype, may lead to different nutritional requirements to ensure the necessary fatty acid supply. A further investigation of the individual differences in the conversion of dietary fatty acid intake to longer chain, biological active metabolites against the background of the *FADS* genotype may enhance the knowledge about the underlying mechanism. Therefore, it would be interesting to include as a further step the fatty acid composition in blood in further analyses.

The development and programming of the immune system is related to PUFA intake during pregnancy and lactation[12,13]. It is known, that the *FADS* genotype influences, upon PUFA levels in blood, the fatty acid composition of breast milk. Therefore, the sufficient supply with PUFA of the mother during pregnancy and lactation is essential for the development. Moreover, it would be highly interesting to investigate the influence of the maternal genotype and thereby account for the variation of PUFA supply during pregnancy and lactation, which is caused by the maternal *FADS* genotype.

In summary, these results suggest that there is an association between the *FADS* gene cluster, dietary fatty acid intake and atopic diseases or lipids in children. The clinical relevance of the *FADS* genotype itself is weak, because the percentage of variance explained is low and therefore cannot be used for prevention purposes, but these results may help to identify possible biological pathways. Thereby, the hypothesis, that there is a causal association between dietary fatty acid intake and atopic diseases or lipids in children is supported. Therefore, the *FADS* genotype should be included in further studies on PUFA and fatty acid related phenotypes and this may help to improve understanding the complex association between PUFAs and allergic inflammation.

References

[1] World Allergy Organization, *White Book on Allergy*. 2011.

[2] P. N. Black and S. Sharpe, "Dietary fat and asthma: is there a connection?," *Eur Respir J*, vol. 10, pp. 6–12, Jan 1997.

[3] S. Sausenthaler, B. Koletzko, and J. Heinrich, "Dietary fat intake and allergic diseases," *Current Nutrition & Food Science*, vol. 2, no. 4, pp. 351–359, 2006.

[4] D. Mozaffarian and J. H. Y. Wu, "Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events.," *J Am Coll Cardiol*, vol. 58, pp. 2047–2067, Nov 2011.

[5] M. Y. Abeywardena and G. S. Patten, "Role of ω3 longchain polyunsaturated fatty acids in reducing cardio-metabolic risk factors.," *Endocr Metab Immune Disord Drug Targets*, vol. 11, pp. 232–246, Sep 2011.

[6] C. M. Milte, N. Sinn, and P. R. C. Howe, "Polyunsaturated fatty acid status in attention deficit hyperactivity disorder, depression, and Alzheimer's disease: towards an omega-3 index for mental health?," *Nutr Rev*, vol. 67, pp. 573–590, Oct 2009.

[7] G. Kohlboeck, C. Glaser, C. Tiesler, H. Demmelmair, M. Standl, M. Romanos, *et al.*, "Effect of fatty acid status in cord blood serum on children's behavioral difficulties at 10 y of age: results from the LISAplus Study.," *Am J Clin Nutr*, vol. 94, pp. 1592–1599, Dec 2011.

[8] B. Koletzko, I. Cetin, J. T. Brenna, and for the Perinatal Lipid Intake Working Group, "Dietary fat intakes for pregnant and lactating women.," *Br J Nutr*, vol. 98, pp. 873–877, Nov 2007.

[9] J. E. Karr, J. E. Alexander, and R. G. Winningham, "Omega-3 polyunsaturated fatty acids and cognition throughout the lifespan: a review.," *Nutr Neurosci*, vol. 14, pp. 216–225, Sep 2011.

[10] M. M. Suárez-Varela, L. G.-M. Alvarez, M. D. Kogan, J. C. Ferreira, A. Martínez Gimeno, I. Aguinaga Ontoso, *et al.*, "Diet and prevalence of atopic eczema in 6 to 7-year-old schoolchildren in Spain: ISAAC phase III.," *J Investig Allergol Clin Immunol*, vol. 20, no. 6, pp. 469–475, 2010.

[11] R. Barros, A. Moreira, J. Fonseca, L. Delgado, M. G. Castel-Branco, T. Haahtela, *et al.*, "Dietary intake of α-linolenic acid and low ratio of n-6:n-3 PUFA are associated with decreased exhaled NO and improved asthma control.," *Br J Nutr*, vol. 106, pp. 441–450, Aug 2011.

[12] P. C. Calder, L.-S. Kremmyda, M. Vlachava, P. S. Noakes, and E. A. Miles, "Is there a role for fatty acids in early life programming of the immune system?," *Proc Nutr Soc*, vol. 69, pp. 373–380, Aug 2010.

[13] L.-S. Kremmyda, M. Vlachava, P. S. Noakes, N. D. Diaper, E. A. Miles, and P. C. Calder, "Atopy risk in infants and children in relation to early exposure to fish, oily fish, or long-chain omega-3 fatty acids: a systematic review.," *Clin Rev Allergy Immunol*, vol. 41, pp. 36–66, Aug 2011.

[14] B. E. Phillipson, D. W. Rothrock, W. E. Connor, W. S. Harris, and D. R. Illingworth, "Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia.," *N Engl J Med*, vol. 312, pp. 1210–1216, May 1985.

[15] L. Calabresi, B. Villa, M. Canavesi, C. R. Sirtori, R. W. James, F. Bernini, *et al.*, "An omega-3 polyunsaturated fatty acid concentrate increases plasma high-density lipoprotein 2 cholesterol and paraoxonase levels in patients with familial combined hyperlipidemia.," *Metabolism*, vol. 53, pp. 153–158, Feb 2004.

[16] D. Mozaffarian, R. Micha, and S. Wallace, "Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials.," *PLoS Med*, vol. 7, p. e1000252, Mar 2010.

[17] W. P. Newman, D. S. Freedman, A. W. Voors, P. D. Gard, S. R. Srinivasan, J. L. Cresanta, *et al.*, "Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis. The Bogalusa Heart Study.," *N Engl J Med*, vol. 314, pp. 138–144, Jan 1986.

[18] G. S. Berenson, S. R. Srinivasan, W. Bao, W. P. Newman, R. E. Tracy, and W. A. Wattigney, "Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study.," *N Engl J Med*, vol. 338, pp. 1650–1656, Jun 1998.

[19] H. C. McGill, C. A. McMahan, A. W. Zieske, G. D. Sloop, J. V. Walcott, D. A. Troxclair, *et al.*, "Associations of coronary heart disease risk factors with the intermediate lesion of atherosclerosis in youth. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group.," *Arterioscler Thromb Vasc Biol*, vol. 20, pp. 1998–2004, Aug 2000.

[20] S. G. O. Johansson, T. Bieber, R. Dahl, P. S. Friedmann, B. Q. Lanier, R. F. Lockey, *et al.*, "Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003.," *J Allergy Clin Immunol*, vol. 113, pp. 832–836, May 2004.

[21] D. Vercelli, "Discovering susceptibility genes for asthma and allergy.," *Nat Rev Immunol*, vol. 8, pp. 169–182, Mar 2008.

[22] M. F. Moffatt, I. G. Gut, F. Demenais, D. P. Strachan, E. Bouzigon, S. Heath, *et al.*, "A large-scale, consortium-based genomewide association study of asthma.," *N Engl J Med*, vol. 363, pp. 1211–1221, Sep 2010.

[23] A. Sala-Vila, E. A. Miles, and P. C. Calder, "Fatty acid composition abnormalities in atopic disease: evidence explored and role in the disease process examined.," *Clin Exp Allergy*, vol. 38, pp. 1432–1450, Sep 2008.

[24] P. C. Calder, "Polyunsaturated fatty acids and inflammation.," *Biochem Soc Trans*, vol. 33, pp. 423–427, Apr 2005.

[25] P. C. Calder, "n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases.," *Am J Clin Nutr*, vol. 83, pp. 1505S–1519S, Jun 2006.

[26] E. Lattka, T. Illig, B. Koletzko, and J. Heinrich, "Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism.," *Curr Opin Lipidol*, vol. 21, pp. 64–69, Feb 2010.

[27] A. Marquardt, H. Stöhr, K. White, and B. H. Weber, "cDNA cloning, genomic structure, and chromosomal localization of three members of the human fatty acid desaturase family.," *Genomics*, vol. 66, pp. 175–183, Jun 2000.

[28] H. Blanchard, P. Legrand, and F. Pédrono, "Fatty Acid Desaturase 3 (Fads3) is a singular member of the Fads cluster.," *Biochimie*, vol. 93, pp. 87–90, Jan 2011.

[29] L. Schaeffer, H. Gohlke, M. Müller, I. M. Heid, L. J. Palmer, I. Kompauer, *et al.*, "Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids.," *Hum Mol Genet*, vol. 15, pp. 1745–1756, Jun 2006.

[30] L. Xie and S. M. Innis, "Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation.," *J Nutr*, vol. 138, pp. 2222–2228, Nov 2008.

[31] A. Baylin, E. Ruiz-Narvaez, P. Kraft, and H. Campos, "alpha-linolenic acid, Delta6-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction.," *Am J Clin Nutr*, vol. 85, pp. 554–560, Feb 2007.

[32] G. Malerba, L. Schaeffer, L. Xumerle, N. Klopp, E. Trabetti, M. Biscuola, *et al.*, "SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease.," *Lipids*, vol. 43, pp. 289–299, Apr 2008.

[33] C. Moltó-Puigmartí, J. Plat, R. P. Mensink, A. Müller, E. Jansen, M. P. Zeegers, *et al.*, "FADS1 FADS2 gene variants modify the association between fish intake and the docosahexaenoic acid proportions in human milk.," *Am J Clin Nutr*, vol. 91, pp. 1368–1376, May 2010.

[34] E. Lattka, P. Rzehak, E. Szabó, V. Jakobik, M. Weck, M. Weyermann, *et al.*, "Genetic variants in the FADS gene cluster are associated with arachidonic acid concentrations of human breast milk at 1.5 and 6 mo postpartum and influence the course of milk dodecanoic, tetracosenoic, and trans-9-octadecenoic acid concentrations over the duration of lactation.," *Am J Clin Nutr*, vol. 93, pp. 382–391, Feb 2011.

[35] B. Koletzko, E. Lattka, S. Zeilinger, T. Illig, and C. Steer, "Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and others polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children.," *Am J Clin Nutr*, vol. 93, pp. 211–219, Jan 2011.

[36] Y. S. Aulchenko, S. Ripatti, I. Lindqvist, D. Boomsma, I. M. Heid, P. P. Pramstaller, *et al.*, "Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts.," *Nat Genet*, vol. 41, pp. 47–55, Jan 2009.

[37] C. Sabatti, S. K. Service, A.-L. Hartikainen, A. Pouta, S. Ripatti, J. Brodsky, *et al.*, "Genome-wide association analysis of metabolic traits in a birth cohort from a founder population.," *Nat Genet*, vol. 41, pp. 35–46, Jan 2009.

[38] D. I. Chasman, G. Paré, S. Mora, J. C. Hopewell, G. Peloso, R. Clarke, *et al.*, "Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis.," *PLoS Genet*, vol. 5, p. e1000730, Nov 2009.

[39] S. Kathiresan, C. J. Willer, G. M. Peloso, S. Demissie, K. Musunuru, E. E. Schadt, *et al.*, "Common variants at 30 loci contribute to polygenic dyslipidemia.," *Nat Genet*, vol. 41, pp. 56–65, Jan 2009.

[40] N. M. G. De Silva, R. M. Freathy, T. M. Palmer, L. A. Donnelly, J. Luan, T. Gaunt, *et al.*, "Mendelian randomization studies do not support a role for raised circulating triglyceride levels influencing type 2 diabetes, glucose levels, or insulin resistance.," *Diabetes*, vol. 60, pp. 1008–1018, Mar 2011.

[41] E. Lattka, T. Illig, J. Heinrich, and B. Koletzko, "FADS gene cluster polymorphisms: important modulators of fatty acid levels and their impact on atopic diseases.," *J Nutrigenet Nutrigenomics*, vol. 2, no. 3, pp. 119–128, 2009.

[42] E. Lattka, T. Illig, J. Heinrich, and B. Koletzko, "Do FADS genotypes enhance our knowledge about fatty acid related phenotypes?," *Clin Nutr*, vol. 29, pp. 277–287, Jun 2010.

[43] C. Glaser, E. Lattka, P. Rzehak, C. Steer, and B. Koletzko, "Genetic variation in polyunsaturated fatty acid metabolism and its potential relevance for human development and health.," *Matern Child Nutr*, vol. 7 Suppl 2, pp. 27–40, Apr 2011.

[44] A. von Berg, U. Krämer, E. Link, C. Bollrath, J. Heinrich, I. Brockow, *et al.*, "Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years.," *Clin Exp Allergy*, vol. 40, pp. 627–636, Apr 2010.

[45] J. Heinrich, G. Bolte, B. Hölscher, J. Douwes, I. Lehmann, B. Fahlbusch, *et al.*, "Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates.," *Eur Respir J*, vol. 20, pp. 617–623, Sep 2002.

[46] B. M. Hartmann, S. Bell, A. Vásquez-Caicedo, A. Götz, J. Erhardt, and C. Brombach, *Der Bundeslebensmittelschlüssel. German Nutrient Data Base.* 2005.

[47] P. Stiegler, S. Sausenthaler, A. E. Buyken, P. Rzehak, D. Czech, J. Linseisen, *et al.*, "A new FFQ designed to measure the intake of fatty acids and antioxidants in children.," *Public Health Nutr*, vol. 13, pp. 38–46, Jan 2010.

[48] P. Rzehak, J. Heinrich, N. Klopp, L. Schaeffer, S. Hoff, G. Wolfram, *et al.*, "Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes.," *Br J Nutr*, vol. 101, pp. 20–26, Jan 2009.

[49] A. Caspi, B. Williams, J. Kim-Cohen, I. W. Craig, B. J. Milne, R. Poulton, *et al.*, "Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism.," *Proc Natl Acad Sci U S A*, vol. 104, pp. 18860–18865, Nov 2007.

[50] S. Robinson, K. Godfrey, C. Osmond, V. Cox, and D. Barker, "Evaluation of a food frequency questionnaire used to assess nutrient intakes in pregnant women.," *Eur J Clin Nutr*, vol. 50, pp. 302–308, May 1996.

4 Paper 1: *FADS* Variants, Dietary Fatty Acid Intake and Atopic Diseases in Children

(Standl et al. Clinical & Experimental Allergy, 2011)

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ORIGINAL ARTICLE **Epidemiology of Allergic Disease**

FADS gene variants modulate the effect of dietary fatty acid intake on allergic diseases in children

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Summary

Background The association between dietary fatty acid intake and the development of atopic diseases has been inconsistent. This could be due to inter-individual genetic differences in fatty acid metabolism.

Objective The aim of the current study was to assess the influence of *FADS1 FADS2* gene cluster polymorphisms on the association between dietary fatty acid intake and atopic diseases and allergic sensitization in 10-year-old children.

Methods The analysis was based on data from two German prospective birth cohort studies. Data on margarine and fatty acid intake were collected using a food frequency questionnaire. Information on atopic diseases was collected using a questionnaire completed by the parents. Specific IgE against common food and inhalant allergens were measured. Six variants of the *FADS1 FADS2* gene cluster (rs174545, rs174546, rs174556, rs174561, rs174575 and rs3834458) were tested. Logistic regression modelling, adjusted for gender, age, maternal education level and study centre, was used to analyse the association between fatty acid intake and atopic diseases stratified by genotype.

Results No significant association was found between the six *FADS* single nucleotide polymorphisms (SNPs) and allergic diseases or atopic sensitization. The total n-3/total n-6 ratio was positive associated with an increased risk of hayfever in homozygous major allele carriers ranging from an adjusted odds ratios of 1.25 (95%-CI: 1.00–1.57) to 1.31 (95%-CI: 1.01–1.69) across the six tested SNPs although this association was not significant anymore after correcting for multiple testing. Daily margarine intake was significantly associated with asthma [1.17 (1.03–1.34) to 1.22 (1.06–1.40)] in individuals carrying the homozygous major allele. This association was also significant after correcting for multiple testing.

Conclusions & Clinical Relevance The association between dietary intake of fatty acids and allergic diseases might be modulated by *FADS* gene variants in children.

Keywords atopy, children, epidemiology, *FADS*, fatty acid intake, FFQ, specific IgE
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Introduction

Dietary factors may be partly responsible for the increasing prevalence of atopic diseases in children. The altered consumption of polyunsaturated fatty acids (PUFA) has been suggested to contribute to this increase. This is due to a reduction in the consumption of animal fat and an increase in the use of margarine and vegetable oils containing proinflammatory n-6 PUFA [1]. However, the results of previous studies are not consistent [2, 3].

Linoleic acid (LA, 18 : 2n-6), the most common dietary n-6 PUFA, is metabolized to γ-linoleic acid (GLA, 18 : 3n-6),

which is converted to arachidonic acid (AA, 20 : 4n-6). AA can act as substrate of inflammatory eicosanoids. N-3 PUFAs are reported to have beneficial effects on allergic inflammation. α-Linolenic acid (ALA, 18 : 3n-3) can be converted to the long-chain fatty acids eisosapentaenoic acid (EPA, 20 : 5n-3), docosapentaenoic acid (DPA, 22 : 5n-3) and docosahexaenoic acid (DHA, 22 : 6n-3), using the same enzymatic pathway as n-6 PUFA. Thereby, n-3 PUFA can competitively inhibit the production of pro-inflammatory AA [4, 5]. This led to the hypothesis that n-6 PUFA intake may enhance the development of allergic diseases in susceptible individuals, mediated at least partly through an increase in IgE synthesis. In contrast, n-3 PUFA is suggested to have protective effects against allergic diseases.

The genes fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*) encode the enzymes delta-5-desaturase and delta-6-desaturase, respectively, which are involved in the fatty acid metabolic pathway [6]. Several studies have shown strong associations between the *FADS* gene cluster and fatty acid levels in serum phospholipids [7, 8], plasma and adipose tissue samples [9], erythrocyte cell membranes [8, 10], breast milk [8] and red blood cell lipids [11]. Carriers of the minor allele exhibit increased levels of desaturase substrates and decreased levels of desaturase products. This might arise from lowered transcriptional levels or diminished conversion rates of the enzymes in individuals carrying the minor alleles.

Inter-individual genetic differences in fatty acid metabolism might be one of the reasons for the inconsistent association between intake of fatty acids and atopic phenotypes [1, 12]. Therefore, we investigated, whether the association between fatty acid intake and atopic diseases could be modified by genetic variants of the *FADS* gene cluster.

Methods

Study population

Data from two ongoing German birth cohort studies were included in this investigation, the German LISAplus (Lifestyle Related Factors on the Immune System and the Development of Allergies in Childhood) and GINIplus (German Infant Nutritional Intervention) studies. LISAplus is a population based birth cohort study. A total of 3097 neonates were recruited between 1997 and 1999 in Munich, Leipzig, Wesel and Bad Honnef. The participants were not pre-selected based on family history of allergic diseases [13]. A total of 5991 mothers and their newborns were recruited into the GINIplus study between September 1995 and June 1998 in Munich and Wesel. Infants with at least one allergic parent and/or sibling were allocated to the interventional study arm investigating the effect of different hydrolysed formulas for allergy prevention in the first year of life [14]. All children without a family history of allergic diseases and children whose parents did not give consent for the intervention were allocated to the non-interventional arm. Detailed descriptions of the LISAplus and GINIplus studies have been published elsewhere ([13] and [14], respectively).

In both studies only individuals with Caucasian German descent were included.

For both studies, approval by the local Ethics Committees (Bavarian General Medical Council, University of Leipzig, Medical Council of North-Rhine-Westphalia) and written consent from participant's families were obtained.

Food frequency questionnaire (FFQ)

The FFQ was developed to measure children's usual food and nutrient intake over the past year, and more specifically to estimate energy, fatty acid and antioxidant intake at 10 years of age. The FFQ comprised a list of 82 food items accompanied by several questions about the preferred fat and energy content of products, preparation methods, diets and food preferences, buying habits and dietary supplement use. To estimate how often foods were eaten by their child on average over the previous year, parents were asked to choose one of nine frequency categories. In addition, common portion sizes were given for each food to enable an estimation of quantities. For foods which were difficult to describe in common household measures, coloured photographs from the European Prospective Investigation into Cancer and Nutrition (EPIC) study showing three different portion sizes were included [15]. The consumption frequencies and portion size estimates were converted to average consumption in grams per day and linked to the German Nutrient Data Base (BLS) version II.3.1 [16]. The design of the FFQ, including the selection of the food item list, validation, and the calculation of food and nutrient intake is described further by Stiegler et al. [17].

For the present analysis, information on fatty acid intake and margarine consumption was used.

Genotyping

Six variants of the *FADS1 FADS2* gene cluster (rs174545, rs174546, rs174556, rs174561, rs174575 and rs3834458) were typed. Five of these single nucleotide polymorphisms (SNPs) (rs174545, rs174546, rs174556, rs174561 and rs3834458) have been previously shown to be in strong linkage disequilibrium (LD) with each other ($r^2 > 0.7$, $D' > 0.9$) [7]. These five SNPs were selected based on previous publications in adult populations [7, 18]. Moreover, we included the SNP rs174575. In addition, applying the tagger server program (http://www.broadinstitute.org/mpg/tagger/) in combination with HapMap we found that with the three SNPs rs174545, rs174546 and rs174556 we

could tag 27 SNPs between basepair positions 61234329 and 61372379 of *FADS1 FADS2*. The efficiency was 10.7 fold although the two further SNPs rs174561 and rs3834458 could not be included as these are not included in the hapmap database. Genotyping of SNPs was realized with the iPLEX (Sequenom, San Diego, CA, USA) method by means of matrix assisted laser desorption ionization-time of flight mass spectrometry method (MALDI-TOF MS, Mass Array; Sequenom, San Diego, CA, USA) in one laboratory according to the manufacturer's instructions. Standard genotyping quality control included 10% duplicate and negative samples. Genotyping discordance rate was below 0.3%.

Definition of outcome variables

Information on allergic diseases was collected using a self-administered questionnaire at 10 years of age completed by the parents. The questions for physician-diagnosed asthma, eczema or hayfever at age 6, 7, 8 or 9 were used to define binary outcome variables for ever having a diagnose.

Allergic sensitization was defined by specific serum IgE concentrations, which were assayed by the CAP-RAST FEIA system (Pharmacia Diagnostics, Freiburg, Germany) according to the manufacturer's instructions. Screening tests were used for testing allergic sensitization against food allergens (fx5: egg, cow milk, wheat, peanut, soybean, and codfish) and inhalant allergens (sx1: *Dermatophagoides pteronyssinus*, cat, dog, rye, timothy grass, *Cladosporium herbarum*, birch, mugwort). The limit of detection for specific IgE was 0.35 kU/L. Children were assigned as IgE positive, if their IgE values exceeded the detection limit in at least one of both RAST tests.

Statistical analysis

Variables of interest were the ratio of n-3 (ALA+EPA+DPA+DHA) to n-6 (LA+AA) fatty acids and daily margarine intake. Due to implausible values of the total n-3/total n-6 ratio, eight subjects were excluded from this analysis.

To evaluate the differences between the genotypes a stratified analysis was used.

Multiple logistic regression analysis was applied to estimate the adjusted odds ratios (aOR) with 95% confidence intervals (CI) for the association between fatty acid intake and the binary health outcomes asthma, hayfever, eczema and IgE positive. Statistical significance was defined by a two-sided alpha level of 5%.

According to Nyholt [19], the number of effective loci of the six SNPs in the *FADS* gene cluster was computed as 2. In order to correct for multiple testing, the alpha level is divided by the two effective loci multiplied by the four phenotypes, which leads to a corrected two-sided alpha level of 0.63%.

The adjusted odds ratios are shown for interquartile range (IQR) increase. Differences between both studies were tested using Fisher's exact test, Wilcoxon rank sum test or Pearson's chi-squared test.

Statistical power was calculated for both margarine consumption and total n-3/total n-6 fatty acid intake. Owing to the distribution of the variables of interest, the fatty acid intake was log transformed and power calculation was approximated with a two sample *t*-test regarding the differences of the means in the group with atopic diseases or allergic sensitization and the control group.

All models are adjusted for gender, age, maternal education level (low, medium and high) and study centre (Munich, Leipzig, Wesel and Bad Honnef).

Statistical analysis was performed using the statistical software R, version 2.9.1 (R Development Core Team, 2009) [20]. For haplotype construction, haplo.em() from the package Haplo Stats, version 1.4.0 was used.

Results

Complete information on dietary fatty acid and margarine intake, *FADS1 FADS2* genotype and allergic diseases was available for 2000 children [1301 (65.05%) children from the GINIplus Study and 699 (34.95%) children from the LISAplus Study]. IgE data existed for 1703 of these children [1102 (64.71%) children from the GINIplus Study and 601 (35.29%) children from the LISAplus Study].

Basic characteristics of the study population are described in the tables. Children from the GINIplus Study were on average older than children in the LISAplus Study (mean age difference of 6 months) (Table 1). Owing to the age difference, total nutrient and dietary fatty acid intake was higher in children from the GINIplus Study. 52.3% of the cohort had mothers with education beyond 10th grade. The prevalence for atopic outcome variables ranged from about 6.5% for asthma to 46.6% for IgE positive. The margarine intake and total n-3/total n-6 ratio were found to be significantly different in the GINIplus and LISAplus Study (*P*-value 0.0001 and 0.002, respectively). In the GINIplus Study, the intake of the fatty acids LA and AA was significantly higher than in the LISAplus Study (*P*-value 0.0109 and 0.0192, respectively).

The genotype and allele frequencies of the six SNPs which were included in the analysis are shown in Table 2. Five of the six SNPs (rs174545, rs174546, rs174556, rs174561 and rs3834458) are in high LD with each other (supporting information, Figure S1). For these five SNPs, the pairwise squared correlations r^2 ranged between 0.82 and 0.99 and Lewontin's *D*' ranged between 0.98 and 1 in the GINIplus Study. For the LISAplus Study r^2 ranged between 0.85 and 1 and *D*' ranged between 0.99 and 1.

For rs174575, the LD is lower. The pairwise correlation r^2 for this SNP ranged between 0.48 and 0.66 in the GINIplus Study and between 0.49 and 0.62 in the LISAplus

Table 1. Basic characteristics of the 10 year follow-up of the study population

	LISAplus ($n = 699$)	GINIplus ($n = 1301$)	Total ($n = 2000$)	
	% or mean (SD)	% or mean (SD)	% or mean (SD)	*P*-value
Boys (%)	54.6	49.8	51.5	0.0391[1]
Age (month)	126 (3)	132 (6)	130 (6)	<0.0001[2]
High maternal education (%)	57.1	49.6	52.3	0.0008[3]
Asthma (DD) (%)	5.5	7	6.5	ns[1]
Hay fever (DD) (%)	11.6	12.6	12.3	ns[1]
Eczema (DD) (%)	6.5	8.9	8.1	ns[1]
IgE positive (%)	47.1	46.3	46.6	ns[1]
Margarine intake (g/d)	3 (5)	4 (7)	4 (7)	0.0001[2]
Total n-3/total n-6 ratio	0.153 (0.033)	0.148 (0.032)	0.149 (0.033)	0.0020[2]
ALA (n-3) (mg)	1099 (421)	1139 (470)	1125 (454)	ns[2]
EPA (n-3) (mg)	44.7 (41.5)	46.5 (59.4)	45.9 (53.8)	ns[2]
DHA (n-3) (mg)	90.7 (68)	93.4 (92.3)	92.4 (84.6)	ns[2]
LA (n-6) (mg)	8262 (3304)	8889 (4264)	8670 (3965)	0.0109[2]
AA (n-6) (mg)	182 (95)	196 (116)	191 (109)	0.0192[2]
DPA (n-6) (mg)	12.7 (9.6)	13.6 (12.9)	13.3 (11.8)	ns[2]

[1]*P*-value derived from Fisher's exact test.

[2]*P*-value derived from Wilcoxon's rank sum test.

[3]*P*-value derived from Pearson's chi-squared test.

ns, nonsignificant; DD, doctor diagnosed; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; AA, arachidonic acid; DPA, docosapentaenoic acid.

Table 2. Characteristics of the SNPs in the *FADS* gene cluster

			Number of subjects with				
			Genoype (%)			Allele (%)	
SNP	Alleles (major/minor) 1/2	*N*	11	12	22	1	2
rs174545	G/C	1817	828 (46%)	790 (43%)	199 (11%)	2446 (67%)	1188 (33%)
rs174546	G/A	1844	845 (46%)	799 (43%)	200 (11%)	2489 (67%)	1199 (33%)
rs174556	G/A	1835	922 (50%)	757 (41%)	156 (9%)	2601 (71%)	1069 (29%)
rs174561	A/G	1855	934 (50%)	765 (41%)	156 (8%)	2633 (71%)	1077 (29%)
rs174575	C/G	1970	1109 (56%)	728 (37%)	133 (7%)	2946 (75%)	994 (25%)
rs3834458	T/del	1967	908 (46%)	852 (43%)	207 (11%)	2668 (68%)	1266 (32%)

Study. Lewontin's *D*' ranged between 0.75 and 0.95 (GINIplus) and between 0.78 and 0.94 (LISAplus).

Median dietary fatty acid intake was not significantly increased in children with and without atopic diseases (Table 3), although children having asthma or eczema have a higher dietary margarine intake and the total n-3/total n-6 ratio is higher in children having hayfever.

The mean margarine intake is higher in homozygous minor allele carriers than in homozygous or heterozygous major allele carriers, also this association is only borderline significant for two SNPs (rs174545 and rs174546). After correction for multiple testing ($\alpha_{corr} = 0.05/2 = 0.025$), this difference is not significant anymore (supporting information, Table S1).

The prevalence of atopic outcomes stratified by genotype for the *FADS* gene cluster was not significantly associated with atopic diseases or allergic sensitization (Table 4). However, when analysing the influence of dietary fatty acid intake on atopic disease risk stratified by the *FADS* genotype, the total n-3/total n-6 ratio was positive associated with an increased risk of hayfever in homozygous major allele carriers ranging from an aOR of 1.25 (95%-CI: 1.00–1.57) to 1.31 (95%-CI: 1.01–1.69) across the six tested SNPs although this association was not significant anymore after correcting for multiple testing (supporting information, Table S2).

The impact of margarine consumption on atopic diseases and allergic sensitization is shown in Table 5. The total n-3/total n-6 ratio was inversely correlated with the daily margarine intake (Spearman's rank correlation $\rho = -0.14$, $P < 0.0001$).

Higher margarine intake was associated with a significantly increased risk for asthma among those who were homozygous for the major allele of each SNP [aOR between 1.17 (95%-CI: 1.03–1.34) and 1.23 (95%-CI: 1.06–1.40)]. This association remained significant for

Table 3. Median dietary fatty acid intake and atopy

	Total n-3/total n-6 ratio		Margarine (g/d)	
	Median	95%-CI	Median	95%-CI
Asthma (DD)				
No (n = 1815)	0.146	(0.144,0.147)	0.95	(0.79,1.11)
Yes (n = 126)	0.149	(0.144,0.155)	2.00	(1.12,2.88)
Hay fever (DD)				
No (n = 1689)	0.146	(0.144,0.147)	1.00	(0.83,1.17)
Yes (n = 236)	0.151	(0.147,0.156)	1.00	(0.56,1.44)
Eczema (DD)				
No (n = 1779)	0.146	(0.144,0.147)	0.93	(0.77,1.09)
Yes (n = 156)	0.149	(0.144,0.154)	1.43	(0.62,2.24)
IgE positive				
No (n = 910)	0.147	(0.145,0.150)	0.79	(0.58,1.01)
Yes (n = 794)	0.146	(0.144,0.149)	0.71	(0.48,0.95)

DD, doctor diagnosed.

three out of six SNPs after correction for multiple testing ($\alpha_{corr} = 0.0063$).

A higher risk for eczema was observed in individuals carrying the homozygous major allele (G/G) of SNP rs174556, although this effect did not reach significance after adjusting for multiple testing.

No association between margarine intake and atopic diseases or allergic sensitization was observed for homozygous or heterozygous minor allele carriers.

The analyses stratified for the GINIplus and LISAplus studies did not indicate heterogeneity (data not shown).

Haplotypes for the six *FADS* SNPs were computed using the EM algorithm (supporting information, Table S3). Only four haplotypes had a frequency $> 1\%$. The most common haplotype (66.7%) is carrying only major alleles and the next frequent haplotype (20.7%) is carrying only minor alleles at all loci.

There were no differences in the constructed haplotypes between the GINIplus and LISAplus studies.

Discussion

The present study investigated the influence of the *FADS1 FADS2* gene cluster on the association between dietary fatty acid intake and atopic diseases and allergic sensitization in 10-year-old children from the GINIplus and LISAplus birth cohort studies. The crude analysis showed no significant association between individuals with atopic characteristics and fatty acid intake. Further, there was no significant difference in the prevalence of atopic diseases dependent on the *FADS* genotype. However, in stratified analyses the total n-3/total n-6 ratio was positively associated with an increased risk for hayfever. This effect was significant for individuals carrying the major allele, but not after correcting for multiple testing. A higher daily margarine intake was a significant risk factor for asthma in major allele carriers.

Table 4. Prevalence of atopic characteristics stratified by genotype for *FADS* gene cluster

	Asthma (DD)		Hayfever (DD)		Eczema (DD)		IgE positive	
	%	(*n*/*N*)	%	(*n*/*N*)	%	(*n*/*N*)	%	(*n*/*N*)
rs174545								
C/C	4.5	(9/199)	12.1	(24/199)	7.5	(15/199)	38.7	(77/199)
C/G	6.2	(49/790)	11.0	(87/790)	8.0	(63/790)	40.3	(318/790)
G/G	7.4	(61/828)	12.3	(102/828)	7.7	(64/828)	39.5	(327/828)
rs174546								
A/A	4.5	(9/200)	12.0	(24/200)	7.5	(15/200)	38.5	(77/200)
A/G	6.1	(49/799)	11.0	(88/799)	8.0	(64/799)	39.9	(319/799)
G/G	7.2	(61/845)	12.4	(105/845)	7.7	(65/845)	39.6	(335/845)
rs174556								
A/A	3.8	(6/156)	10.3	(16/156)	9.6	(15/156)	39.7	(62/156)
A/G	5.8	(44/757)	11.0	(83/757)	7.1	(54/757)	39.0	(295/757)
G/G	7.5	(69/922)	12.9	(119/922)	8.0	(74/922)	40.7	(375/922)
rs174561								
G/G	5.1	(8/156)	9.6	(15/156)	9.6	(15/156)	39.7	(62/156)
A/G	5.5	(42/765)	10.8	(83/765)	7.3	(56/765)	39.3	(301/765)
A/A	7.3	(68/934)	12.7	(119/934)	8.1	(76/934)	40.0	(374/934)
rs174575								
G/G	5.3	(7/133)	12.8	(17/133)	9.0	(12/133)	36.8	(49/133)
C/G	5.4	(39/728)	10.9	(79/728)	8.2	(60/728)	42.3	(308/728)
C/C	6.9	(77/1109)	12.4	(137/1109)	7.4	(82/1109)	38.7	(429/1109)
rs3834458								
del/del	3.9	(8/207)	11.6	(24/207)	7.7	(16/207)	39.6	(82/207)
del/T	5.9	(50/852)	11.3	(96/852)	8.3	(71/852)	40.7	(347/852)
T/T	7.3	(66/908)	12.6	(114/908)	7.3	(66/908)	39.3	(357/908)

N: Total number of children stratified by *FADS* genotype.
n: Number of cases stratified by *FADS* genotype.
DD, doctor diagnosed.

Comparison with other studies

Sausenthaler et al. [2] reviewed findings from studies estimating the association of dietary fat intake with allergic diseases and allergic sensitization. Although the mechanism seems biological plausible, the evidence that n-6 PUFA intake is a risk factor and n-3 PUFA intake is a protective factor for allergic diseases remains scarce and the results are not conclusive. However, margarine intake has been positively linked to an increased risk for allergic sensitization and atopic diseases in children [2]. It was suggested that the high content of n-6 LA in margarine may have a negative effect on allergic disease development. However, it is not clear why a large proportion of studies analysing n-6 PUFA intake failed to find any association with atopic outcomes. One reason might be inter-individual genetic differences in fatty acid metabolism. In fact, our study indicates that *FADS* genotypes modulate the association between fatty acid intake and atopic diseases.

In our study, margarine consumption was positively associated with asthma, but only in individuals who are homozygous for the major allele. This is in line with

Table 5. Results of logistic regression models of daily margarine intake and atopic outcomes stratified by genotype

	Margarine (g/d)			Margarine (g/d)			Margarine (g/d)		
	C/C (n = 199)			C/G (n = 790)			G/G (n = 828)		
rs174545	aOR_{IQR}*	95%-CI	P-value†	aOR_{IQR}*	95%-CI	P-value†	aOR_{IQR}*	95%-CI	P-value†
Asthma (DD)	0.47	(0.16,1.40)	0.1747	1.02	(0.83,1.24)	0.8690	1.22	(1.06,1.40)	0.0051
Hay fever (DD)	1.19	(0.71,1.99)	0.5042	1.04	(0.88,1.23)	0.6288	1.03	(0.89,1.20)	0.6817
Eczema (DD)	1.14	(0.74,1.76)	0.5413	1.01	(0.83,1.22)	0.9586	1.12	(0.98,1.28)	0.1085
IgE positive	1.07	(0.84,1.36)	0.6090	1.10	(0.97,1.24)	0.1372	0.97	(0.88,1.08)	0.6303
	A/A (n = 200)			A/G (n = 799)			G/G (n = 845)		
rs174546	aOR_{IQR}*	95%-CI	P-value†	aOR_{IQR}*	95%-CI	P-value†	aOR_{IQR}*	95%-CI	P-value†
Asthma (DD)	0.47	(0.15,1.44)	0.1856	1.01	(0.83,1.23)	0.9332	1.22	(1.06,1.40)	0.0051
Hay fever (DD)	1.19	(0.71,2.00)	0.4988	1.04	(0.89,1.23)	0.5948	1.03	(0.88,1.19)	0.7286
Eczema (DD)	1.15	(0.75,1.78)	0.5173	0.99	(0.82,1.20)	0.9440	1.12	(0.97,1.28)	0.1188
IgE positive	1.09	(0.86,1.40)	0.4725	1.08	(0.96,1.22)	0.1923	0.97	(0.87,1.08)	0.5537
	A/A (n = 156)			A/G (n = 757)			G/G (n = 922)		
rs174556	aOR_{IQR}*	95%-CI	P-value†	aOR_{IQR}*	95%-CI	P-value†	aOR_{IQR}*	95%-CI	P-value†
Asthma (DD)	0.79	(0.32,1.97)	0.6118	0.96	(0.77,1.20)	0.7286	1.20	(1.05,1.37)	0.0073
Hay fever (DD)	0.75	(0.29,1.92)	0.5492	1.09	(0.93,1.28)	0.2650	1.00	(0.86,1.16)	0.9831
Eczema (DD)	1.09	(0.71,1.69)	0.6858	0.95	(0.76,1.17)	0.6116	1.15	(1.00,1.31)	0.0441
IgE positive	1.03	(0.79,1.33)	0.8426	1.08	(0.96,1.22)	0.2186	0.99	(0.89,1.09)	0.7887
	G/G (n = 156)			A/G (n = 765)			A/A (n = 934)		
rs174561	aOR_{IQR}*	95%-CI	P-value†	aOR_{IQR}*	95%-CI	P-value†	aOR_{IQR}*	95%-CI	P-value†
Asthma (DD)	0.67	(0.24,1.84)	0.4333	1.04	(0.85,1.28)	0.6880	1.17	(1.03,1.34)	0.0194
Hay fever (DD)	0.76	(0.30,1.96)	0.5713	1.09	(0.93,1.27)	0.2965	1.01	(0.87,1.17)	0.9437
Eczema (DD)	1.14	(0.74,1.74)	0.5602	0.97	(0.80,1.19)	0.7957	1.12	(0.98,1.28)	0.0898
IgE positive	1.02	(0.79,1.33)	0.8521	1.09	(0.97,1.23)	0.1623	0.98	(0.88,1.08)	0.6512

	G/G ($n = 133$)			C/G ($n = 728$)			C/C ($n = 1109$)		
rs174575	aOR_{IQR}*	95%-CI	*P*-value†	aOR_{IQR}*	95%-CI	*P*-value†	aOR_{IQR}*	95%-CI	*P*-value†
Asthma (DD)	0.68	(0.22,2.11)	0.4992	0.96	(0.77,1.20)	0.7192	1.17	(1.03,1.32)	0.0165
Hay fever (DD)	1.45	(0.76,2.76)	0.2626	1.07	(0.91,1.24)	0.4193	1.02	(0.89,1.16)	0.7801
Eczema (DD)	1.00	(0.51,1.95)	0.9991	1.01	(0.85,1.21)	0.8880	1.07	(0.93,1.22)	0.3492
IgE positive	1.00	(0.67,1.49)	0.9851	1.04	(0.93,1.16)	0.5261	1.01	(0.92,1.11)	0.7864
	del/del ($n = 207$)			del/T ($n = 852$)			T/T ($n = 908$)		
rs3834458	aOR_{IQR}*	95%-CI	*P*-value†	aOR_{IQR}*	95%-CI	*P*-value†	aOR_{IQR}*	95%-CI	*P*-value†
Asthma (DD)	0.57	(0.19,1.69)	0.3061	0.95	(0.77,1.16)	0.6053	1.22	(1.07,1.40)	0.0036
Hay fever (DD)	1.19	(0.72,1.96)	0.5021	1.07	(0.93,1.23)	0.3651	1.01	(0.87,1.17)	0.8917
Eczema (DD)	1.09	(0.71,1.67)	0.6999	0.92	(0.75,1.12)	0.3900	1.14	(1.00,1.30)	0.0536
IgE positive	1.00	(0.79,1.28)	0.9730	1.09	(0.98,1.22)	0.1118	0.96	(0.86,1.06)	0.4227

*Odds ratios per interquartile range increase adjusted for gender, age, maternal education and study centre. IQR (Margarine (g/d)) = 4.29 g/d.
†5% significance level adjusted for multiple testing: $\alpha_{corr} = 0.0063$.
DD, doctor diagnosed.

previous studies [2]. Although the fatty acid intake was calculated based on the dietary intake, we could not find any significant association between the total n-3/total n-6 ratio and asthma. It is likely that the negative effect of margarine consumption on allergic diseases is not exclusively caused by the high content of n-6 fatty acids.

In contrast to the theory by Black and Sharpe [1], a higher total n-3/total n-6 ratio did not show protective effects on atopic diseases and allergic sensitization. Actually, a higher total n-3/total n-6 ratio was linked to an increased risk for hayfever in individuals carrying the homozygous major allele. However, homozygous minor allele carriers also had an increased risk for hayfever, but this was nonsignificant, possibly due to loss of power from the reduced sample size.

Moltó-Puigmartí et al. [21] reported that *FADS* gene variants modify the association between fish intake and DHA contents in human milk lipids. The percentage contribution of DHA to total milk lipids was similar in women with low fish intake for all genotypes, whereas DHA increased with higher fish consumption in carriers of major but not of homozygous minor alleles. Lu et al. [22] examined the influence of the *FADS1* genotype on the association between dietary intake of n-6 and n-3 PUFAs and plasma concentrations of total, HDL- and non-HDL-cholesterol. Significant associations were observed in major allele carriers, but only in the groups with high dietary intake of n-6 and n-3 PUFAs and not in the groups with low intake. Both studies observed effects of a higher dietary intake only in individuals carrying the major allele. In the current study we also found significant effects only in homozygous major allele carriers.

In our study, margarine intake was positively associated with the development of atopic diseases. It was previously suggested that the high content of n-6 LA in margarine is associated with allergic diseases and symptoms. LA is a precursor of AA, which can act as a substrate of inflammatory eicosanoids. Thirty percent of the variance of AA is explained by genetic predisposition. In individuals carrying the major allele of the *FADS* gene cluster, a higher percentage of LA is metabolized to AA [7]. Therefore, it is hypothesized that the negative effect of margarine consumption on the development of atopic diseases is stronger in major allele carriers.

It is hypothesized that n-6 PUFA may increase the IgE synthesis and thus enhance the development of allergic diseases [4, 5]. Our data does not support this hypothesis. There was no association between fatty acid intake and increased specific IgE against food or inhalant allergens.

While the association between *FADS* genotype and fatty acid composition in serum phospholipids or plasma is well established [6], the association between *FADS* variants and atopic diseases is less clear. A study in adults reported a decreased risk for allergic rhinitis and atopic eczema in minor allele carriers [7], although the

associations did not reach the significance level after adjusting for multiple testing. Singmann et al. [23] investigated the association between five SNPs in the *FADS1 FADS2* gene cluster and doctor-diagnosed atopic diseases in 6-year-old children from the GINIplus and LISAplus birth cohort studies. None of the atopic outcomes were associated with any of the SNPs. Rzehak et al. [24] combined data from the LISAplus study in Munich and the KOALA study in Dutch children. There were no significant association between *FADS1 FADS2* variants and parental reported eczema during the first 2 years of life.

A review by Lattka et al. [25] reported a modulating effect of *FADS* genotypes on fatty acid related phenotypes such as intelligence development, risk of myocardial infarction and metabolic syndrome. This illustrates that *FADS* genotypes have a modulating effect on fatty acid metabolism and may explain the findings of this study.

The haplotype construction revealed four haplotypes with frequencies $> 1\%$. The two most frequent haplotypes are the same haplotypes Schaeffer et al. [7] computed in the 5-Locus haplotype analysis. The frequencies of these two haplotypes were also very similar to the haplotypes provided by Schaeffer et al. [7] (66.7% vs. 68.8% and 20.7% vs. 25.7%).

Strength and limitations

Blood levels of the fatty acids LA and ALA and their derivatives are influenced mainly by diet, but also by genetic variants [26]. Consequently, we assessed both dietary intake and the genotype. The FFQ used in the present study measured dietary intake over the past 12 months. We could not measure fatty acid blood levels over a year due to study constraints, so it was not possible to compare fatty acid intake to blood levels.

To evaluate the effect of the single *FADS* genotypes we stratified the data by alleles for analysis. Owing to this stratification the sample size was reduced, especially in the group of homozygous minor allele carriers. Depending on the different magnitudes of the prevalence of atopic outcomes, the retrospective assessment of the statistical power was heterogeneous. Additionally, the analysed *FADS* variants do not cover the complete *FADS* gene cluster as they were selected for genotyping based on previous publications [7].

Population stratification is present in Northern Europe [27] and also in Germany [28]. Although only Caucasians were included in both studies and the analyses were adjusted for study centre, population substructure might still be present. Steffens et al. [28] observed only low levels of population sub-structuring within the German population, therefore we do not think that a potential population substructure would affect our results substantially.

A major strength of our investigation compared with other studies is the assessment of the physician-diagnosed atopic diseases such as asthma, hayfever and eczema. Further, specific IgE against common food and inhalant allergens from blood samples were measured.

Conclusion and clinical relevance

The association between dietary intake of fatty acids and allergic diseases might be modulated by *FADS* gene variants in children.

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Contributors

M. S. carried out the statistical analysis and wrote the manuscript. J. H. and S. S. were involved in development of the statistical analysis plan and interpreting the results. E. L. and B. K. helped to interpret the results by revising the manuscript. N. K. performed the typing of the *FADS* variants. S. K., C. P. B., H.-E. W., A. v. B., D. B., U. K., B. S., S. R., O. H. and J. H. designed and/or conducted the study and revised the manuscript. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

GINIplus Study Group and LISAplus Study Group

GINIplus Study Group

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LISAplus Study Group

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Conflicts of interest

The authors' work was independent of the funders, who had no role in the study design, analysis of data, writing of the manuscript, or decision to submit for publication.

References

1 Black PN, Sharpe S. Dietary fat and asthma: is there a connection? *Eur Respir J* 1997; **10**:6–12.
2 Sausenthaler S, Koletzko B, Heinrich J. Dietary fat intake and allergic diseases. *Curr Nutr Food Sci* 2006; **2**:351–9.
3 Sala-Vila A, Miles EA, Calder PC. Fatty acid composition abnormalities in atopic disease: evidence explored and role in the disease process examined. *Clin Exp Allergy* 2008; **38**:1432–50.
4 Sprecher H. Biochemistry of essential fatty acids. *Prog Lipid Res* 1981; **20**:13–22.
5 Calder PC, Miles EA. Fatty acids and atopic disease. *Pediatr Allergy Immunol* 2000; **11** (Suppl 13):29–36.
6 Lattka E, Illig T, Koletzko B, Heinrich J. Genetic variants of the *FADS1 FADS2* gene cluster as related to essential fatty acid metabolism. *Curr Opin Lipidol* 2010; **21**:64–9.
7 Schaeffer L, Gohlke H, Müller M *et al.* Common genetic variants of the *FADS1 FADS2* gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum Mol Genet* 2006; **15**:1745–56.
8 Xie L, Innis SM. Genetic variants of the *FADS1 FADS2* gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. *J Nutr* 2008; **138**:2222–8.
9 Baylin A, Ruiz-Narvaez E, Kraft P, Campos H. alpha-Linolenic acid, Delta6-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction. *Am J Clin Nutr* 2007; **85**:554–60.
10 Malerba G, Schaeffer L, Xumerle L *et al.* SNPs of the *FADS* gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. *Lipids* 2008; **43**:289–9.
11 Koletzko B, Lattka E, Zeilinger S, Illig T, Steer C. Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the avon longitudinal study of parents and children. *Am J Clin Nutr* 2011; **93**:211–9.
12 Lattka E, Illig T, Heinrich J, Koletzko B. *FADS* gene cluster polymorphisms: important modulators of fatty acid levels and their impact on atopic diseases. *J Nutrigenet Nutrigenom* 2009; **2**:119–28.
13 Heinrich J, Bolte G, Hölscher B *et al.* Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur Respir J* 2002; **20**:617–23.
14 von Berg A, Koletzko S, Grübl A *et al.* The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol* 2003; **111**:533–40.
15 Bohlscheid-Thomas S, Hoting I, Boeing H, Wahrendorf J. Reproducibility and relative validity of food group intake in a food frequency questionnaire developed for the German part of the EPIC project. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol* 1997; **26** (Suppl 1):S59–S70.
16 Hartmann BM, Bell S, Vásquez-Caicedo AL, Götz A, Erhardt J, Brombach C.. Der Bundeslebensmittelschlüssel. German Nutrient Data Base. Karlsruhe. 2005
17 Stiegler P, Sausenthaler S, Buyken AE *et al.* A new FFQ designed to measure the intake of fatty acids and antioxidants in **children**. *Public Health Nutr* 2010; **13**:38–46.
18 Rzehak P, Heinrich J, Klopp N *et al.* Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (*FADS1 FADS2*) gene cluster and the fatty acid composition of erythrocyte membranes. *Br J Nutr* 2009; **101**:20–6.
19 Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 2004; **74**:765–9.
20 R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria; 2009. ISBN 3-900051-07-0
21 Moltó-Puigmartí C, Plat J, Mensink RP *et al. FADS1 FADS2* gene variants modify the association between fish intake and the docosahexaenoic acid proportions in human milk. *Am J Clin Nutr* 2010; **91**:1368–76.
22 Lu Y, Feskens EJ, Dollé ME *et al.* Dietary n-3 and n-6 polyunsaturated fatty acid intake interacts with *FADS1* genetic variation to affect total and HDL-cholesterol concentrations in the Doetinchem Cohort Study. *Am J Clin Nutr* 2010; **92**:258–65.
23 Singmann P, Rzehak P, Berdel D, Wichmann HE, Heinrich J. No association between *FADS* polymorphisms and atopic diseases in children from the GINI and LISA birth cohorts. *Allergy* 2010; **65**:1627–9.
24 Rzehak P, Thijs C, Standl M *et al.* Variants of the *FADS1 FADS2* gene cluster, blood levels of polyunsaturated fatty acids and eczema in children within the first 2 years of life. *PLoS ONE* 2010; **5**:e13261.

25 Lattka E, Illig T, Heinrich J, Koletzko B. Do *FADS* genotypes enhance our knowledge about fatty acid related phenotypes? *Clin Nutr* 2010; **29**:277–8.
26 Glaser C, Heinrich J, Koletzko B. Role of *FADS1* and *FADS2* polymorphisms in polyunsaturated fatty acid metabolism. *Metabolism* 2010; **59**:993–9.
27 Salmela E, Lappalainen T, Fransson I *et al*. Genome-wide analysis of single nucleotide polymorphisms uncovers population structure in Northern Europe. *PLoS One* 2008; **3**:e3519.
28 Steffens M, Lamina C, Illig T *et al*. SNP-based analysis of genetic substructure in the German population. *Hum Hered* 2006; **62**:20–9.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Pairwise LD pattern between the six SNPs in the *FADS* gene cluster measured by Lewontin's D' (a. LISAplus study, c. GINIplus study) and r^2 (b. LISAplus study, d. GINIplus study).
Table S1. Margarine intake [g/d] stratified by genotype.
Table S2. Logistic regression analyses of total n-3/total n-6 ratio and atopic outcomes.
Table S3. Haplotype characteristics for the haplotypes constructed based on the six SNPs in the *FADS1 FADS2* gene cluster with frequency $> 1\%$.

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Paper 1: Supplementary Material

Supplementary Material

Supplementary Table S1 Margarine intake [g/d] stratified by genotype

	Homozygous minor allele	Heterozygous allele	Homozygous major allele	p-value[1]
rs174545				
Mean (25%Q.,75%Q.)	4.21 (0.08,4.71)	3.99 (0.00,4.57)	3.60 (0.00,4.03)	0.0469
rs174546				
Mean (25%Q.,75%Q.)	4.12 (0.08,4.71)	4.04 (0.00,4.62)	3.59 (0.00,4.03)	0.0470
rs174556				
Mean (25%Q.,75%Q.)	4.15 (0.08,4.43)	4.05 (0.00,4.86)	3.59 (0.00,4.00)	0.0951
rs174561				
Mean (25%Q.,75%Q.)	3.97 (0.07,4.33)	4.11 (0.00,5.00)	3.60 (0.00,4.00)	0.1005
rs174575				
Mean (25%Q.,75%Q.)	4.01 (0.14,4.71)	3.99 (0.00,4.41)	3.65 (0.00,4.13)	0.0859
rs3834458				
Mean (25%Q.,75%Q.)	4.01 (0.07,4.45)	4.04 (0.00,4.57)	3.54 (0.00,4.00)	0.0838

[1] p-value derived from Kruskal-Wallis test

Supplementary Table S2 Logistic regression analyses of total n-3/total n-6 ratio and atopic outcomes

rs174545	total n-3/total n-6 ratio C/C (n=199)			total n-3/total n-6 ratio C/G (n=790)			total n-3/total n-6 ratio G/G (n=828)		
	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]
Asthma (DD)	1.44	(0.51,4.06)	0.4948	0.97	(0.67,1.41)	0.8777	1.27	(0.91,1.75)	0.1567
Hay fever (DD)	1.46	(0.78,2.71)	0.2346	1.06	(0.80,1.41)	0.6862	1.29	(1.00,1.68)	0.0526
Eczema (DD)	1.89	(0.85,4.20)	0.1195	0.93	(0.67,1.30)	0.6795	0.98	(0.70,1.37)	0.9114
IgE positive	0.88	(0.57,1.35)	0.5610	0.91	(0.75,1.11)	0.3601	0.91	(0.74,1.10)	0.3245

rs174546	A/A (n=200)			A/G (n=799)			G/G (n=845)		
	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]
Asthma (DD)	1.33	(0.47,3.73)	0.5873	0.98	(0.68,1.42)	0.9227	1.28	(0.92,1.77)	0.1375
Hay fever (DD)	1.46	(0.78,2.71)	0.2359	1.07	(0.81,1.42)	0.6444	1.31	(1.01,1.69)	0.0410
Eczema (DD)	1.84	(0.83,4.08)	0.1340	0.92	(0.67,1.28)	0.6348	0.97	(0.70,1.36)	0.8748
IgE positive	0.86	(0.56,1.32)	0.4852	0.93	(0.76,1.12)	0.4379	0.93	(0.76,1.12)	0.4398

rs174556	A/A (n=156)			A/G (n=757)			G/G (n=922)		
	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]
Asthma (DD)	0.84	(0.23,3.12)	0.7995	0.93	(0.62,1.38)	0.7087	1.30	(0.96,1.75)	0.0903
Hay fever (DD)	1.32	(0.65,2.70)	0.4399	1.04	(0.77,1.40)	0.8118	1.31	(1.03,1.66)	0.0291
Eczema (DD)	1.88	(0.85,4.14)	0.1168	0.97	(0.68,1.38)	0.8700	0.94	(0.69,1.28)	0.6734
IgE positive	0.85	(0.53,1.35)	0.4804	0.96	(0.78,1.18)	0.7072	0.88	(0.74,1.06)	0.1876

rs174561	G/G (n=156)			A/G (n=765)			A/A (n=934)		
	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]
Asthma (DD)	1.35	(0.47,3.92)	0.5795	0.85	(0.57,1.29)	0.4523	1.30	(0.96,1.76)	0.0879
Hay fever (DD)	1.50	(0.71,3.15)	0.2881	1.04	(0.77,1.41)	0.7800	1.30	(1.03,1.65)	0.0296
Eczema (DD)	1.97	(0.90,4.31)	0.0897	0.96	(0.68,1.36)	0.8260	0.94	(0.69,1.27)	0.6765
IgE positive	0.88	(0.55,1.40)	0.5908	0.99	(0.81,1.22)	0.9440	0.88	(0.74,1.06)	0.1814

rs174575	G/G (n=133)			C/G (n=728)			C/C (n=1109)		
	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]
Asthma (DD)	1.00	(0.30,3.37)	0.9971	1.08	(0.72,1.62)	0.7172	1.18	(0.89,1.58)	0.2515
Hay fever (DD)	1.40	(0.68,2.91)	0.3616	1.06	(0.79,1.44)	0.6871	1.25	(1.00,1.57)	0.0518
Eczema (DD)	1.31	(0.56,3.08)	0.5302	0.95	(0.67,1.35)	0.7805	0.96	(0.72,1.29)	0.8039
IgE positive	0.87	(0.51,1.46)	0.5889	0.92	(0.75,1.13)	0.4409	0.94	(0.79,1.11)	0.4685

rs3834458	del/del (n=207)			del/T (n=852)			T/T (n=908)		
	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]	aOR_{IQR}[$]	95%-CI	p-value[$$]
Asthma (DD)	0.67	(0.22,2.07)	0.4853	1.08	(0.76,1.54)	0.6725	1.27	(0.93,1.73)	0.1309
Hay fever (DD)	1.46	(0.79,2.71)	0.2238	1.05	(0.80,1.38)	0.7171	1.29	(1.01,1.65)	0.0430
Eczema (DD)	1.74	(0.81,3.72)	0.1560	0.94	(0.69,1.28)	0.6712	0.95	(0.68,1.32)	0.7515
IgE positive	0.88	(0.58,1.33)	0.5348	0.92	(0.76,1.11)	0.3830	0.95	(0.79,1.14)	0.5780

[$] **Odds ratios per interquartile range increase adjusted for gender, age, maternal education and study centre. IQR(total n-3/total n-6 ratio) = 0.041**

[$$] **5% significance level adjusted for multiple testing: α_{corr}=0.0063**

DD doctor diagnosed

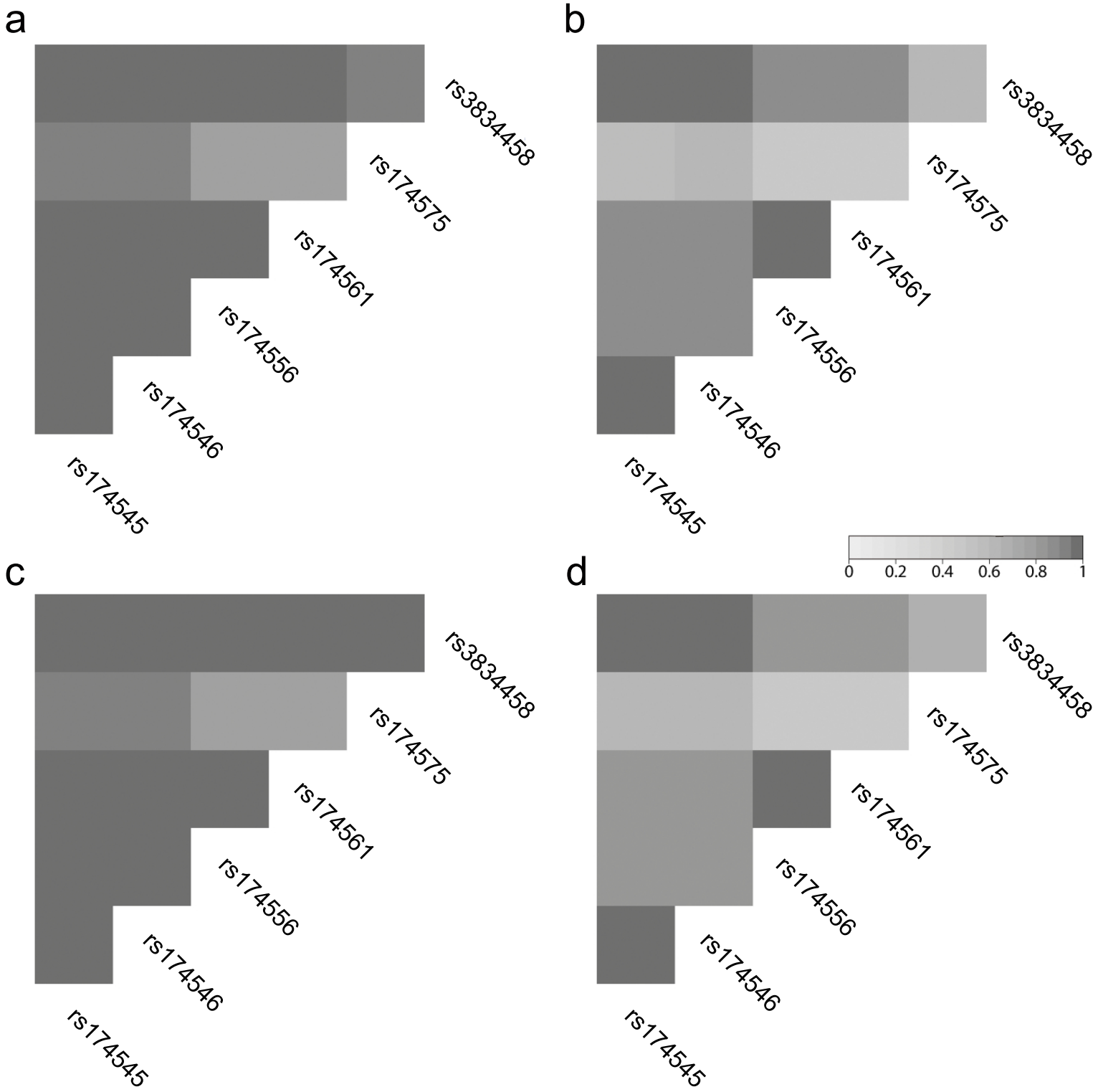
Supplementary Table S3 Haplotype characteristics for the haplotypes constructed based on the six SNPs in the *FADS1 FADS2* gene cluster with frequency >1%.

	Haplotype	Alleles (1: major/ 2: minor)	Frequency (%)
LISAplus			
Major allele haplotype	GGGACT	111111	67.1
Minor allele haplotype	CAAGGdel	222222	20.4
Haplotype 1	CAAGCdel	222212	8.2
Haplotype 2	CAGAGdel	221122	2.7
GINIplus			
Major allele haplotype	GGGACT	111111	66.5
Minor allele haplotype	CAAGGdel	222222	20.9
Haplotype 1	CAAGCdel	222212	7.9
Haplotype 2	CAGAGdel	221122	3.4
Total			
Major allele haplotype	GGGACT	111111	66.7
Minor allele haplotype	CAAGGdel	222222	20.7
Haplotype 1	CAAGCdel	222212	8.0
Haplotype 2	CAGAGdel	221122	3.1

Legend to Figure

Supplementary Figure 1

Pairwise LD pattern between the six SNPs in the *FADS* gene cluster measured by Lewontin's D' (a. LISAplus study, c. GINIplus study) and r^2 (b. LISAplus study, d. GINIplus study).


a
rs3834458
rs174575
rs174561
rs174556
rs174546
rs174545
b
rs3834458
rs174575
rs174561
rs174556
rs174546
rs174545
0
0.2
0.4
0.6
0.8
1
c
rs3834458
rs174575
rs174561
rs174556
rs174546
rs174545
d
rs3834458
rs174575
rs174561
rs174556
rs174546
rs174545

5 Paper 2: *FADS* Variants, Exclusive Breastfeeding and Asthma in Children
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FADS gene cluster modulates the effect of breastfeeding on asthma. Results from the GINIplus and LISAplus studies

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Abstract

Background: The protective effect of breastfeeding (BF) on the development of asthma has been widely recognized, even if not all results have been consistent. Gene variants of the *FADS* gene cluster have a major impact on fatty acid composition in blood and in breast milk. Therefore, we evaluated the influence of the *FADS1 FADS2* gene cluster polymorphisms on the association between BF and asthma.

Methods: The analysis was based on data ($N = 2245$) from two German prospective birth cohort studies. Information on asthma and BF during the first 6 months was collected using questionnaires completed by the parents. Logistic regression modelling was used to analyse the association between exclusive BF and ever having asthma stratified by genotype.

Results: In the stratified analyses, BF for 3 or 4 months after birth had a protective effect for heterozygous and homozygous carriers of the minor allele (adjusted odds ratio between 0.37 (95% CI: 0.18–0.80) and 0.42 (95% CI: 0.20–0.88). Interaction terms of BF with genotype were significant and ranged from −1.17 (*P*-value: 0.015) to −1.33 (0.0066). Moreover, heterozygous and homozygous carriers of the minor allele who were exclusively breastfed for 5 or 6 months after birth had a reduced risk of asthma [0.32 (0.18–0.57) to 0.47 (0.27–0.81)] in the stratified analyses. For individuals carrying the homozygous major allele, BF showed no significant effect on the development of asthma.

Conclusions: The association between exclusive BF and asthma is modified by the genetic variants of *FADS* genotypes in children.

Breastfeeding (BF) is widely recognized to have beneficial effects on asthma and atopy (1), although not all results are conclusive (2) and the underlying biological mechanism is not entirely clear. Among other factors in breast milk, the composition of polyunsaturated fatty acids (PUFA) of breast milk has been proposed to cause the protective effect (3).

Linoleic acid (LA, 18:2n-6), the most common dietary n-6 PUFA, is metabolized to arachidonic acid (AA, 20:4n-6). Arachidonic acid can act as substrate of inflammatory eicosanoids. Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), products of the metabolism of the essential n-3 fatty acid α-linolenic acid (ALA,

18:3n-3), have been suggested to have beneficial effects on allergic inflammation. n-6 and n-3 fatty acids use the same enzymatic pathway (4, 5). This led to the hypothesis that n-6 PUFA intake may enhance the development of allergic diseases in susceptible individuals. In contrast, n-3 PUFA are suggested to have protective effects against allergic diseases (6).

The fatty acid desaturase 1 and 2 genes (*FADS1* and *FADS2*) encode the enzymes delta-5-desaturase and delta-6-desaturase, respectively, which regulate the conversion of the precursor essential fatty acids to long chain metabolites (7). Several studies have shown strong associations between the *FADS* gene cluster and fatty acid levels in serum phospholipids (8, 9), plasma and adipose tissue samples (10), erythrocyte cell membranes (9, 11), breast milk (9, 12, 13) and red blood cell lipids (14). Carriers of the minor allele exhibit increased levels of desaturase substrates and decreased levels of desaturase products. This might arise from lowered transcriptional levels or diminished conversion rates of the enzymes in individuals carrying the minor alleles.

Inter-individual genetic differences in fatty acid metabolism might be one of the reasons for the controversial association between BF and atopic phenotypes (3). Therefore, we investigated whether the association between exclusive BF and later development of asthma could be modified by genetic variants of the *FADS* gene cluster.

Methods

Study population

Data from two ongoing German birth cohort studies were included in this investigation, the German LISAplus (Lifestyle Related Factors on the Immune System and the Development of Allergies in Childhood) and GINIplus (German Infant Nutritional Intervention) studies. LISAplus is a population-based birth cohort study. A total of 3097 neonates were recruited between 1997 and 1999 in Munich, Leipzig, Wesel and Bad Honnef. The participants were not preselected based on family history of allergic diseases (15). A total of 5991 mothers and their newborns were recruited to the GINIplus study between September 1995 and June 1998 in Munich and Wesel. Infants with at least one allergic parent and/or sibling were allocated to the interventional study arm investigating the effect of different hydrolysed formulas for allergy prevention in the first year of life (16). All children without a family history of allergic diseases and children whose parents did not give consent for the intervention were allocated to the noninterventional arm. Detailed descriptions of the LISAplus and GINIplus studies have been published elsewhere [(15) and (16), respectively].

In both studies, only individuals with Caucasian German descent were included.

For both studies, approval by the local ethics committees (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia) and written consent from participant's families were obtained.

Definition of exclusive breastfeeding

Questions on infant feeding during the first 6 months of life were answered by the parents during the 1-year follow-up in the GINIplus study and during the 6-month follow-up in the LISAplus study. The parents were asked for each of the first 6 months of life whether the newborns were exclusively breastfed, exclusively bottle-fed or both breastfed and bottle-fed. Mutually exclusive categories were built based on the number of months the children were exclusively breastfed after birth (no exclusive BF, up to 2 months, up to 4 months or more than 4 months of exclusive BF). Children who were exclusively bottle-fed or mixed breastfed and bottle-fed were categorized as the reference group 'No exclusive BF' to reach sufficient numbers for the reference group.

Genotyping

Six single-nucleotide polymorphisms (SNPs) of the *FADS1 FADS2* gene cluster (rs174545, rs174546, rs174556, rs174561, rs174575 and rs3834458) were typed. Five of these variants (rs174545, rs174546, rs174556, rs174561 and rs3834458) have been previously shown to be in strong linkage disequilibrium (LD) with each other ($r^2 > 0.7$, D' > 0.9) (8). These five SNPs were selected based on the previous publications in adult populations (8, 17). Moreover, we included the SNP rs174575. In addition, applying the tagger server program (http://www.broadinstitute.org/mpg/tagger/) in combination with HapMap, we found that with the three SNPs rs174545, rs174546 and rs174556 we could tag 27 SNPs between base pair positions 61234329 and 61372379 of *FADS1 FADS2*. The efficiency was 10.7-fold although the two further SNPs rs174561 and rs3834458 could not be included as these are not included in the HapMap database. Genotyping of SNPs was realized with the iPLEX (Sequenom, San Diego, CA, USA) method by means of matrix-assisted laser desorption ionization–time of flight mass spectrometry method (MALDI-TOF MS, Mass Array; Sequenom) in one laboratory according to the manufacturer's instructions. Standard genotyping quality control included 10% duplicate and negative samples. Genotyping discordance rate was below 0.3%.

Definition of outcome variables

Information on ever having physician-diagnosed asthma was collected using self-administered questionnaires completed by the parents. The questionnaires were completed at 6, 12, 18 and 24 months and 4, 5, 6 and 10 years of age in the LISAplus study and 1, 2, 3, 4, 6 and 10 years in the GINIplus study. Each questionnaire asked for information pertaining to the timeframe since the previous follow-up. Based on these questions for asthma for each year of age up to 10 years, a binary outcome variable for ever having a diagnosis was defined. Blood was collected at age 6 and 10 years. Specific serum IgE concentrations were assayed by the CAP-RAST FEIA system (Pharmacia Diagnostics, Freiburg, Germany) according to the manufacturer's instructions. Screening tests were used to test allergic sensitization against food allergens

(fx5: egg, cow milk, wheat, peanut, soybean and codfish) and inhalant allergens (sx1: *Dermatophagoides pteronyssinus*, cat, dog, rye, timothy grass, *Cladosporium herbarum*, birch and mugwort). The limit of detection for allergen-specific IgE was 0.35 kU/l.

Atopic asthma was defined as ever having physician-diagnosed asthma and having an IgE value exceeding the detection limit in at least one of both RAST tests at 6 or 10 years of age. Nonatopic asthma was defined as ever having physician-diagnosed asthma and having an IgE value below the detection limit in both RAST tests administered at 6 and 10 years of age.

Statistical analysis

Preliminary analyses showed similar results for the homozygous and heterozygous minor allele carriers. Therefore, we assumed a dominant model and compared homozygous or heterozygous minor allele carriers with homozygous major allele carriers.

Multiple logistic regression analysis stratified by genotype was applied to estimate the adjusted odds ratios (aOR) with 95% confidence intervals (CI) for the association between exclusive BF and asthma. Additionally, the interaction between *FADS* genotype and category of BF was tested.

Statistical significance was defined by a two-sided alpha level of 5%. According to Nyholt (18), the number of effective loci of the six SNPs in the *FADS* gene cluster was computed as 2. To correct for multiple testing, the alpha level is divided by the number of effective loci, which leads to a corrected two-sided alpha level of 5%/2 = 2.5%.

All models are adjusted for gender, age, maternal education level (low, medium and high), study centre (Munich, Leipzig, Wesel and Bad Honnef), presence of older siblings (yes/no) and study (GINI intervention, GINI nonintervention, LISA). In bivariate analyses, testing was performed using Pearson's chi-squared test. Statistical analysis was performed using the statistical software R, version 2.13.1 (http://www.R-project.org) (19).

Results

Complete information on BF, *FADS1 FADS2* genotype and asthma was available for 2245 children [1456 (65%) children from the GINIplus study and 789 (35%) children from the LISAplus study]. Basic characteristics of the study population are presented in Table 1. Forty-nine per cent of the neonates were exclusively breastfed for at least 5 months after birth. The prevalence for doctor-diagnosed asthma up to 10 years of age is in total 11%.

The genotype and allele frequencies of the six SNPs that were included in the analysis are shown in Table 2. Five of the six SNPs (rs174545, rs174546, rs174556, rs174561 and rs3834458) are in high LD with each other. For these five SNPs, the pairwise squared correlations r^2 ranged between 0.84 and 0.99 and Lewontin's D' ranged between 0.99 and 1. For rs174575, the LB is lower. The pairwise correlation r^2 for this SNP ranged between 0.49 and 0.64 and Lewontin's D' ranged between 0.77 and 0.95.

Table 1 Basic characteristics of the study population

	LISAplus (n = 789)	GINIplus (n = 1456)	Total (n = 2245)
Boys	56%	50%	52%
Intervention group	0%	50%	32%
High maternal education	58%	50%	53%
Presence of older siblings	47%	48%	48%
Study centre			
München	53%	56%	55%
Leipzig	25%	0%	9%
Bad Honnef	13%	0%	5%
Wesel	9%	44%	32%
Breastfeeding (BF)			
Number of months of exclusive BF	18%	26%	23%
1–2	13%	11%	12%
3–4	18%	16%	17%
5–6	51%	47%	48%
Asthma (DD)	9%	12%	11%
Atopic asthma (DD)	7%	8%	8%
Nonatopic asthma (DD)	1%	3%	2%

The association between asthma and exclusive BF is presented in Table 3. Asthma prevalence is decreasing with increasing duration of exclusive BF (P = 0.0172). Table 4 shows the asthma prevalence stratified by genotype. The asthma prevalence is lower in minor allele carriers than in homozygous major allele carriers, although this effect is nonsignificant.

Figure 1 shows the association between asthma prevalence and the number of months of exclusive BF stratified by genotype for each of the six SNPs. The asthma prevalence is reduced in children who were exclusively breastfed for at least 3 months and are carrying the minor allele, whereas no effect is observed in homozygous major allele carriers.

The results of logistic regression models of exclusive BF on asthma stratified by genotype show similar effects (Table 5). Individuals carrying the minor allele have a significant decreased asthma risk if they are exclusively breastfed for 3 or 4 months [aOR between 0.37 (95% CI: 0.18–0.80) and 0.42 (95% CI: 0.20–0.88)] or more than 5 months [0.32 (0.18–0.57) to 0.47 (0.27–0.81)]. These associations remained significant after correction for multiple testing (α_{corr} = 0.025).

In children carrying at least one minor allele of the *FADS* variants rs174545, rs174546, rs174556, rs174561 and rs3834458, asthma risk reduction is strongest for exclusive BF for 3 or 4 months after birth. Only for minor allele carriers of one SNP, rs174575, the asthma risk is further decreased for further extended exclusive BF. Additionally, adjusting for parental atopy did not modify these associations substantially.

The results of a stratified analysis for atopic (Supplementary Table S2) and nonatopic asthma (Supplementary Table S3) did not change substantially. In an additional model, the

Table 2 Characteristics of the SNPs in the *FADS* gene cluster

SNP	Alleles (major/minor) 1/2	*N*	Number of subjects with Genotype (%) 11	12/22	Allele (%) 1	2
rs174545	G/C	2047	931 (45%)	1116 (55%)	2757 (67%)	1337 (33%)
rs174546	G/A	2076	946 (46%)	1130 (54%)	2799 (67%)	1353 (33%)
rs174556	G/A	2069	1033 (50%)	1036 (50%)	2927 (71%)	1211 (29%)
rs174561	A/G	2082	1040 (50%)	1042 (50%)	2951 (71%)	1213 (29%)
rs174575	C/G	2212	1236 (56%)	976 (44%)	3300 (75%)	1124 (25%)
rs3834458	T/del	2211	1016 (46%)	1195 (54%)	2995 (68%)	1427 (32%)

SNP, single-nucleotide polymorphisms.

Table 3 Prevalence of doctor-diagnosed asthma stratified by number of months of exclusive breastfeeding

	Number of months of exclusive BF	1–2	3–4	5–6	
	% (*n*/*N*)	% (*n*/*N*)	% (*n*/*N*)	% (*n*/*N*)	*P*-value*
Asthma ever (DD)					
No	86.0 (442/513)	87.0 (233/268)	89.0 (338/379)	91.0 (988/1085)	0.0172
Yes	14.0 (71/513)	13.0 (35/268)	11.0 (41/379)	9.0 (97/1085)	

BF, breastfeeding.
*Chi-squared test.

Table 4 Prevalence of doctor-diagnosed asthma stratified by genotype

	Asthma ever (DD)	
	% (*n*/*N*)	*P*-value*
rs174545		
Allele 12/22	10.5 (117/1116)	0.3372
Allele 11	11.9 (111/931)	
rs174546		
Allele 12/22	10.4 (118/1130)	0.3105
Allele 11	11.9 (113/946)	
rs174556		
Allele 12/22	10.0 (104/1036)	0.1190
Allele 11	12.3 (127/1033)	
rs174561		
Allele 12/22	10.1 (105/1042)	0.1790
Allele 11	12.0 (125/1040)	
rs174575		
Allele 12/22	10.2 (100/976)	0.4944
Allele 11	11.2 (139/1236)	
rs3834458		
Allele 12/22	10.0 (120/1195)	0.2062
Allele 11	11.8 (120/1016)	

*Chi-squared test.

interaction between *FADS* genotype and exclusive BF was tested. In Supplementary Table S1, only the interactive effects are presented. These effects also show a highly decreased risk of asthma in children carrying at least one minor allele who are breastfed for more than 3 months. After correction for multiple testing ($\alpha_{corr} = 0.025$), the interaction is significant for children breastfed for 3 to 4 months for all of the six tested SNPs [interaction effect ranging from −1.17 ($P = 0.015$) to −1. 33 ($P = 0.0066$)]. The asthma risk is also decreased in minor allele carriers who are breastfed for more than 5 months [−0.64 ($P = 0.0861$) to −1.33 ($P = 0.0005$)]. After correction for multiple testing, the interaction is significant for four SNPs (rs174545, rs174546, rs174575 and rs3834458).

Discussion

The present study investigated the modulating effect of the *FADS* gene cluster on the association between exclusive BF and the development of asthma up to 10 years of age. Exclusive BF for more than 3 months was found to reduce the risk of asthma in homozygous or heterozygous carriers of the minor allele. In homozygous major allele carriers, no effect of the duration of exclusive BF on the development of asthma was observed. We identified a strong interaction of the association between exclusive BF and asthma by *FADS* gene polymorphism in children.

Comparison with other studies

We could not find another study that investigated the association between BF, *FADS* genotype and atopy. Two studies reported a modulating effect of the *FADS* genotype on the association between BF and IQ (20, 21). Caspi et al. (20)

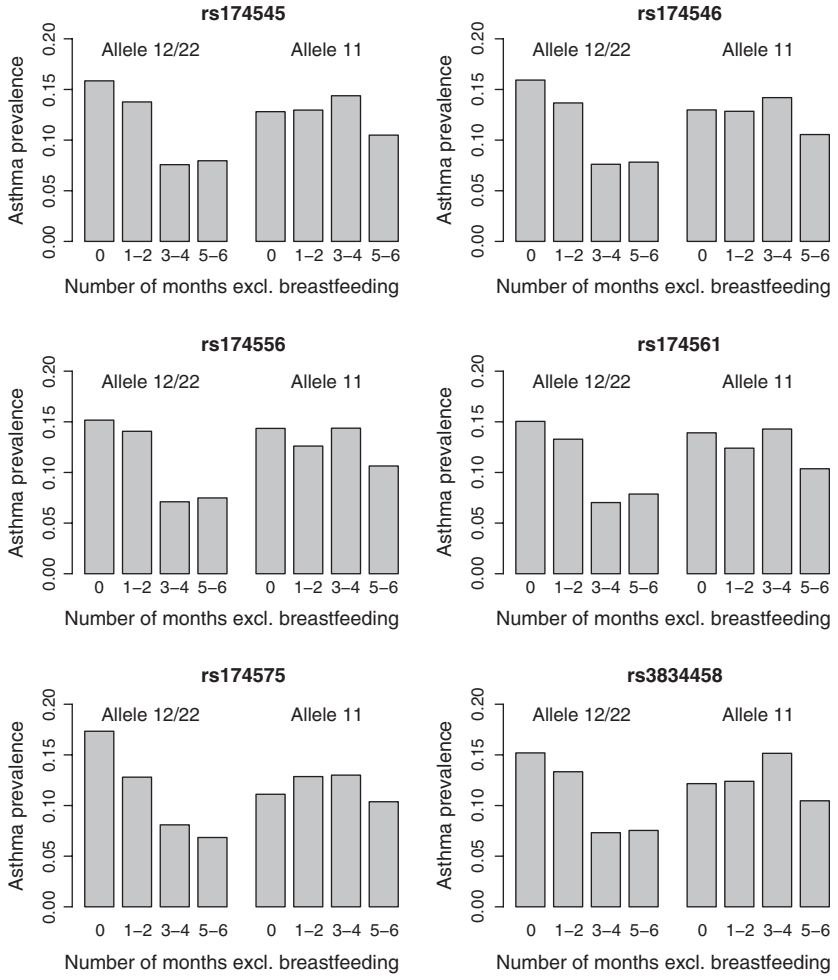

Figure 1 Asthma prevalence stratified by *FADS* genotype and breastfeeding (1: major allele, 2: minor allele).

found no significant differences in IQ in homozygous minor allele carriers of rs174575 but a beneficial effect of BF on IQ scores in homozygous or heterozygous major allele carriers. In contrast, Steer et al. (21) could not replicate the results by Caspi et al. In this study, BF was associated with higher IQ scores at 8 years of age in homozygous minor allele carriers.

While the association between *FADS* genotype and fatty acid composition in serum phospholipids or plasma is well established (7), the association between *FADS* variants and atopic diseases or other fatty acid-related phenotypes is less clear (22, 23). Singmann et al. (24) investigated the association between five SNPs in the *FADS1 FADS2* gene cluster and doctor-diagnosed atopic diseases in 6-year-old children from the GINIplus and LISAplus birth cohort studies. Schaeffer et al. (8) reported a decreased risk of allergic rhinitis and atopic eczema in minor allele carriers in adults, although the associations did not reach the significance level after adjusting for multiple testing.

More than 3 months of exclusive BF was protective for the development of asthma in minor allele carriers, but there was no significant difference whether minor allele carriers were exclusively breastfed for 3–4 months or more than 4 months. This is in line with the results of the review by Kramer et al. (1). There was no difference in the development of allergies after exclusive BF for 6 months compared with 3–4 months. We did not find a significant association for only 1 or 2 months of exclusive BF in our study. As the reference group is not defined by exclusive bottle-feeding only, the difference between these two groups may be too small to be detected as statistical significant.

Additionally, the innate immune system of neonates is skewed towards Th2 responses. The stimulation of the immune system in early childhood redirects the balance between Th1 and Th2 cells (25). So, it might be possible that the beneficial effects of BF occur during the development of the immune system after the third month.

The sensitivity analysis for atopic and nonatopic asthma did not show different effects for atopic and nonatopic asthma. Looking at the point estimates for both outcomes, there seems to be a stronger protective effect in particular for minor allele carriers exclusively breastfed for 3–4 months for nonatopic asthma, but the numbers are much too small for statistical approval.

Table 5 Results of logistic regression models of breastfeeding (BF) on asthma stratified by genotype, adjusted for gender, study centre, maternal education level, study (GINI intervention, GINI nonintervention, LISA) and presence of older siblings (reference category: never exclusive breastfeeding)

		Never exclusive BF	1–2 months exclusive BF		3–4 months exclusive BF		5–6 months exclusive BF	
	N	aOR	aOR (95% CI)	*P*-value*	aOR(95% CI)	*P*-value*	aOR (95% CI)	*P*-value*
rs174545								
Allele 12/22	1073	1	0.89 (0.48, 1.66)	0.7120	**0.38** (0.19, 0.76)	0.0062	**0.41** (0.24, 0.69)	0.0007
Allele 11	905	1	1.33 (0.61, 2.89)	0.4686	1.47 (0.75, 2.92)	0.2639	1.07 (0.60, 1.91)	0.8177
rs174546								
Allele 12/22	1085	1	0.90 (0.48, 1.68)	0.7462	**0.38** (0.19, 0.77)	0.0073	**0.41** (0.24, 0.68)	0.0006
Allele 11	919	1	1.33 (0.62, 2.89)	0.4640	1.48 (0.75, 2.92)	0.2600	1.09 (0.61, 1.94)	0.7725
rs174556								
Allele 12/22	997	1	0.98 (0.51, 1.87)	0.9413	**0.37** (0.18, 0.80)	0.0107	**0.41** (0.24, 0.72)	0.0018
Allele 11	1000	1	1.16 (0.56, 2.38)	0.6947	1.37 (0.73, 2.57)	0.3308	0.95 (0.56, 1.62)	0.8609
rs174561								
Allele 12/22	1003	1	1.02 (0.53, 1.95)	0.9624	**0.39** (0.18, 0.83)	0.0148	**0.47** (0.27, 0.81)	0.0065
Allele 11	1008	1	1.14 (0.55, 2.34)	0.7224	1.38 (0.73, 2.59)	0.3200	0.94 (0.55, 1.59)	0.8039
rs174575								
Allele 12/22	934	1	0.81 (0.41, 1.59)	0.5388	**0.42** (0.20, 0.88)	0.0224	**0.32** (0.18, 0.57)	0.0001
Allele 11	1204	1	1.44 (0.74, 2.81)	0.2844	1.32 (0.72, 2.41)	0.3706	1.17 (0.71, 1.94)	0.5372
rs3834458								
Allele 12/22	1149	1	0.94 (0.51, 1.73)	0.8338	**0.40** (0.20, 0.81)	0.0104	**0.42** (0.25, 0.71)	0.0011
Allele 11	988	1	1.25 (0.59, 2.68)	0.5609	1.44 (0.75, 2.76)	0.2745	1.07 (0.61, 1.86)	0.8189

aOR, adjusted odds ratios; 1, major allele; 2, minor allele.
*Estimates reaching significance after correcting for multiple testing (α_{corr} = 0.05/2 = 0.025) are marked in bold.

The underlying biological mechanism that causes the association between BF, *FADS1 FADS2* genotype and asthma is not completely clear although there are a number of biologically plausible indicators (3, 26). Minor allele carriers have a lower proportion of products of the fatty acid metabolism and therefore a lower proportion of AA, a product of the n-6 pathway which may reduce the risk of asthma. But this is highly speculative as the underlying biological mechanisms that cause these associations are unknown.

Strength and limitations

The fatty acid composition of the breast milk varies depending on the *FADS* genotype of the mother (9, 12, 13). The breast milk of women carrying the homozygous minor allele contains lower proportions of products of the fatty acid metabolism compared to the breast milk of woman carrying the major allele. As we do not know the genotype of the mother, we cannot take the variation of the fatty acid composition in the breast milk into account.

The prevalence of doctor-diagnosed asthma is in total 11% up to 10 years of age. Owing to this low prevalence, the outcome variable was defined as ever having a diagnosis, and it was not possible to apply a more appropriate longitudinal model. Indeed, Scholtens et al. (27) could show in a longitudinal analysis that BF for more than 16 weeks decreases the risk of asthma until 8 years of age, but no age-dependent differences.

Additionally, the percentage of exclusively bottle-fed neonates was very low in our study. Therefore, the reference category 'No exclusive BF' covers all children that were exclusively bottle-fed and both breastfed and bottle-fed after birth. Thus, the effect estimates of our study might even underestimate the true magnitude, if a bottle-fed-only group could have been used. Further, the asthma definition is based on parental report of a doctor diagnosis for each year up to 10 years of age, but there was no clinical ascertainment of the parentally reported diagnosis. A major strength of our study is the prospective design and the long-term follow-up until 10 years of a large study population.

Conclusion

The association between exclusive BF and asthma is modified by the genetic variants of *FADS* genotypes in children. Our results suggest that only minor allele carriers benefit from exclusive BF in regard to asthma development, while homozygous major allele carriers have no advantage in this respect. This might explain the partly inconsistent results from previous studies on BF and asthma prevalence, which suggests the inclusion of genetic data in future studies.

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Authors contributions

MS carried out the statistical analysis and wrote the manuscript. JH and SS were involved in the development of the statistical analysis plan and interpreting the results. EL and BK helped to interpret the results by revising the manuscript. NK performed the typing of the *FADS* variants. SK, CPB, HEW, AvB, DB, UK, BS, IL, OH and JH designed and/or conducted the study and revised the manuscript. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

Conflict of interest statement

None declared.

Supporting Information

Additional Supporting Information may be found in the online version of this article found at: http://www.wileyonlinelibrary.com

Table S1. Results of logistic regression models of exclusive breastfeeding (BF) and SNP interaction on asthma, adjusted for gender, study centre, maternal education level, study (GINI intervention, GINI non-intervention, LISA) and presence of older siblings (reference category: never excl. BF and major allele).

Table S2. Results of logistic regression models of breastfeeding (BF) on atopic asthma stratified by genotype, adjusted for gender, study centre, maternal education level, study (GINI intervention, GINI non-intervention, LISA) and presence of older siblings (reference category: never exclusive breastfeeding).

Table S3. Results of logistic regression models of breastfeeding (BF) on non-atopic asthma stratified by genotype, adjusted for gender, study centre, maternal education level, study (GINI intervention, GINI non-intervention, LISA) and presence of older siblings (reference category: never exclusive breastfeeding).

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References

1. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev* 2002;**1**:CD003517.
2. Fewtrell M, Wilson DC, Booth I, Lucas A. Six months of exclusive breast feeding: how good is the evidence? *BMJ* 2011;**342**:c5955.
3. Friedman NJ, Zeiger RS. The role of breast-feeding in the development of allergies and asthma. *J Allergy Clin Immunol* 2005;**115**:1238–1248.
4. Sprecher H. Biochemistry of essential fatty acids. *Prog Lipid Res* 1981;**20**:13–22.
5. Calder PC, Miles EA. Fatty acids and atopic disease. *Pediatr Allergy Immunol* 2000;**11**(Suppl 13):29–36.
6. Sausenthaler S, Koletzko B, Heinrich J. Dietary fat intake and allergic diseases. *Curr Nutr Food Sci* 2006;**2**:351–359.
7. Lattka E, Illig T, Koletzko B, Heinrich J. Genetic variants of the *FADS1 FADS2* gene cluster as related to essential fatty acid metabolism. *Curr Opin Lipidol* 2010;**21**:64–69.
8. Schaeffer L, Gohlke H, Müller M, Heid IM, Palmer LJ, Kompauer I et al. Common genetic variants of the *FADS1 FADS2* gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum Mol Genet* 2006;**15**:1745–1756.
9. Xie L, Innis SM. Genetic variants of the *FADS1 FADS2* gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in

breast milk during lactation. *J Nutr* 2008;**138**:2222–2228.
10. Baylin A, Ruiz-Narvaez E, Kraft P, Campos H. alpha-Linolenic acid, Delta6-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction. *Am J Clin Nutr* 2007;**85**:554–560.
11. Malerba G, Schaeffer L, Xumerle L, Klopp N, Trabetti E, Biscuola M et al. SNPs of the *FADS* gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. *Lipids* 2008;**43**:289–299.
12. Lattka E, Rzehak P, Szabó É, Jakobik V, Weck M, Weyermann M et al. Genetic variants in the FADS gene cluster are associated with arachidonic acid concentrations of human breast milk at 1.5 and 6 mo postpartum and influence the course of milk dodecanoic, tetracosenoic, and trans-9-octadecenoic acid concentrations over the duration of lactation. *Am J Clin Nutr* 2011;**93**:382–391.
13. Moltó-Puigmartí C, Plat J, Mensink RP, Müller A, Jansen E, Zeegers MP et al. *FADS1 FADS2* gene variants modify the association between fish intake and the docosahexaenoic acid proportions in human milk. *Am J Clin Nutr* 2010;**91**:1368–1376.
14. Koletzko B, Lattka E, Zeilinger S, Illig T, Steer C. Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children. *Am J Clin Nutr* 2011;**93**:211–219.
15. Heinrich J, Bolte G, Hölscher B, Douwes J, Lehmann I, Fahlbusch B et al. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur Respir J* 2002;**20**:617–623.
16. von Berg A, Koletzko S, Grübl A, Filipiak-Pittroff B, Wichmann HE, Bauer CP et al. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol* 2003;**111**:533–540.
17. Rzehak P, Heinrich J, Klopp N, Schaeffer L, Hoff S, Wolfram G et al. Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (*FADS1 FADS2*) gene cluster and the fatty acid composition of erythrocyte membranes. *Br J Nutr* 2009;**101**:20–26.
18. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 2004;**74**:765–769.
19. R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2011. ISBN 3-900051-07-0.
20. Caspi A, Williams B, Kim-Cohen J, Craig IW, Milne BJ, Poulton R et al. Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism. *Proc Natl Acad Sci USA* 2007;**104**:18860–18865.
21. Steer CD, Smith GD, Emmett PM, Hibbeln JR, Golding J. *FADS2* polymorphisms modify the effect of breastfeeding on child IQ. *PLoS ONE* 2010;**5**:e11570.
22. Lattka E, Illig T, Heinrich J, Koletzko B. *FADS* gene cluster polymorphisms: important modulators of fatty acid levels and their impact on atopic diseases. *J Nutrigenet Nutrigenomics* 2009;**2**:119–128.
23. Lattka E, Illig T, Heinrich J, Koletzko B. Do *FADS* genotypes enhance our knowledge about fatty acid related phenotypes? *Clin Nutr* 2010;**29**:277–287.
24. Singmann P, Rzehak P, Berdel D, Wichmann HE, Heinrich J. No association between *FADS* polymorphisms and atopic diseases in children from the GINI and LISA birth cohorts. *Allergy* 2010;**65**:1627–1629.
25. Belderbos M, Levy O, Bont L. Neonatal innate immunity in allergy development. *Curr Opin Pediatr* 2009;**21**:762–769.
26. Oddy WH. Breast feeding and childhood asthma. *Thorax* 2009;**64**:558–559.
27. Scholtens S, Wijga AH, Brunekreef B, Kerkhof M, Hoekstra MO, Gerritsen J et al. Breast feeding, parental allergy and asthma in children followed for 8 years. The PIAMA birth cohort study. *Thorax* 2009;**64**:604–609.

Paper 2: Supplementary Material

Supplementary Table 1 Results of logistic regression models of exclusive breastfeeding (BF) and SNP interaction on asthma, adjusted for gender, study centre, maternal education level, study (GINI intervention, GINI non-intervention, LISA) and presence of older siblings (reference category: never excl. BF and major allele)

	1-2 months excl. bf			3-4 months excl. bf			5-6 months excl. bf		
	Estimate	**Sd**	**p-value[$]**	**Estimate**	**Sd**	**p-value[$]**	**Estimate**	**Sd**	**p-value[$]**
rs174545									
Allele 12 /22 vs. Allele 11	-0.34	0.50	0.4914	**-1.33**	0.49	0.0066	**-0.91**	0.38	0.0168
rs174546									
Allele 12 /22 vs. Allele 11	-0.34	0.50	0.4964	**-1.31**	0.49	0.0069	**-0.95**	0.38	0.0122
rs174556									
Allele 12 /22 vs. Allele 11	-0.13	0.49	0.7858	**-1.30**	0.50	0.0087	-0.79	0.38	0.0365
rs174561									
Allele 12 /22 vs. Allele 11	-0.07	0.49	0.8861	**-1.27**	0.50	0.0108	-0.64	0.37	0.0861
rs174575									
Allele 12 /22 vs. Allele 11	-0.56	0.48	0.2414	**-1.17**	0.48	0.0150	**-1.33**	0.38	0.0005
rs3834458									
Allele 12 /22 vs. Allele 11	-0.26	0.49	0.5984	**-1.24**	0.48	0.0095	**-0.87**	0.37	0.0187

[$] Significance level after correcting for multiple testing: $\alpha_{corr}=0.05/2=0.025$

1: major allele, 2: minor allele

Supplementary Table 2 Results of logistic regression models of breastfeeding (BF) on atopic asthma stratified by genotype, adjusted for gender, study centre, maternal education level, study (GINI intervention, GINI non-intervention, LISA) and presence of older siblings (reference category: never exclusive breastfeeding)

		never excl. BF	1-2 months excl. BF			3-4 months excl. BF			5-6 months excl. BF		
	N	aOR	aOR	95%-CI	p-value[$]	aOR	95%-CI	p-value[$]	aOR	95%-CI	p-value[$]
rs174545											
Allele 12 / 22	1039	1	0.74	(0.34,1.63)	0.4548	0.43	(0.19,0.96)	0.0394	**0.44**	(0.23,0.81)	0.0082
Allele 11	885	1	1.33	(0.57,3.08)	0.5060	1.05	(0.47,2.30)	0.9127	1.11	(0.59,2.08)	0.7442
rs174546											
Allele 12 / 22	1051	1	0.75	(0.34,1.66)	0.4794	0.44	(0.19,0.98)	0.0446	**0.43**	(0.23,0.80)	0.0078
Allele 11	899	1	1.33	(0.58,3.08)	0.5000	1.05	(0.48,2.32)	0.8955	1.13	(0.61,2.12)	0.6948
rs174556											
Allele 12 / 22	967	1	0.72	(0.31,1.65)	0.4332	0.42	(0.18,0.98)	0.0453	**0.38**	(0.20,0.74)	0.0045
Allele 11	976	1	1.29	(0.58,2.84)	0.5337	1.07	(0.51,2.27)	0.8573	1.10	(0.61,1.98)	0.7440
rs174561											
Allele 12 / 22	973	1	0.72	(0.31,1.66)	0.4390	0.42	(0.18,0.98)	0.0446	**0.41**	(0.21,0.78)	0.0070
Allele 11	984	1	1.27	(0.58,2.79)	0.5548	1.08	(0.51,2.30)	0.8328	1.08	(0.60,1.95)	0.7951
rs174575											
Allele 12 / 22	905	1	0.79	(0.34,1.84)	0.5881	0.56	(0.24,1.32)	0.1850	**0.35**	(0.17,0.71)	0.0037
Allele 11	1175	1	1.24	(0.59,2.60)	0.5690	0.93	(0.46,1.88)	0.8482	1.10	(0.64,1.90)	0.7333
rs3834458											
Allele 12 / 22	1112	1	0.85	(0.39,1.83)	0.6698	0.47	(0.21,1.07)	0.0715	**0.44**	(0.23,0.83)	0.0110
Allele 11	967	1	1.17	(0.52,2.64)	0.7028	1.03	(0.49,2.15)	0.9420	1.02	(0.56,1.84)	0.9513

[$] Significance level after correcting for multiple testing: $\alpha_{corr}=0.05/2=0.025$

1: major allele, 2: minor allele

Supplementary Table 3 Results of logistic regression models of breastfeeding (BF) on non-atopic asthma stratified by genotype, adjusted for gender, study centre, maternal education level, study (GINI intervention, GINI non-intervention, LISA) and presence of older siblings (reference category: never exclusive breastfeeding)

		never excl. BF	1-2 months excl. BF			3-4 months excl. BF			5-6 months excl. BF		
	N	aOR	aOR	95%-CI	p-value[$]	aOR	95%-CI	p-value[$]	aOR	95%-CI	p-value[$]
rs174545											
Allele 12 / 22	997	1	1.08	(0.35,3.38)	0.8893	0.30	(0.06,1.47)	0.1384	0.50	(0.19,1.33)	0.1645
Allele 11	814	1	1.27	(0.11,14.83)	0.8470	4.87	(0.87,27.16)	0.0712	1.42	(0.26,7.76)	0.6871
rs174546											
Allele 12 / 22	1009	1	1.09	(0.35,3.40)	0.8797	0.31	(0.06,1.49)	0.1439	0.49	(0.18,1.31)	0.1538
Allele 11	827	1	1.28	(0.11,14.86)	0.8431	4.80	(0.86,26.74)	0.0734	1.43	(0.26,7.84)	0.6817
rs174556											
Allele 12 / 22	929	1	1.74	(0.50,6.03)	0.3811	0.25	(0.03,2.11)	0.2005	0.80	(0.27,2.39)	0.6915
Allele 11	900	1	0.48	(0.05,4.33)	0.5160	2.11	(0.58,7.59)	0.2543	0.53	(0.14,1.95)	0.3397
rs174561											
Allele 12 / 22	934	1	2.17	(0.59,7.95)	0.2413	0.32	(0.04,2.83)	0.3052	1.16	(0.37,3.64)	0.8004
Allele 11	909	1	0.47	(0.05,4.23)	0.5033	2.10	(0.58,7.56)	0.2581	0.53	(0.14,1.94)	0.3369
rs174575											
Allele 12 / 22	873	1	0.85	(0.25,2.94)	0.8027	0.16	(0.02,1.32)	0.0880	0.36	(0.12,1.05)	0.0622
Allele 11	1094	1	2.42	(0.33,17.81)	0.3862	5.07	(0.98,26.16)	0.0523	2.89	(0.61,13.66)	0.1807
rs3834458											
Allele 12 / 22	1075	1	0.98	(0.32,2.99)	0.9686	0.29	(0.06,1.39)	0.1226	0.54	(0.22,1.36)	0.1914
Allele 11	890	1	2.52	(0.15,42.45)	0.5202	8.55	(0.93,78.24)	0.0574	3.62	(0.42,31.22)	0.2411

[$] Significance level after correcting for multiple testing: $\alpha_{corr}=0.05/2=0.025$

1: major allele, 2: minor allele

6 Paper 3: Meta-analysis of Genome-wide Association Studies on Atopic Dermatitis

(Paternoster* & Standl* et al. Nature Genetics, 2012)

Original title:	Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis
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Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis

Atopic dermatitis (AD) is a commonly occurring chronic skin disease with high heritability. Apart from filaggrin (*FLG*), the genes influencing atopic dermatitis are largely unknown. We conducted a genome-wide association meta-analysis of 5,606 affected individuals and 20,565 controls from 16 population-based cohorts and then examined the ten most strongly associated new susceptibility loci in an additional 5,419 affected individuals and 19,833 controls from 14 studies. Three SNPs reached genome-wide significance in the discovery and replication cohorts combined, including rs479844 upstream of *OVOL1* (odds ratio (OR) = 0.88, $P = 1.1 \times 10^{-13}$) and rs2164983 near *ACTL9* (OR = 1.16, $P = 7.1 \times 10^{-9}$), both of which are near genes that have been implicated in epidermal proliferation and differentiation, as well as rs2897442 in *KIF3A* within the cytokine cluster at 5q31.1 (OR = 1.11, $P = 3.8 \times 10^{-8}$). We also replicated association with the *FLG* locus and with two recently identified association signals at 11q13.5 (rs7927894; $P = 0.008$) and 20q13.33 (rs6010620; $P = 0.002$). Our results underline the importance of both epidermal barrier function and immune dysregulation in atopic dermatitis pathogenesis.

Atopic dermatitis, or eczema, is one of the most common chronic inflammatory skin diseases, with prevalence rates of up to 20% in children and 3% in adults. It commonly starts during infancy and frequently precedes or co-occurs with food allergy, asthma and rhinitis[1].

Atopic dermatitis has a broad spectrum of clinical manifestations and is characterized by dry skin, intense pruritus and a typical age-related distribution of inflammatory lesions that are frequently superinfected by bacteria and viruses[1]. Profound alterations in skin barrier function and immunologic abnormalities are considered to be key components affecting the development and severity of atopic dermatitis, but the exact cellular and molecular mechanisms remain incompletely understood[1].

There is substantial evidence supporting the idea of a strong genetic component in atopic dermatitis; however, it is not well understood how genetic susceptibility contributes to the development of this condition[2,3]. To date, only null mutations in the *FLG* gene encoding the epidermal structural protein filaggrin have been established as major risk factors[4,5].

The only genome-wide association study (GWAS) of atopic dermatitis in European populations identified a new susceptibility locus at 11q13.5 downstream of *C11orf30* (ref. 6). A second GWAS, recently carried out in a Chinese Han population, identified two new susceptibility loci, one of which (rs6010620 at 20q13.33) also showed evidence for association in a German sample[7]. In a collaborative effort to identify additional risk genes for atopic dermatitis, we conducted a well-powered, two-stage genome-wide association meta-analysis for the EAGLE Consortium.

In the discovery analysis of 5,606 individuals with atopic dermatitis (cases) and 20,565 controls from 16 population-based cohorts of European descent (**Supplementary Tables 1** and **2**), there was little evidence for population stratification at the study level (genomic inflation factor, $\lambda_{GC} \leq 1.08$) or at the meta-analysis level ($\lambda_{GC} = 1.02$), and we detected an excess of association signals beyond those expected by chance (**Supplementary Figs. 1** and **2**).

SNPs from two regions reached genome-wide significance ($P < 5 \times 10^{-8}$) in the discovery meta-analysis (**Fig. 1** and **Supplementary Table 3**): rs7000782 (8q21.13 near *ZBTB10*; OR = 1.14, $P = 1.6 \times 10^{-8}$) and rs9050 (1q21.3 near *TCHH*; OR = 1.33, $P = 1.9 \times 10^{-8}$). Given the proximity of rs9050 to the well-established atopic dermatitis susceptibility gene *FLG*[4,5], we evaluated whether the observed association was due to linkage disequilibrium (LD) with *FLG* mutations. Despite low correlation between rs9050 and the two most prevalent *FLG* mutations in ALSPAC cases ($r^2 = 0.257$ for p.Arg501* (c.1501C>T) and $r^2 = 0.001$

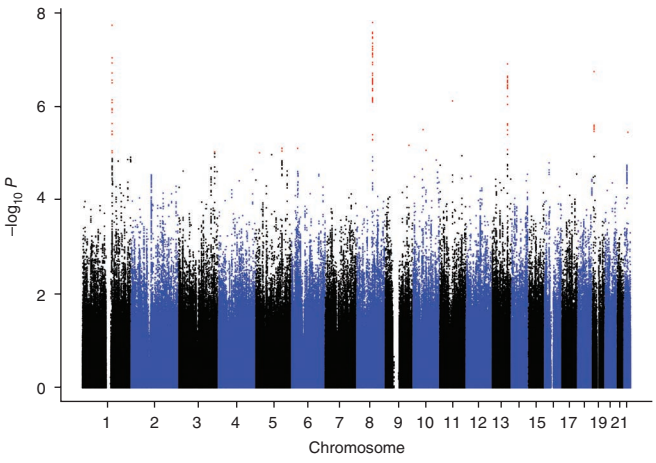

Figure 1 Manhattan plot for the discovery genome-wide association meta-analysis of atopic dermatitis after excluding all SNPs with minor allele frequency (MAF) <1% and r^2 <0.3 or proper info <0.4. $\lambda_{GC} = 1.017$. SNPs with $P < 1 \times 10^{-5}$ are shown in red.

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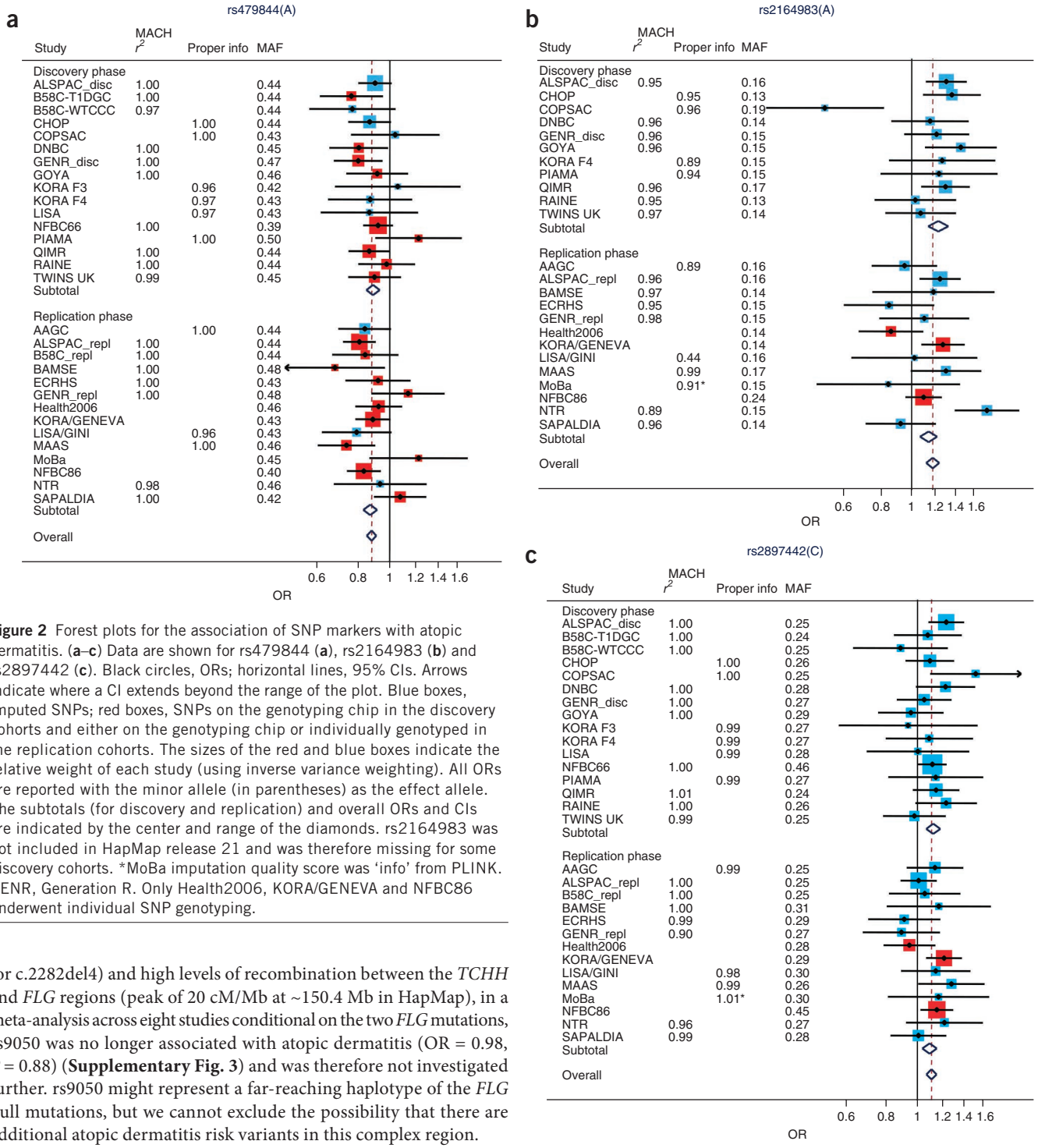

Figure 2 Forest plots for the association of SNP markers with atopic dermatitis. (**a**–**c**) Data are shown for rs479844 (**a**), rs2164983 (**b**) and rs2897442 (**c**). Black circles, ORs; horizontal lines, 95% CIs. Arrows indicate where a CI extends beyond the range of the plot. Blue boxes, imputed SNPs; red boxes, SNPs on the genotyping chip in the discovery cohorts and either on the genotyping chip or individually genotyped in the replication cohorts. The sizes of the red and blue boxes indicate the relative weight of each study (using inverse variance weighting). All ORs are reported with the minor allele (in parentheses) as the effect allele. The subtotals (for discovery and replication) and overall ORs and CIs are indicated by the center and range of the diamonds. rs2164983 was not included in HapMap release 21 and was therefore missing for some discovery cohorts. *MoBa imputation quality score was 'info' from PLINK. GENR, Generation R. Only Health2006, KORA/GENEVA and NFBC86 underwent individual SNP genotyping.

for c.2282del4) and high levels of recombination between the *TCHH* and *FLG* regions (peak of 20 cM/Mb at ~150.4 Mb in HapMap), in a meta-analysis across eight studies conditional on the two *FLG* mutations, rs9050 was no longer associated with atopic dermatitis (OR = 0.98, $P = 0.88$) (**Supplementary Fig. 3**) and was therefore not investigated further. rs9050 might represent a far-reaching haplotype of the *FLG* null mutations, but we cannot exclude the possibility that there are additional atopic dermatitis risk variants in this complex region.

The 11q13.5 locus previously reported to be associated in the only other European GWAS on atopic dermatitis to date[6] was confirmed in our meta-analysis (rs7927894: OR = 1.07, 95% confidence interval (CI)1.02–1.12, $P = 0.008$) (**Supplementary Fig. 4**). Association with the rs6010620 variant, which was reported in a recent Chinese GWAS[7], was similarly confirmed (OR = 1.09, 95% CI 1.03–1.15, $P = 0.002$).

Of the 15 loci reported to be associated with asthma or total serum immunoglobulin E (IgE) levels in a recent GWAS[8], two showed suggestive evidence for association with atopic dermatitis (*IL13*: rs1295686, $P = 0.0008$ and rs20541, $P = 0.0007$; *STAT6*: rs167769, $P = 0.0379$) (**Supplementary Table 4**).

After excluding the rs9050 SNP, we attempted to replicate the remaining 10 most strongly associated loci ($P < 1 \times 10^{-5}$ in the discovery meta-analysis) (**Fig. 2**, **Table 1**, **Supplementary Fig. 5** and **Supplementary Table 3**) in 5,419 cases and 19,833 controls from 14 studies (**Supplementary Tables 1** and **2**). Three of the ten SNPs showed significant association after conservative Bonferroni correction ($P < 0.05/10 = 0.005$) in the replication meta-analysis (with the same direction of effect as in the discovery meta-analysis), including rs479844 near *OVOL1*, rs2164983 near *ACTL9* and rs2897442 in intron 8 of *KIF3A* (**Fig. 2** and **Table 1**). All three SNPs reached

Table 1 Discovery and replication results for loci associated with atopic dermatitis

Chr.	SNP	Position (bp)	Gene	Effect allele	Other allele	Effect allele freq.	Stage	*N*	OR (95% CI)	*P* value	P_{het}
11	rs479844	65,308,533	*OVOL1*	A	G	0.44	I	26,151	0.89 (0.85–0.93)	7.8×10^{-7}	
							II	25,098	0.87 (0.83–0.92)	2.4×10^{-8}	
							I + II	51,249	0.88 (0.85–0.91)	1.1×10^{-13}	0.23
19	rs2164983[a]	8,650,381	*ACTL9*	A	C	0.15	I	17,403	1.22 (1.13–1.32)	1.8×10^{-7}	
							II	22,996	1.11 (1.04–1.19)	0.002	
							I + II	40,399	1.16 (1.10–1.22)	7.1×10^{-9}	0.004
5	rs2897442	132,076,926	*KIF3A*	C	T	0.29	I	26,164	1.12 (1.07–1.18)	7.8×10^{-6}	
							II	25,064	1.09 (1.04–1.15)	0.001	
							I + II	51,228	1.11 (1.07–1.15)	3.8×10^{-8}	0.52

Results are for the fixed-effect, inverse variance meta-analysis, with genomic control applied to the individual studies in the discovery meta-analysis. Chr., chromosome; Stage I, discovery screen; stage II, replication; stages I + II, combined analysis; *N*, number of subjects in each analysis. The heterogeneity *P* value (P_{het}) for overall heterogeneity between all discovery and replication studies was generated using Cochran's *Q* test for heterogeneity. All ORs are given with the minor allele representing the effect allele.
[a]rs2164983 was not included in HapMap release 21 and was therefore missing for some discovery cohorts. This SNP showed evidence of heterogeneity ($P = 0.004$). The random-effects combined (I + II) result for this SNP was OR = 1.14 (95%CI 1.05–1.24), $P = 0.001$.

genome-wide significance in the combined meta-analysis of the discovery and replication sets: rs479844 with OR = 0.88, $P = 1.1 \times 10^{-13}$; rs2164983 with OR = 1.16, $P = 7.1 \times 10^{-9}$; and rs2897442 with OR = 1.11, $P = 3.8 \times 10^{-8}$. In contrast, rs7000782, which had reached genome-wide significance in the discovery analysis, showed no evidence of association in the replication cohort ($P = 0.296$). There was no evidence of interaction between the three replicated SNPs (**Supplementary Table 5**).

rs479844 (at 11q13.1) is located <3 kb upstream of *OVOL1*. The pattern of LD is complex at this locus, but there is a low recombination rate between rs479844 and *OVOL1* in Europeans (**Supplementary Fig. 2**). *OVOL1* belongs to a highly conserved family of genes involved in regulation of the development and differentiation of epithelial tissues and germ cells[9–11]. The encoded OVOL1 protein functions as a c-Myc repressor in keratinocytes, is activated by the β-catenin–LEF1 complex during epidermal differentiation and is a downstream target of the Wg-Wnt and TGF-β–BMP7–Smad4 developmental signaling pathways[10,12,13]. Apart from their role in the organogenesis of skin and skin appendages[14,15], these pathways are also implicated in postnatal regulation of epidermal proliferation and differentiation[16–18]. Disruption of *Ovol1* in mice leads to keratinocyte hyperproliferation, hair shaft abnormalities, kidney cysts and defective spermatogenesis[10,11]. In addition, OVOL1 regulates loricrin (*LOR*) expression, thereby preventing premature terminal differentiation[10]. Thus, it is reasonable to speculate that variation at this locus might influence epidermal proliferation and/or differentiation, which are known to be disturbed in atopic dermatitis. Analysis of transcript levels of all genes within 500 kb of rs479844 in Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) from 949 ALSPAC individuals revealed a significant association ($P = 7 \times 10^{-5}$) between rs479844 and the nearby *DKFZp761E198* locus, which encodes a hypothetical protein. Likewise, analysis of SNP-transcript pairs in the Multiple Tissue Human Expression Resource (MuTHER) pilot database of skin genome-wide expression quantitative trait loci (eQTL) from 160 samples[19] provided suggestive evidence for an association with *DKFZp761E198* in the same direction in one of the twin sets (**Supplementary Fig. 6**). Further investigations are needed to clarify whether the causal variant(s) at this locus exerts its effect by regulating the expression of the *DKFZp761E198* transcript.

rs2164983 (at 19p13.2) is located in an intergenic region 70 kb upstream of *ADAMTS10* and 18 kb downstream of *ACTL9* (encoding a hypothetical protein). ADAMTS proteins are a group of complex, secreted zinc-dependent metalloproteinases, which bind to and cleave extracellular matrix components and are involved in connective tissue remodeling and extracellular matrix turnover[20,21]. Actin proteins have well-characterized cytoskeletal functions, are important for the maintenance of epithelial morphology and cell migration and have also been implicated in nuclear activities[22–24]. The low recombination rate between rs2164983 and *ACTL9* and the recombination peak between this SNP and *ADAMTS10* in the HapMap reference set of Utah residents of Northern and Western European descent (CEU) (**Supplementary Fig. 2**) suggests the functional variant may be located within the *ACTL9* region. There was no evidence for association between rs2164983 and altered expression of genes within 500 kb of this marker in either the ALSPAC LCL eQTL analysis or the MuTHER skin eQTL data (**Supplementary Fig. 6**).

rs2897442 is located in intron 8 of *KIF3A*, which encodes a subunit of the kinesin II complex required for the assembly of primary cilia, which are essential for Hedgehog signaling and are implicated in β-catenin–dependent Wnt signaling to induce expression of a variety of genes that influence proliferation and apoptosis[25,26]. Of note, *KIF3A* is located at 5q31, which is characterized by a complex LD pattern and contains a cluster of cytokine and immune-related genes. This chromosomal location has been linked to several autoimmune and inflammatory diseases, including psoriasis[27,28], Crohn's disease[29,30] and asthma[8,29,31] (**Supplementary Table 4**). In particular, distinct functional *IL13* (interleukin 13) variants have been associated with asthma susceptibility[32]. Although rs2897442 is within the *KIF3A* gene, there is little recombination between this locus and *IL4* (encoding interleukin 4). There is a recombination peak between this region and *IL13* (**Supplementary Fig. 7a**); however, there also seems to be a secondary association signal in the *IL13-RAD50* region, and when making association conditional on rs2897442 in the discovery meta-analysis, the signal in the *IL13-RAD50* region is still present, providing evidence of two independent signals (**Supplementary Fig. 7b**). In an attempt to further refine the association at this locus, we analyzed Immunochip data from 1,553 German atopic dermatitis cases and 3,640 population controls, of whom 767 cases and 983 controls were part of the replication stage. The Immunochip is a custom-content Illumina iSelect array that focuses on genes involved in autoimmune disorders and offers an increased resolution at 5q31. In the population tested, the strongest association signal was seen for the rs848 SNP in *IL13* ($P = 1.93 \times 10^{-10}$), which is in high LD with the functional *IL13* variant rs20541 ($r^2 = 0.979$; $D' = 0.995$). Additional significantly associated signals were observed for a cluster of tightly linked variants in *IL4* (lead SNP rs66913936: $P = 2.58 \times 10^{-8}$) and *KIF3A* (rs2897442: $P = 8.84 \times 10^{-7}$) (**Supplementary Fig. 8** and **Supplementary Tables 6** and **7**). Whereas rs2897442 showed only weak LD with rs848

($r^2 = 0.160$; $D' = 0.483$), it was strongly correlated with rs66913936 ($r^2 = 0.858$; $D' = 0.982$). Likewise, pairwise genotype-conditioned analyses showed that the significant association of rs2897442 with atopic dermatitis was abolished upon conditioning for rs66913936, whereas there an association signal remained after conditioning for rs848 (**Supplementary Tables 6** and **7**). Analysis of the expression levels of all genes within 500 kb of rs2897442 in LCLs derived from ALSPAC individuals revealed a modest association between rs2897442 and *IL13* transcript levels ($P = 2.7 \times 10^{-3}$). No association with transcript levels for any gene within 500 kb of the proxy variant, rs2299009, ($r^2 = 1$) was seen in the MuTHER skin eQTL data (**Supplementary Fig. 6**). However, this result does not exclude the possibilities that this variation may cause a regulatory effect in another tissue or physiological state, that this variant or causative variants in LD may be involved in long-range control of more distant genes[33] or that different functional effects, such as alternative splicing, may be affected.

It is well known that genes that participate in the same pathway tend to be located adjacent to one another in the human genome and are coordinately regulated[34]. Thus, our results and previous findings suggest that there are distinct effects at the 5q31 locus, which might involve loci that are part of a regulatory block in this region. Further efforts, including detailed sequencing and functional exploration, are necessary to fully explore this locus.

The rs2164983, rs1327914 and rs10983837 variants showed evidence of heterogeneity in the meta-analysis ($P < 0.01$). The overall random-effects results for these variants were OR = 1.14 (95% CI 1.05–1.24), $P = 0.001$; OR = 1.06 (95% CI 1.00–1.13), $P = 0.058$; and OR = 1.11 (95% CI 0.98–1.20), $P = 0.155$, respectively. Stratified analysis showed that the effects of rs2164983 and rs1327914 were stronger in the childhood atopic dermatitis cohorts (OR = 1.23, $P = 2.9 \times 10^{-9}$ and OR = 1.12, $P = 2.5 \times 10^{-4}$) than in studies that included atopic dermatitis cases of any age (OR = 1.17, $P = 0.002$ and OR = 1.02, $P = 0.584$; P values for the differences = 0.031 and 0.028, respectively) (**Supplementary Fig. 9**). This stratification did not fully explain the heterogeneity for rs2164983 (in the childhood-only cohorts, the P value for heterogeneity was still <0.01). In the COPSAC cohort, results were noticeably in the opposite direction, and excluding this study gave a heterogeneity P value of 0.069 (OR = 1.17, $P = 8.1 \times 10^{-10}$). However, the COPSAC cases are diagnostically and demographically comparable to those in the other cohorts, and, thus, there is no obvious reason why this cohort should give such a different result. Neither stratification by age of diagnosis nor by whether a physician's diagnosis was required for definition as a case explained the heterogeneity observed for rs10983837. Stratified analyses also indicated a stronger effect of rs2897442 in studies with a more stringent definition of atopic dermatitis (reported physician's diagnosis) (OR = 1.14, $P = 7.0 \times 10^{-9}$) compared to studies in which atopic dermatitis was defined using only self-reported histories of the disease (OR = 1.05, $P = 0.119$) (**Supplementary Fig. 9**). These observations highlight the importance of careful phenotyping and support the claim that atopic dermatitis encompasses distinct disease entities rather than being one illness, as is reflected by the current, relatively broad and inclusive concept of this condition. It is anticipated that the results of molecular studies will enable a more precise classification of atopic dermatitis.

In summary, in this large-scale GWAS of 11,025 atopic dermatitis cases and 40,398 controls, we have identified and replicated two newly identified risk loci for atopic dermatitis near genes that have annotations suggesting roles in epidermal proliferation and differentiation, supporting the importance of abnormalities in skin barrier function in the pathobiology of atopic dermatitis. In addition, we observed an association signal of genome-wide significance from within the cytokine cluster at 5q31.1, which seemed to be composed of two distinct signals, one centered at *IL13-RAD50* and the other at *IL4-KIF3A*, both of which showed moderate association with *IL13* expression. We further observed a signal in the epidermal differentiation complex, representing the *FLG* locus, and replicated the association with the 11q13.5 variant identified in the only other (smaller) published European GWAS of atopic dermatitis to date. Our results are consistent with the hypothesis that atopic dermatitis is caused by both epidermal barrier abnormalities and immunological features. Further studies are needed to identify the causal variants at the associated loci and to understand the mechanisms through which they confer risk for atopic dermatitis.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AAGC provided results for the replication analysis, and GOYA provided results for the discovery analysis.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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1. Bieber, T. Atopic dermatitis. *N. Engl. J. Med.* **358**, 1483–1494 (2008).
2. Brown, S.J. & McLean, W.H.I. Eczema genetics: current state of knowledge and future goals. *J. Invest. Dermatol.* **129**, 543–552 (2009).
3. Morar, N., Willis-Owen, S.A.G., Moffatt, M.F. & Cookson, W.O. The genetics of atopic dermatitis. *J. Allergy Clin. Immunol.* **118**, 24–34 (2006).

4. Palmer, C.N.A. *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat. Genet.* **38**, 441–446 (2006).
5. Rodríguez, E. *et al.* Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. *J. Allergy Clin. Immunol.* **123**, 1361–1370 e7 (2009).
6. Esparza-Gordillo, J. *et al.* A common variant on chromosome 11q13 is associated with atopic dermatitis. *Nat. Genet.* **41**, 596–601 (2009).
7. Sun, L.D. *et al.* Genome-wide association study identifies two new susceptibility loci for atopic dermatitis in the Chinese Han population. *Nat. Genet.* **43**, 690–694 (2011).
8. Moffatt, M.F. *et al.* A large-scale, consortium-based genomewide association study of asthma. *N. Engl. J. Med.* **363**, 1211–1221 (2010).
9. Li, B. *et al. Ovol1* regulates meiotic pachytene progression during spermatogenesis by repressing Id2 expression. *Development* **132**, 1463–1473 (2005).
10. Nair, M. *et al.* Ovol1 regulates the growth arrest of embryonic epidermal progenitor cells and represses c-myc transcription. *J. Cell Biol.* **173**, 253–264 (2006).
11. Dai, X. *et al.* The *ovo* gene required for cuticle formation and oogenesis in flies is involved in hair formation and spermatogenesis in mice. *Genes Dev.* **12**, 3452–3463 (1998).
12. Kowanetz, M., Valcourt, U., Bergström, R., Heldin, C.H. & Moustakas, A. Id2 and Id3 define the potency of cell proliferation and differentiation responses to transforming growth factor β and bone morphogenetic protein. *Mol. Cell. Biol.* **24**, 4241–4254 (2004).
13. Li, B. *et al.* The LEF1/β-catenin complex activates *movo1*, a mouse homolog of *Drosophila ovo* required for epidermal appendage differentiation. *Proc. Natl. Acad. Sci. USA* **99**, 6064–6069 (2002).
14. Owens, P., Han, G., Li, A.G. & Wang, X.J. The role of Smads in skin development. *J. Invest. Dermatol.* **128**, 783–790 (2008).
15. Widelitz, R.B. Wnt signaling in skin organogenesis. *Organogenesis* **4**, 123–133 (2008).
16. Buschke, S. *et al.* A decisive function of transforming growth factor-β/Smad signaling in tissue morphogenesis and differentiation of human HaCaT keratinocytes. *Mol. Biol. Cell* **22**, 782–794 (2011).
17. Maganga, R. *et al.* Secreted Frizzled related protein-4 (sFRP4) promotes epidermal differentiation and apoptosis. *Biochem. Biophys. Res. Commun.* **377**, 606–611 (2008).
18. Romanowska, M. *et al.* Wnt5a exhibits layer-specific expression in adult skin, is upregulated in psoriasis, and synergizes with type 1 interferon. *PLoS ONE* **4**, e5354 (2009).
19. Nica, A.C. *et al.* The architecture of gene regulatory variation across multiple human tissues: the MuTHER study. *PLoS Genet.* **7**, e1002003 (2011).
20. Apte, S.S. A disintegrin-like and metalloprotease (reprolysin-type) with thrombospondin type 1 motif (ADAMTS) superfamily: functions and mechanisms. *J. Biol. Chem.* **284**, 31493–31497 (2009).
21. Porter, S., Clark, I.M., Kevorkian, L. & Edwards, D.R. The ADAMTS metalloproteinases. *Biochem. J.* **386**, 15–27 (2005).
22. Pollard, T.D. The cytoskeleton, cellular motility and the reductionist agenda. *Nature* **422**, 741–745 (2003).
23. Pollard, T.D. & Borisy, G.G. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* **112**, 453–465 (2003).
24. Winder, S.J. Structural insights into actin-binding, branching and bundling proteins. *Curr. Opin. Cell Biol.* **15**, 14–22 (2003).
25. Goetz, S.C. & Anderson, K.V. The primary cilium: a signalling centre during vertebrate development. *Nat. Rev. Genet.* **11**, 331–344 (2010).
26. Mosimann, C., Hausmann, G. & Basler, K. β-catenin hits chromatin: regulation of Wnt target gene activation. *Nat. Rev. Mol. Cell Biol.* **10**, 276–286 (2009).
27. Chang, M. *et al.* Variants in the 5q31 cytokine gene cluster are associated with psoriasis. *Genes Immun.* **9**, 176–181 (2008).
28. Nair, R.P. *et al.* Genome-wide scan reveals association of psoriasis with IL-23 and NF-κB pathways. *Nat. Genet.* **41**, 199–204 (2009).
29. Li, Y. *et al.* The 5q31 variants associated with psoriasis and Crohn's disease are distinct. *Hum. Mol. Genet.* **17**, 2978–2985 (2008).
30. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678 (2007).
31. Weidinger, S. *et al.* Genome-wide scan on total serum IgE levels identifies *FCER1A* as novel susceptibility locus. *PLoS Genet.* **4**, e1000166 (2008).
32. Vercelli, D. Discovering susceptibility genes for asthma and allergy. *Nat. Rev. Immunol.* **8**, 169–182 (2008).
33. Kleinjan, D.A. & van Heyningen, V. Long-range control of gene expression: emerging mechanisms and disruption in disease. *Am. J. Hum. Genet.* **76**, 8–32 (2005).
34. Sproul, D., Gilbert, N. & Bickmore, W.A. The role of chromatin structure in regulating the expression of clustered genes. *Nat. Rev. Genet.* **6**, 775–781 (2005).

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ONLINE METHODS

Discovery analysis. For the discovery analysis, we used 5,606 atopic dermatitis cases and 20,565 controls of European descent from 16 population-based cohorts, 10 of which were birth cohorts. Local research ethics committees approved the individual studies, and informed consent was obtained from all participants when necessary (see the **Supplementary Note** for full details of ethics and consent procedures for each study). Additional details on sample recruitment and phenotypes and summary details for each collection are given in the **Supplementary Note** and in **Supplementary Table 1**. Genome-wide genotyping was performed independently in each cohort with the use of various standard genotyping technologies (see **Supplementary Table 2**). Imputation was independently conducted for each study with reference to HapMap release 21 or 22 CEU phased genotypes, and association analysis was performed using logistic regression models based on an expected allelic dosage model for SNPs, adjusting for sex- and ancestry-informative principal components as necessary. SNPs with MAF <1% and poor imputation quality (r^2 <0.3, if using MACH, or proper info <0.4, if using the IMPUTE imputation algorithm) were excluded. After genomic control at the level of the individual studies, we combined association data for ~2.5 million imputed and genotyped autosomal SNPs into an inverse variance fixed-effects additive model meta-analysis. There was little evidence for population stratification at the study level ($\lambda_{GC} \leq 1.08$; **Supplementary Table 2**) or at the meta-analysis level ($\lambda_{GC} = 1.02$), and the quantile-quantile plot of the meta-statistic showed a marked excess of detectable association signals well beyond those expected by chance (**Supplementary Fig. 1**).

Replication analysis. For replication, we selected the most strongly associated SNPs from the 10 most strongly associated loci in the discovery meta-analysis (all with $P < 1 \times 10^{-5}$ in stage I; **Table 1**). These SNPs were analyzed using *in silico* data from 11 GWAS sample sets not included in the discovery meta-analysis and *de novo* genotyping data from an additional 3 studies (**Supplementary Tables 1** and **2**), for a maximum possible replication sample size of 5,419 cases and 19,833 controls, all of European descent. Association analyses were again conducted for each study using a logistic regression model with similar covariate adjustments, based on expected allelic dosage for the *in silico* studies and allele counts in the *de novo* genotyping studies, and the results underwent meta-analysis with Stata 11.1 software (Statacorp LP). We applied a threshold of $P < 5 \times 10^{-8}$ for genome-wide significance and tested for overall heterogeneity of the discovery and replication studies using the Cochran's *Q* statistic.

Immunochip analysis. In this analysis, we evaluated 1,553 German atopic dermatitis cases and 3,640 German population controls. Cases were obtained from German university hospitals (Technical University Munich, as part of the GENEVA study, and University of Kiel). Atopic dermatitis was diagnosed on the basis of a skin examination by experienced dermatologists according to standard criteria, which included the presence of chronic or chronically relapsing pruritic dermatitis with the typical morphology and distribution[6]. Controls were derived from the Kooperative Gesundheitsforschung in der Region Augsburg (KORA) population-based surveys[35] and the previously described population-based Popgen Biobank[36]. Of these samples, 767 of the cases and 983 of the controls were also part of the replication stage. Samples with >10% missing data, individuals from pairs of unexpected duplicates or relatives and individuals with outlier heterozygosities of greater than ±5 s.d. from the mean were excluded. The remaining Immunochip samples were tested for population stratification using the principal-components stratification method, as implemented in EIGENSTRAT[37]. The results of principal-component analysis revealed no evidence for population stratification. SNPs that had >5% missing data, MAF <1% or exact Hardy-Weinberg equilibrium ($P_{controls} < 1 \times 10^{-4}$) were excluded. Association *P* values were calculated using χ^2 tests (degree of freedom (d.f.) = 1) and conditional association was analyzed using logistic regression, with both implemented in PLINK[38], from which we also derived OR values and their respective CIs.

ALSPAC expression analysis. RNA was extracted from LCLs generated from 997 unrelated ALSPAC individuals using an RNeasy extraction kit (Qiagen) and was amplified using the Illumina TotalPrep-96 RNA Amplification kit (Ambion). Expression was evaluated using Illumina HT-12 v3 BeadChip arrays. Each individual sample was run with two replicates. Expression data were normalized by quantile normalization between replicates and then by median normalization across individuals. For 949 ALSPAC individuals, both expression levels and imputed genome-wide SNP data were available (see ALSPAC replication cohort genotyping). For each of the three replicated SNPs that were associated with atopic dermatitis (rs479844, rs2164983 and rs2897442), we used linear regression in Mach2QTL to investigate the association between each SNP and any transcript within 500 kb of this SNP.

35. Holle, R., Happich, M., Löwel, H. & Wichmann, H.E. & MONICA/KORA Study Group. KORA—a research platform for population based health research. *Gesundheitswesen* **67** (suppl. 1), S19–S25 (2005).
36. Krawczak, M. *et al.* PopGen: population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships. *Community Genet.* **9**, 55–61 (2006).
37. Price, A.L. *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* **38**, 904–909 (2006).
38. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).

Paper 3: Supplementary Material

META-ANALYSIS OF GENOME-WIDE ASSOCIATION STUDIES IDENTIFIES THREE NEW RISK LOCI FOR ATOPIC DERMATITIS

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SUPPLEMENTARY MATERIAL

i. Supplementary Note

Study sample descriptions

Collaborating consortia members

Acknowledgements

Funding

ii. Supplementary Tables

Supplementary Table 1. Study characteristics - discovery & replication

Supplementary Table 2. Study genetic & analysis methods - discovery & replication

Supplementary Table 3. Discovery and replication results of the top 11 SNPs for AD

Supplementary Table 4. AD association results from the discovery meta-analysis for the 15 loci associated with asthma or total serum IgE levels in a recent GWAS.

Supplementary Table 5. Meta-analysis results for interactions between the three identified loci.

Supplementary Table 6. Immunochip association results for region 5q31.1

Supplementary Table 7. Immunochip linkage disequilibrium for region 5q31.1

iii. Supplementary Figures

Supplementary Figure 1. QQ plot for the discovery genome-wide association meta-analysis

Supplementary Figure 2. Regional association plots for the top 11 regions

Supplementary Figure 3. FLG adjusted meta-analysis

Supplementary Figure 4. 11q13 regional association plot and forest plot

Supplementary Figure 5. Forest plots of the association of the 7 SNPs which did not meet genome-wide significance.

Supplementary Figure 6. MuTHER pilot skin eQTL data for probes within 1Mb of the SNP (a) rs479844, (b) rs2164983 and (c) rs2897442 for 160 female twins.

Supplementary Figure 7. Regional association plots for 5q31.1 in the discovery cohorts (a) no conditional SNPs and (b) conditional on rs2897442.

Supplementary Figure 8. Regional association plot of markers within the cytokine cluster on 5q31.1.

Supplementary Figure 9. Stratified forest plots for SNPs associated with AD or with evidence of heterogeneity.

iv. Supplementary References

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SUPPLEMENTARY NOTE

Note on nomenclature

The extant nosology of atopic disease is confusing, and terms such as *atopic dermatitis, eczema, atopic eczema, endogenous eczema* and *flexural dermatitis* are frequently used interchangeably in the literature. Recently, a World Allergy Organization (WAO) report suggested the use of *eczema* as preferable to *atopic dermatitis*[1]. However, in this article, we continued to use the term atopic dermatitis, as many studies used for this project were designed prior to the WAO report and because this is the term used in many questionnaires on which the results presented here are based.

STUDY SAMPLE DESCRIPTIONS

Australian Asthma Genetics Consortium (AAGC) replication cohort

As part of the AAGC, we performed a GWAS of asthma in 7,197 unrelated individuals of European ancestry ascertained from the Australian population as described in detail elsewhere {Ferreira, submitted}. For this analysis, we tested 10 SNPs for association with AD status in 3,881 individuals (49% males, mean age 35 years, range 3 to 89), including 269 who reported having had AD at any point in their lifetime diagnosed by a doctor and 3,612 AD-free controls. These individuals participated in one of five studies: QIMR (N=3,132), CAPS (N=53), LIWA (N=474), MESCA (N=64) or TAHS (N=158). The QIMR individuals included in the AAGC analysis are unrelated to those included in the QIMR discovery cohort described below. Genotyping was performed with Illumina 610K or 370K arrays and stringent quality control filters applied as described in Supplementary Table 2. Imputation to HapMap 3 (all 11 populations, Feb 2009 release) and 1000 Genomes Project (CEU, Mar 2010 release) SNPs was performed with Impute2 and SNPs tested for association with disease status using logistic regression in PLINK, with sex and array type included as a covariate. Participants provided informed consent to participate in this study, which was approved by the respective ethics committees.

The Avon Longitudinal Study of Parents and Children (ALSPAC)

The Avon Longitudinal Study of Parents and their Children (ALSPAC) is a longitudinal population-based birth cohort that recruited pregnant women residing in Avon, UK, with an expected delivery data between 1st April 1991 and 31st December 1992. 14,541 pregnant women were initially enrolled with 14,062 children born (see [2] and website http://www.alspac.bris.ac.uk). Biological samples including DNA have been collected for 10,121 of the children from this cohort. Ethical approval was obtained from the ALSPAC Law and Ethics committee and relevant local ethics committees, and written informed consent provided by all parents. Questionnaire data has been collected regularly, with extensive questions, including those relating to AD. In this study we included data from the following questions, asked when the children were approximately 81, 91, 103 months, 10, 13 and 14 years [possible answers]:

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1. Has your child in the past 12 months had eczema? [yes, saw a Dr; Yes, but did not see a Dr; No, did not have]
2. Has a doctor ever actually said that your child has eczema? (10 & 14 years only) [yes; no]

We defined cases as those individuals who answered 'Yes, and saw a Dr' to Q1 or 'yes' to Q2. We defined controls as those individuals who answered 'no' to Q2 at age 14 years.

Discovery Cohort Genotyping and Statistical Analysis

Subjects were genotyped using either Illumina 317K or 610K genome-wide SNP genotyping platforms by the Wellcome Trust Sanger Institute, Cambridge, UK and the Centre National de Génotypage, Evry, France. A common set of SNPs were extracted and the resulting raw genome-wide data was subjected to standard quality control methods. Individuals were excluded on the basis of having incorrect gender assignments; minimal (0.34) or excessive (0.36) heterozygosity; disproportionate levels of individual missingness (>3%) and evidence of cryptic relatedness (PI HAT > 0.11). The remaining individuals were assessed for evidence of population stratification by multidimensional scaling analysis, using CEU, Yoruba, Japanese and Chinese individuals as reference ethnic groups. The underlying population stratification was thereafter controlled for by using EIGENSTRAT derived ancestry informative covariates. SNPs with a minor allele frequency of < 0.5% and call rate of < 97% were removed. Furthermore, only SNPs which passed an exact test of Hardy-Weinberg equilibrium (P > 5E-7) were considered for analysis. The resulting dataset consisted of 3233 individuals and 285,531 SNPs. Missing genotypes were subsequently imputed with MACH 1.0 Markov Chain Haplotyping software, using CEPH individuals from phase two of the HapMap project as a reference set (release 22). The final imputed dataset consisted of 3233 subjects, each with 2,483,534 imputed markers. 2811 of which also had AD phenotype information (909 cases and 1902 controls).

Genome-wide association analysis of AD was carried out in MACH2DAT [3,4] regressing expected allelic dosage on case-control status, including sex as a covariate.
R501X and 2282del4 have been genotyped in a previous study on 2634 subjects[5]. In the FLG adjusted analysis, these were included as covariates (using an additive model).

Replication Cohort Genotyping and Statistical Analysis

Subjects were genotyped using the Illumina HumanHap550 quad genome-wide SNP genotyping platform by 23andMe subcontracting the Wellcome Trust Sanger Institute, Cambridge, UK and the Laboratory Corporation of America, Burlington, NC, US. Individuals were excluded on the basis of having incorrect gender assignments; minimal or excessive heterozygosity (<0.32 and >0.345 for the Sanger data and <0.31 and >0.33 for the LabCorp data); disproportionate levels of individual missingness (>3%); evidence of cryptic relatedness (>10% IBD) and being of non-European ancestry. The resulting dataset consisted of 9233 individuals. SNPs with a

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minor allele frequency of < 1% and call rate of < 95% were removed. Furthermore, only SNPs which passed an exact test of Hardy-Weinberg equilibrium (P > 5E-7) were considered for analysis. Genotypes were subsequently imputed with MACH 1.0.16 Markov Chain Haplotyping software, using CEPH individuals from phase 2 of the HapMap project as a reference set (release 22). Of the 9233 ALSPAC genotyped individuals, 2903 also had AD phenotype information (895 cases and 2008 controls) and were not included in the ALSPAC discovery set.

Replication association analysis of the 10 SNPs was carried out as per the discovery cohort methods.

BAMSE

BAMSE is a Swedish birth cohort study. A total number of 4,089 newborn infants were recruited between 1994 and 1996 in the Stockholm area[6]. The first questionnaire data, dealing with parental allergic diseases, socio-economic status and residential characteristics, was obtained when the children were about 2 months. Similar questionnaires with a focus on the children's symptoms related to asthma and allergic diseases including eczema were answered by the parents when the children were approximately 1, 2, 4 and 8 years old. At 8 years of age, all children were invited to clinical testing, and blood samples were obtained from 2,480 children (~60%). DNA was extracted from 2,033 samples after exclusion of samples with too little blood, lack of questionnaire data, or if parental consent to genetic analysis of the sample was not obtained. From these samples, all children with a doctor's diagnosis of asthma (ever) and children with no history of eczema or other allergic diseases (controls) underwent GWAS genotyping[7]. Among asthmatics, all children with doctor's diagnosis of eczema (ever) were identified and after QC, a total of 100 eczema (ever) cases and 246 controls were included in this study.

BAMSE genotyping was conducted as part of the GABRIEL consortium. Genotyping in GABRIEL was carried out at Centre National de Génotypage (Evry, France) using the Illumina Human610 quad array (Illumina, Inc., San Diego, CA)[7]. An ancestry analysis was performed using EIGENSTRAT, and putative non-Caucasian samples were flagged as outliers and eliminated from subsequent analyses. Imputation to HapMap CEU release 22 was conducted using MACH v.1.0.16 with option MLE (original genotypes were only replaced if the underlying reference haplotypes strongly contradict the input genotypes). Samples from the British 1958 birth cohort (B58C) with greater than 95% genotyping success rate were selected to estimate model parameters of error rates and recombination rates for step 1 of the imputation procedure. In step 2, all GABRIEL cohorts were imputed, no SNP/sample QC filters were applied to individual cohorts prior to this.

Genome-wide association analysis of AD was carried out in ProbAbel[8] regressing expected allelic dosage on case-control status, including sex as a covariate. The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden.

British 1958 birth cohort (B58C)

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The British 1958 birth cohort is an ongoing follow-up of all persons born in England, Scotland and Wales during one week in 1958. At age 7 years, a history of eczematous rashes was obtained by interview with a parent, and the presence of visible AD on skin examination was recorded by a school medical officer[9]. For the purpose of this meta-analysis, cases were defined by a positive interview response for either AD during the first year of life, or AD after the first year (ie. ages 1-7), or both. (The results of skin examination were not used to define cases.) Controls were defined as children with no parentally reported history of AD by age 7, and no record of AD on skin examination at age 7.

At the age of 44-45 years, the cohort were followed up with a biomedical examination and blood sampling [10], from which a DNA collection was established as a nationally representative reference panel (http://www.b58cgene.sgul.ac.uk/). The discovery phase of the analysis used two non-overlapping subsets of the DNA collection which were selected as controls for use by the Wellcome Trust Case-Control Consortium (WTCCC) [11] and the Type 1 Diabetes Genetics Consortium (T1DGC)[12]. Genotyping by the WTCCC used the Affymetrix 500K array and the T1DGC used the Illumina 550K array. Imputations using the HapMap 2 (release 21) template were performed using SNPTEST for the WTCCC subset and MACH for the T1DGC subset. Within-cohort logistic regression analyses for AD were performed using Quicktest for the WTCCC subset and ProbAbel for the T1DGC subset.

In silico replication analyses were performed using Illumina 550K/610K genotypes deposited by the GABRIEL consortium [7] and by the WTCCC on cohort members that had not been included in the discovery sets. Imputations for the replication set using the HapMap 2 (release 21) template were performed using MACH and within-cohort logistic regression analyses for eczema were performed using ProbAbel.

CHOP

CHOP patients and controls were recruited at the Children's Hospital of Philadelphia between 2006 and 2010. All subjects were of self-reported Caucasian origin and resident in the Greater Philadelphia area. Ethical approval for this study was obtained from the Institutional Revue Board of the Children's Hospital of Philadelphia. The study included 519 patients with physician-diagnosed eczema and 1004 disease-free controls without eczema. Cases were defined by the presence of the ICD9 code for eczema (691.8) in their electronic medical records. All CHOP samples were genotyped on either the Illumina HH550 or HH610 BeadChips (Illumina, San Diego) at the Center for Applied Genomics.

In addition to self-reported ancestry, Principal Component Analysis was carried on all cases and controls using smartPCA to reduce the risk of population stratification. Mean age of the case cohort was 9 years and 51% were males and 49% females.

Genotyping QC measures, imputation, analysis

Prior to imputation, quality control was carried out in *plink* resulting in the exclusion of 10,930 SNPs with call rates <95%, 22,252 SNPs with a minor allele frequency (MAF) <1% and 13,181 SNPs with Hardy Weinberg

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equilibrium $P<10^{-5}$; the genomic inflation factor (GIF) was 1.05. Imputation was carried out using Impute version 1, and the HapMap release 22 haplotypes as a reference. Statistical analysis was carried out using SNPTEST, assuming an additive model and taking genotype uncertainty into account.

COPSAC

The COPSAC birth cohort study is a prospective clinical study of a birth cohort of 411 infants born to mothers with a history of asthma. The newborns were enrolled at the age of 1 month, the recruitment of which was previously described in detail[13-15]. The study was approved by the Ethics Committee for Copenhagen (KF 01-289/96) and The Danish Data Protection Agency (2008-41-1754) and informed consent was obtained from both parents. The families used doctors employed at the clinical research unit, and not the family practitioner, for diagnosis and treatment of AD and other skin-related symptoms. Skin lesions were described at both scheduled visits at 6-monthly intervals and acute visits with skin symptoms according to pre-defined morphology and localization; AD was defined based on the Hanifin-Rajka criteria as previously detailed[16-18].

High throughput genome-wide SNP genotyping were performed using the Illumina Infinium™ II HumanHap550 v1, v3 or quad BeadChip platform (Illumina, San Diego), at the Children's Hospital of Philadelphia's Center for Applied Genomics, as described previously[19].

Statistical analysis was carried out using SNPTEST, assuming an additive model and taking genotype uncertainty into account.

Danish National Birth Cohort (DNBC)

DNBC is a population-based cohort of more than 100,000 pregnancies, recruited in the years 1996-2002[20]. Extensive phenotype information was collected by computer-assisted telephone interviews twice during pregnancy as well as 6 and 18 months after delivery. An additional questionnaire-based follow-up survey was conducted when the children reached 7 years of age. Cases with early onset AD were identified from the 18 months telephone interview data using an algorithm specifically developed for this purpose[21]. In addition, children with a positive response to both of the following two questions from the 7 year survey were included in the case group: 1) "Has a doctor ever said that your child had AD, also known as allergic rash?" and 2) "Has your child ever had an itchy rash which was coming and going for at least 6 months?". Finally, children with ICD10 diagnosis code L20 in the Danish Hospital Discharge Register were also included in the case group. Controls were required not to have any AD or AD symptoms recorded in interview, questionnaire, or register data. GWAS data were generated for 3,840 individuals from the DNBC (mothers and their children) in a study of prematurity and its complications (Principal investigator Jeff Murray) within the Gene Environment Association Studies (GENEVA) consortium. AD information and genome-wide genotype and imputed data were available for 1,641 children. Imputation was carried out with MACH, using HapMap CEU release 22 as the reference panel. Logistic regression analysis for AD was performed with MACH2DAT, using imputed allele dosages and including sex as a covariate.

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The DNBC study protocol was approved by the Danish Scientific Ethical Committee and the Danish Data Protection Agency.

ECRHS

Details of the methods of ECRHS I and ECRHS II, a multicentre international cohort study, have been published elsewhere[22,23]. Participants within the ECRHS were eligible for inclusion in this analysis if they were identified by random sampling of those who fulfilled the following criteria 1) lived in centres that took part in genome-wide genotyping initiative under the auspices of GABRIEL[7] AND 2) were initially selected to take part in the ECRHS clinical measurements as part of the random sample (ie not specifically selected for inclusion because of any pre-existing disease). Cases were those answering positively to the questions 'Have you ever had an itchy rash that was coming and going for at least 6 months?' AND yes to 'Have you had this itchy rash in the last 12 months?' during ECRHS II (aged 27-58). Further information on the distribution of eczema within the cohort is available[24].

Genotyping and imputation was carried out within the GABRIEL consortium, details in BAMSE methods (page S5). Genome-wide association analysis of AD was carried out in ProbABEL regressing expected allelic dosage on case-control status, adjusted for sex, recruitment centre and first two principal components informative of European ancestry.

Each participating centre obtained ethical permission from the appropriate local committee.

Generation R

The Generation R Study is a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, The Netherlands[25,26]. All children were born between April 2002 and January 2006, and currently followed until young adulthood. Of all eligible children in the study area, 61% were participating in the study at birth[26]. Cord blood samples including DNA have been collected at birth. Postnatal data about eczema was annually collected by questionnaires at the ages of 1 to 5 years. Response rates for the questionnaires were 71%, 76%, 72%, 73% and 74%, respectively[26]. For the current study, 1,115 children were included in the discovery analysis (males, n = 594 (53%)). A total number of 620 children were available for the replication analyses (males, n = 299 (48%). Questions about eczema were 'Has your child in the past 12 months had eczema [yes, saw a doctor; Yes, but did not see a doctor; No, did not have] (age 1 to 4 years)?' and 'Has your child ever had eczema [yes; no] (age 5)?'. We defined cases as those children of whom parents answered their child 'Yes, had eczema and saw a doctor' or 'Yes, ever had eczema'. We defined controls as those children of whom parents answered their child 'No, never had eczema' and 'Yes, had eczema but did not see a doctor/No, did not have eczema'. The current study used the first set of Generation R samples of Northern European Ancestry. Samples were genotyped using Illumina Infinium II HumanHap610 Quad Arrays following standard manufacturer's protocols. Intensity files were analyzed using the Beadstudio Genotyping Module software v.3.2.32 and genotype calling based on default cluster files. Any sample displaying call rates below 97.5%, excess of autosomal heterozygosity (F<mean-4SD) and mismatch between called and phenotypic

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gender were excluded. In addition, individuals identified as genetic outliers by the IBS clustering analysis (> 3 standard deviations away from the HapMap CEU population mean) an one of 2 pairs of identical twins (IBD probabilities =1) were excluded from the analysis. After quality control (QC) 2,729 children were included in the analyses. Genotypes were imputed for all polymorphic SNPs from phased haplotypes in autosomal chromosomes of the HapMap CEU Phase II panel (release 22, build 36) oriented to the positive (forward) strand. Genotyped SNPs with minor allele frequency < 0.01, SNP Call Rate < 0.98 and HWE P-value < $1x10^{-6}$ were filtered. After marker pruning 503,248 SNPs were used for imputation (MACH v 1.0.16) of 2,543,887 SNPs. Association analysis for directly genotyped data were carried out in PLINK implemented on BCSNPmax and for imputed data were ran using MACH2DAT implemented in the GRIMP[27] user interface platform. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC 217.595/2002/20). Written informed consent was obtained from all participants.

Genetics of Overweight Young Adults (GOYA) women's study

In total, 91,387 pregnant women were recruited to the Danish National Birth Cohort during 1996-2002, 67,853 of whom gave birth to a live born infant and had provided a blood sample during pregnancy. The GOYA study includes a subset of these women, selected for genome-wide genotyping according to their BMI and is described in full elsewhere[28,29]. The 4% (2,451) of the women with the largest residuals from the regression of BMI on age and parity and a random sample of similar size (2,450) drawn from the remaining distribution were selected for genotyping. Pertinent to this study, the women were asked questions about eczema during a telephone interview at ~16 weeks of gestation. The questions were:

1. Have you ever had any skin disease?
2. Was the skin disease diagnosed by a doctor?
3. What kind of skin disease?

Cases were defined as those that answered "yes" to Qs 1 and 2, and 'AD' to Q3. Controls were defined as those that answered "no" to Q1.

The GOYA study was approved by the regional scientific ethics committee and by the Danish Data Protection Board.

Genome-wide genotyping on the Illumina 610k quad chip was carried out at the Centre National de Génotypage (CNG), Evry, France. We excluded SNPs with minor allele frequency <1%, >5% missing genotypes or which failed an exact test of Hardy-Weinberg equilibrium (HWE) in the controls (p<10-7). We also excluded any individual who did not cluster with the CEU individuals (Utah residents with ancestry from northern and western Europe) in a multidimensional scaling analysis seeded with individuals from the International HapMap release 22, who had >5% missing data, outlying heterozygosity of >35% or <30.2%, both samples in the case of genetic duplicates, one of each pair of genetically related individuals, individuals with sex discrepancies and individuals whose genotyping was discordant with a previous project. After data cleaning, 3,908 women and 545,349 SNPs remained. We carried out imputation to HapMap release 22 (CEU individuals) using Mach 1.0, Markov Chain Haplotyping[3,4].

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Logistic genome-wide association analysis for AD (with no covariates) was carried out in MACH2DAT[3,4].

The Danish Glostrup Cohort (Health2006)

Between June 2006 and May 2008, a cross-sectional study was performed in the general population in Copenhagen, the Capital of Denmark. A random sample of 7931 subjects aged 18–69 years old was obtained from the Danish Central Personal Register, Ministry of Internal Affairs. All were Danish adults with Danish citizenship and born in Denmark. A total of 3471 (44%) subjects participated in a general health examination and 3329 (95.9%) responded to the question about atopic dermatitis. The participation rate was higher among older age-groups than among younger age groups in both genders[30]. The Ethical Committee of Copenhagen County approved the study (KA-20060011). A written informed consent form was obtained from all participants prior to the beginning of the study.

All participants were mailed a standard invitation letter and a questionnaire about health, lifestyle, and socioeconomic factors. AD was defined by the U.K. Working Party's diagnostic criteria for atopic dermatitis as a history of an itchy skin condition plus a minimum of two of four minor criteria[31].

Genotyping of SNPs was performed by the PCR KASPar genotyping system (KBiosciences, Hoddesdon, UK). None of the SNPs deviated from HW equilibrium ($p>0.05$ for all SNPs). Lowest call rate for SNPs was 0.98.

Data analyses were performed using the Statistical Products and Service Solutions package (SPSS Inc., Chicago, IL, U.S.A.) for Windows (release 15.0).

KORA

The Cooperative Health Research in the Region of Augsburg (KORA) study is a series of independent population-based epidemiological surveys and follow-up studies of participants living in the region of Augsburg, Southern Germany[32]. All participants are of German nationality identified through the registration office and informed consent has been given by all participants. The study has been approved by the local ethics committee. Participants were examined in 1994/95 (KORA S3) or 1999/2001 (KORA S4) and in the follow-up examinations in 2004/05 (KORA F3) and 2006/08 (KORA F4). All KORA subjects had completed a standardized questionnaire which next to demographic data included the basis allergy questions of the European Community Respiratory Health Survey (ECRHS) on respiratory health[22]. AD was diagnosed based on a reported physician's diagnosis in the past. For the genome-wide association study we genotyped 1,644 randomly selected participants of KORA F3 using Affymetrix 500K and 1,814 randomly selected participants of KORA F4 using Affymetrix 6.0[33]. Genome-wide association analysis of AD was carried out using logistic regression in SNPTEST V2 (http://www.stats.ox.ac.uk/~marchini/software/gwas/snptest.html), including sex as a covariate.

For replication purpose 1100 AD cases of self-reported German ethnicity were obtained from the GENEVA (Genetic evaluation of atopic dermatitis) study from the Department of Dermatology and Allergy, Technical

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University Munich[34]. AD was diagnosed on the basis of a skin examination by experienced dermatologists according to standard criteria in the presence of a chronic or chronically relapsing pruritic dermatitis with the typical morphology and distribution[35]. KORA controls were selected of the remaining KORA F4 sample which was not included in the GWAS analysis. De novo replication analysis was carried with R 2.12.2 (http://www.R-project.org) using logistic regression adjusted for sex. Genetic information entered the model as allele counts.

LISA/GINI

The influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany PLUS the influence of traffic emissions and genetics (LISAplus) Study is a population based birth cohort study. A total of 3097 healthy, fullterm neonates were recruited between 1997 and 1999 in Munich, Leipzig, Wesel and Bad Honnef. The participants were not pre-selected based on family history of allergic diseases[36].

A total of 5991 mothers and their newborns were recruited into the German Infant study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development (GINIplus) between September 1995 and June 1998 in Munich and Wesel. Infants with at least one allergic parent and/or sibling were allocated to the interventional study arm investigating the effect of different hydrolysed formulas for allergy prevention in the first year of life[37]. All children without a family history of allergic diseases and children whose parents did not give consent for the intervention were allocated to the non-interventional arm. Detailed descriptions of the LISAplus and GINIplus studies have been published elsewhere [36] and [37], respectively).

Information on ever having physician-diagnosed AD was collected using self-administered questionnaires completed by the parents. The questionnaires were completed at 6, 12, 18 and 24 months and 4, 5, 6 years of age in the LISAplus study and 1, 2, 3, 4 and 6 years in the GINIplus study asking for each year of age since the previous follow-up. DNA was collected at the age 6 and 10 years. For both studies, approval by the local Ethics Committees and written consent from participant's families were obtained.

In the discovery analysis, 379 children from the LISAplus study from Munich were included (number of boys: 227 (57%)). DNA was analysed using the Affymetrix Human SNP Array 5.0 for each individual. Genome-wide data was called using BRLMM-P algorithm and imputed in IMPUTE[38].

Genome-wide association analysis of AD was carried out in SNPTEST V1 (http://www.stats.ox.ac.uk/~marchini/software/gwas/snptest.html) regressing expected allelic dosage on case-control status, including sex as a covariate.

For replication, 665 children from Munich from both studies were included (499 (75%) children from the GINIplus study and 166 (25%) children from the LISAplus study)). 583 individuals (499 from the GINIplus study and 84 from the LISA study) were analysed using the Affymetrix Human SNP Array 5.0 and 82 individuals from the LISAplus study were analysed using Affymetrix Human SNP Array 6.0. Genotypes were called using BRLMM-P algorithm (5.0), respectively BIRDSEED V2 algorithm (6.0), imputed in IMPUTE2[39] and genome-wide

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association analysis of AD was carried out in SNPTEST V2 (http://www.stats.ox.ac.uk/~marchini/software/gwas/snptest.html) regressing expected allelic dosage on case-control status, including sex as a covariate.

Manchester Asthma and Allergy Study (MAAS)

The Manchester Asthma and Allergy Study is an unselected, population-based prospective study which follows the development of atopic disorders in a cohort of children described in detail elsewhere[40-44]. The setting is the maternity catchment area of Wythenshawe and Stepping Hill Hospitals, comprising of 50 square miles of South Manchester and Cheshire, UK, a stable mixed urban-rural population. Study was approved by the Local Research Ethics Committee. Informed consent was obtained from all parents.

Screening & Recruitment

All pregnant women were screened for eligibility at antenatal visits (8th-10th week of pregnancy). The study was explained to the parents, and informed consent for initial questionnaires and skin prick testing was obtained. Both parents completed a questionnaire about their and their partner's history of asthma and allergic diseases and smoking habits.

If the pregnant woman's partner was not present at the antenatal clinic visit, an invitation was sent for him to attend an open-access evening clinic for skin prick testing and questionnaire. Once both parents had completed questionnaires and skin prick testing, a full explanation of the proposed future follow-up for the child was given. Of the 1499 couples who met the inclusion criteria (<10 weeks of pregnancy, maternal age >18 years, questionnaire and skin test data available for both parents), 288 declined to take part in the study. A total of 1185 participants had at least some evaluable data.

Follow-up

The children have been followed prospectively, and attended review clinics at ages 1, 3, 5 and 8 years (±4 weeks).

Definitions of outcomes

AD: Information on the age of onset of parentally-reported AD was collected using an interviewer-administered validated ISAAC questionnaire to collect information on parentally reported symptoms, physician-diagnosed illnesses and treatments received.

In this analysis eczema was defined as a positive answer to the question "Has your child ever suffered from eczema?". The association study was carried out in the 761 MAAS individuals for which both genotype and phenotype data was available.

Genotyping

DNA samples were genotyping on an illumina 610 quad chip. The illumina genotypes were called using the Illumina GenCall application following the manufacturer's instructions. Quality control criteria for samples

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included: 97% call rate, exclusion of samples with an outlier autosomal heterozygosity (scree-plot visualisation) gender validation and sequenome genotype concordance. Quality control criteria for SNPs included a 95% call rate, HWE > $5.9x10^{-7}$, minor allele frequency > 0.005. Genotypes were imputated with IMPUTE version 2.1.2 with 1000 genomes and hapmap phase 3 reference genotypes. Association analysis was carried out using SNPTEST version 2.1 using frequentist with the score method.

The Norwegian Mother and Child Cohort Study (MoBa)

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health[45,46]. Participants were recruited from all over Norway from 1999-2008, and 38.5% of invited women consented to participate. The cohort now includes 108,000 children, 90,700 mothers and 71,500 fathers. Blood samples were obtained from both parents during pregnancy and from mothers and children (umbilical cord) at birth. Follow-up is conducted by questionnaires at regular intervals and by linkage to national health registries.

The current study is based on version 4 of the quality-assured data files and included participants that were recruited between 1999-2005. Informed consent was obtained from each MoBa participant upon recruitment. The study was approved by The Regional Committee for Medical Research Ethics in South-Eastern Norway and the Norwegian Data Inspectorate.

The cases were indentified from questionnaires at the child's age 6, 18 and 36 months defined by the following questions: Does your child have or has he/she had any of the following health problems? Atopic eczema was listed as one of several items here. If yes was entered on the first question, a second were asked: has the mother and child health care centre or someone else referred your child for further specialist investigation Our cases were restricted to unique cases across age 6, 18 and 36 months with yes on both questions. Consequently the controls were unique controls across age 6, 18 and 36 months with no on both questions.

The genotype platform used were Illumina 660W and imputed SNPs were only included if the met the recommended threshold for imputation quality (PLINK INFO >0.8). Logistic regression analyses for AD was performed using an additive model in PLINK, including sex as a covariate. The children included in this study are originally genotyped for a case control study (n=1200 children) of spontaneous preterm delivery.

The Northern Finland Birth Cohort 1966 (NFBC66)

The Northern Finland Birth Cohort 1966 is a prospective follow-up study of children from the two northernmost provinces of Finland[47]. Women with expected delivery dates in 1966 were recruited through maternity health centres[48]. Cohort members living in northern Finland or in the capital area were invited to a clinical examination as well as questionnaire at age 31 years. DNA was extracted from blood samples given at the clinical examination[49]. For the purpose of this meta-analysis, we included data from the following questions:

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1. Have you had eczema (infantile, atopic or allergic)?
2. If yes, have you ever been treated by a doctor

Individuals who answered yes to both questions were defined as cases (1208). Individuals that answered no to the first question were defined as controls (2294). Genotyping was completed at the Broad Institute Biological Sample Repository in participants with available DNA using Illumina HumanCNV370DUO Analysis BeadChip array for 339,629 SNPs. We excluded 3,345 SNPs from analysis because HWE was not met at a level p <0.0001, 55 because of low call rate (<95%) and 7,681 because the MAF was <1%, leaving 329,091 SNPs for the association analysis. Imputation was conducted using the algorithm implemented in IMPUTE and association analysis using quicktest[50]. Informed consent for the use of the data including DNA was obtained from all subjects. The study was approved by the ethics committees in Oulu (Finland) and Oxford (UK) universities in accordance with the Declaration of Helsinki.

Northern Finland Birth Cohort 1986 (NFBC86)

The Northern Finland Birth Cohort 1986 comprises 9432 live-born children with an expected date of birth between July 1, 1985, and June 30, 1986 from the two northernmost provinces of Finland, Oulu and Lapland. The cohort covers over 99% of all the deliveries in the target area during that time (N=9,362 mothers with N=9,432 liveborn children)[51]. At the age of 16, the cohort members were sent a postal questionnaire including questions on eczema, and 80% returned it. At the same time, they were invited to a clinical examination with 74% taking part in it. DNA was extracted from blood samples given at the clinical examination for 6,266 subjects. For the purpose of this meta-analysis, we included data from 1717 individuals that answered the following questions:

1. Have you ever had eczema which has been called infantile eczema, atopic eczema or allergic eczema?
2. Diagnosed or treated by a doctor?

Individuals who answered yes to both questions were defined as cases (316). Individuals that answered no to the first question were defined as controls (1401). Genotyping was performed by KBiosciences (Hoddesdon) using their own system of fluorescence-based competitive allele-specific PCR (KASPar) with genotype success rate >97%. Association analysis was conducted using quicktest[50]. Informed consent for the use of the data including DNA was obtained from all subjects at the age of 16 years. The study was approved by ethics committees in Oulu (Finland) university in accordance with the Declaration of Helsinki.

Netherlands Twin Register (NTR)

The Netherlands Twin Register (NTR) is a large population based study that registers approximately 40% of all multiple births in the Netherlands since 1986[52]. At age 5 of the children, a survey is sent out in which the

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parents of the twins are asked to indicate for each child separately whether a doctor has ever diagnosed eczema. A similar question concerns doctor diagnosed baby eczema[53].

Blood and/or buccal samples for DNA extraction were collected for a subsample of the NTR in several projects. Genotyping was performed on the Affymetrix Human SNP Array 6.0 in the Avera Institute, Sioux Falls, South Dakota (USA). Genotypes were called using the BIRDSEED V2 algorithm and imputed in BEAGLE in the MD Anderson Cancer Center, Houston, Texas (USA). After QC, one individual of each family was selected. If both twins were cases or controls, one individual was picked at random, otherwise the case was selected. A total of 123 cases and 306 controls were included in the study. Logistic regression analyses were performed using an additive model in PLINK, including sex as a covariate. The study was approved by the Medical Ethical Committee of the VU Medical Centre, Amsterdam, the Netherlands (IRB00002991).

PIAMA

PIAMA is a birth cohort study consisting of two parts: a placebo controlled intervention study in which the effect of mite impermeable mattress covers was studied and a natural history study in which no intervention took place. Details of the study design have been published previously[54]. Recruitment took place in 1996-1997. A screening questionnaire was distributed to pregnant women visiting one of 52 prenatal clinics at three regions in the Netherlands. A total of 10,232 pregnant women completed a validated screening questionnaire. Mothers reporting a history of asthma, current hay fever or allergy to pets or house dust mite were defined as allergic. Based on this screening, 7862 women were invited to participate, of whom 4,146 women (1327 allergic and 2819 nonallergic) gave written informed consent. The response rates to the annual questionnaires ranged from 3030 (92%) at age 1 to 2732 (83%) at age 8 years. DNA was collected from 2162 children at age 4 and/or 8 years. Genome-wide genotyping was performed within the framework of the Gabriel Consortium[7]. For this, DNA samples from 213 children with parental reported doctor diagnosed asthma ever at age 8 years and from 213 controls without doctor diagnosed asthma or wheeze ever at age 8 years were provided. From these children, 186 cases of eczema and 167 controls were selected for the current study. Cases of eczema were defined as parental reported doctor diagnosed eczema ever at age 2 years or doctor diagnosed eczema in the last 12 months at ages 3, 4, 5, 6 ,7 or 8 years. Controls were defined as children whose parents denied the presence of doctor diagnosed eczema in the last 12 months at all ages. Genotyping was performed with an Illumina Human610 quad array. SNPs were excluded that fullfilled one or more of the following criteria: p-value for test of Hardy-Weinberg equilibrium $\leq$ 1E -7, genotyping call rate <95% or MAF < 1%. SNPs were imputed with IMPUTE version 2 software using HAPMAP CEU release #22 b36. Genome-wide association analyses were performed using SNPTEST version 1.1.5. The Medical Ethical Committees of the participating institutes approved the study.

QIMR discovery cohort

We recently performed a GWAS of asthma in 2,832 unrelated individuals of European ancestry ascertained from the Australian population as described in detail elsewhere[55]. Of these, 2,148 individuals (34% males, mean age 32 years, range 10 to 92) reported information on their AD status in health questionnaires, including

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482 individuals who reported having had AD at any point in their lifetime (32% diagnosed by a doctor) and 1,666 AD-free controls. Genotyping was performed with Illumina 610K or 370K arrays and stringent quality control filters applied as described in Supplementary Table 2. Imputation of HapMap 2 SNPs (CEU release 21) was performed with MACH and SNPs tested for association with disease status using logistic regression in PLINK, with sex included as a covariate. Participants provided informed consent to participate in this study, which was approved by the QIMR ethics committee.

Western Australian Pregnancy (Raine) cohort

Recruitment of the Western Australian Pregnancy (Raine) cohort has previously been described in detail[56-58]. In brief, between 1989 and 1991 2,900 pregnant women were recruited prior to 18-weeks gestation into a randomised controlled trial to evaluate the effects of repeated ultrasound in pregnancy. Recruitment predominantly took place at King Edward Memorial Hospital (Perth, Western Australia). Women were randomised to repeat ultrasound measurements at 18, 24, 28, 34 and 38 weeks gestation or to a regular ultrasound assessment at 18-weeks. Children have been comprehensively phenotyped from birth to 21 years of age (average ages of one, two, three, six, eight, ten, 14, 17 and currently 21) by trained members the Raine research team. Data collection included questionnaires completed by the child's primary carer and by the adolescent from age 14, physical assessments by trained assessors at all follow up years, DNA collection from the year 14 follow-up. Information on ever having AD diagnosed by a paediatrician or GP was collected using a questionnaire at 6 and 8 years of age. The study was conducted with appropriate institutional ethics approval, and written informed consent was obtained from all mothers and the children from age 18-years. The cohort has been shown to be representative of the population presenting to the antenatal tertiary referral centre in Western Australia[56]. Genotyping was performed using the Illumina 660w quad array and imputation of HapMap 2 (CEU release 22) SNPs was performed using MACH. Association testing was performed using MACH2DAT.

SAPALDIA

SAPALDIA data are derived from among 6,055 SAPALDIA cohort subjects that participated in both, the baseline (1991) and follow-up (2002) examinations and agreed to providing blood for genetic analysis.

SAPALDIA is a population-based cohort that originally recruited subjects aged 18 to 60 from population registries in eight Swiss communities representing the three largest language groups (German, French, Italian) as well as different levels of air pollution, altitude and degrees of urbanization[59,60]. At both baseline and follow-up examination subjects underwent spirometry as well as a detailed interview on respiratory health and allergies, smoking history, lifestyle factors and anthropometry. At follow-up, 8,047 of 9,651 baseline subjects re-participated in at least one part of the study and a formal biobank was established. AD was defined as positive answer to the question "Have you ever had atopic dermatitis or any other kind of skin allergy?" at either examination. The basis for this study formed control subjects and a random sample of all asthmatics (sampled proportionally to the overall asthma prevalence in the study) that were part of a nested asthma case-

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control sample subjected for genomewide genotyping in the context of the GABRIEL genome-wide association study on asthma[7]. Genotyping and imputation was carried out within the GABRIEL consortium, details in BAMSE methods (page S5).

Association analysis was performed in ProbABEL. All study participants gave written informed consent, and the study was approved by the national and respective cantonal ethics committees.

The Department of Twin Research and Genetic Epidemiology at King's College London (TwinsUK)

The TwinsUK adult twin registry based at St Thomas' Hospital in London is a volunteer cohort of over 12,000 identical and non-identical twins[61] recruited since 1993. The cohort is predominantly female (92%). Twins largely volunteered unaware of the study in which they would subsequently be included, gave fully informed consent under a protocol reviewed by the St Thomas' Hospital Local Research Ethics Committee.

Subjects were genotyped using Illumina's Human Hap 300k Duo and Human Hap610 Quad. Genotyping was performed in part at the Wellcome Trust Sanger Institute (Hinxton, UK) and in part at the Center for Inherited Disease Research, NIH, Baltimore, MD, United States. Genotypes were quality controlled and were excluded from the analysis for low genotype rate defined as less than 95% for alleles with a minor allele frequency (MAF) of 0.05 and above or less than 99% for loci with a MAF of 0.05 or below or for Hardy-Weinberg disequilibrium ($p<0.0001$). Individual samples were included in the analysis if they were of non-admixed Caucasian descent, did not show lack or excess heterozygocity, had high (defined as in excess of 99% success rate) individual genotypes available.

Genotypes were imputed using IMPUTE 2.0 using Linkage Disequilibrium patterns observed in the HapMap 2 CEU population as a template. A total of 1,236 unrelated subjects for which both genetic and phenotypic information was available was analyzed using PLINK.

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GOYA - Genetics of Overweight Young Adults

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Health2006

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LISA/GINI

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MoBa

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NFBC66 and NFBC86

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RAINE

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SAPALDIA

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Supplementary Table 1. Study characteristics - discovery & replication

Cohort	Type	N	Percent male	Mean age @ interview	Atopic dermatitis question	Physician diagnosis required	Case response	case #	Control response	control #
Discovery cohorts										
ALSPAC	Birth cohort	2811	50%	81m, 91m, 103m, 10y, 13y, 14y	1. Has your child in the past 12 months had eczema?		1. Yes, and saw a Dr			
				10y, 14y	2. Has a doctor ever actually said that your child has eczema?		2. Yes		2. No (@ 14 y)	
						yes	Yes to 1 or 2 at any timepoint	909		1902
B58C-WTCCC	Birth cohort	1285	50%	7y	1. Parent interview: History of atopic dermatitis in first year?		1. Yes			
				7y	2. Parent interview: History of atopic dermatitis after first year?		2. Yes			
				7y	3. Medical examination					
						no	Yes to 1 or 2	103	No to 1 and 2 and 3	1182
B58C-T1DGC	Birth cohort	2186	48%	7y	1. Parent interview: History of atopic dermatitis in first year?		1. Yes			
				7y	2. Parent interview: History of atopic dermatitis after first year?		2. Yes			
				7y	3. Medical examination					
						no	Yes to 1 or 2	188	No to 1 and 2 and 3	1998
CHOP	Population based cohort	1523	51%	9y	ICD9 diagnosis in electronic medical record	yes		519		1004
COPSAC	Birth cohort	332	49%	0-6y	Diagnosis prospectively by dermatologist at the research unit based on Hanifin-Rajka criteria	yes	Diagnosed atopic dermatitis	171	No atopic dermatitis diagnosis and followed up to 6 yr	161
DNBC	Birth cohort	1641	52%	18m	1. Has your child had itchy rash?		1. Yes			
				18m	2. Has a doctor told you that your child had atopic dermatitis?		2. Yes			
				18m	3. Was the rash recurrent?		3. Yes			
				18m	4. In which 0.5 month periods did your child have the rash?		4. Rash for at least 4 consecutive 0.5-month periods			

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				18m	5. Where was the rash located?		5. Localization in elbow creases, behind the knees, face, wrists/hands or generalized/4 or more localizations			
				7y	6. Has your child ever had atopic dermatitis?		6. Yes			
				7y	7. Has your child ever had an itchy rash which was coming and going for at least 6 months?		7. Yes			
					9. Hospital Discharge Record of ICD10 code L20					
						no	[Yes to 1 or 2 and Yes to 3 or 4 and 5] or [Yes to 6 and 7] or 9	225	No AD or AD symptoms recorded in interview, questionnaire, or register data	1416
Generation R	Birth cohort	1115	53%	1y, 2y, 3y, 4y	1. Has your child had atopic dermatitis in the last 12 months for which he/she attended a general practitioner/hospital?					
				5y	2. Has your child ever had atopic dermatitis?					
						no	Yes to 1 (at any timepoint) or 2	676	No to 1 and 2	439
GOYA	Mothers from birth cohort	3359	0%	29y	1. Have you ever had any skin disease?	yes	Yes to 1 and 2 and 'atopic dermatitis' to 3	180	No to 1	3179
					2. Was the skin disease diagnosed by a doctor?					
					3. What kind of skin disease?					
KORA F3	Cohort study	1375	49%	61y	1. Did a physician ever diagnose you with atopic eczema?	yes	Yes	42	No	1333
KORA F4	Cohort study	1791	49%	61y	1. Did you ever have atopic dermatitis/eczema?					
					2. If yes, was it diagnosed by a physician?					
						yes	Yes to 1 and 2	101	No to 1 or 2	1690
LISA	Birth cohort	379	57%	6m, 12m, 18m, 24m	1. Did a physician diagnose your child having atopic dermatitis in the past 6 months?		1. Yes			
				4y, 5y, 6y	2. Did a physician diagnose your child having atopic dermatitis in the past 12 months?		2. Yes			
						yes	Yes to 1 or 2 at any timepoint	93	No to all of the time points	286
NFBC66	Birth cohort	3502	47%	31y	1. Have you had eczema (infantile, atopic or allergic)?		1. Yes			
					2. if yes, have you ever been treated by a doctor		2. Yes			
						yes	Yes to 1 and 2	1208	No to 1	2294

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PIAMA	Birth cohort	353	53%	3m,1y,2y,3y,4y,5y,6y, 7y,8y	1. Has your child ever had atopic dermatitis?		1. Yes			
				2y,3y,4y,6y,7y,8y	2. Did a doctor ever diagnose atopic dermatitis in your child?		2. Yes			
				2y,3y,4y,6y,7y,8y	3. Did your child have atopic dermatitis during the past 12 months?		3. Yes			
						yes	Yes to 1 and 2 and 3	186	No to 1 and 2 and 3 at ages 2-8	167
RAINE	Birth cohort	1135	53%	5y	1. Do you think your child has ever had atopic dermatitis? Has anyone ever told you your child has atopic dermatitis?[yes and who(paediatrician, GP, child health nurse, naturopath, friend, relative)		1. Yes			
				8y	2. Has your child had atopic dermatitis in the last 12 month?					
						yes	Yes to 1 and were diagnosed by a paediatrician/GP	245	No to 1 and 2 (subject excluded in case of missing).subjects who answered 'Yes' to 1 and were diagnosed to someone other than a paediatrician/GP were coded as missing	890
QIMR	Population based cohort	2148 (adolescent=765, asthma=55, adult=1328)	34%	Mean=32, SD=15, range=10-92	1. Adolescent/Asthma study: Have you (your child) ever had eczema confirmed by a doctor?		1. Yes		1. No	
					2. Adult study: How often have you had any eczema? [“Only as a child”, “Quite often”, “Sometimes”, “Often”, "Never"]		2. Yes to “Only as a child”, “Quite often”, “Sometimes” or “Often”		2. Yes to “Never“	
						no	Yes to 1 or 2	482	No to 1 or 2	1666
Twins UK	cohort study	1236	8%	46 years	1. Have you ever had eczema?	no	Yes	278	No	958

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Replication cohorts										
AAGC		3881	49%	Mean=35, SD=17, range=3-89						
	Population base cohort	QIMR			1. QIMR study: Have you (your child) ever had eczema confirmed by a doctor?	yes	1. Yes	241	1. No	2891
	Birth cohort	CAPS			2. CAPS study: Has your child ever had eczema confirmed by a doctor?	yes	2. Yes	28	2. No	25
	Population based cohort	LIWA						0		474
	Birth cohort	MESCA						0		64
	Birth cohort	TAHS						0		158
						yes	Yes to 1 or 2 at any timepoint	269		3612
ALSPAC	Birth cohort	2903	50%	81m, 91m, 103m, 10y, 13y, 14y	1. Has your child in the past 12 months had eczema?		1. Yes, and saw a Dr			
				10y, 14y	2. Has a doctor ever actually said that your child has eczema?		2. Yes		2. No (@ 14 y)	
						yes	Yes to 1 or 2 at any timepoint	895		2008
BAMSE	Birth cohort	346	Cases: 62%	1y, 2y, 4y, 8y	1. Has a doctor diagnosed your child as having atopic dermatitis after the age of x year		1. Yes			
			Controls:48%	1y	2. Has a doctor ever diagnosed your child as having atopic dermatitis up to 1 year of age		2. Yes			
						yes	Yes to 1 and/or 2	100	No to both Q at all times	246
B58C-REPL	Birth cohort	2090	51.3%	7y	1. Parent interview: History of atopic dermatitis in first year?		1. Yes			
				7y	2. Parent interview: History of atopic dermatitis after first year?		2. Yes			
				7y	3. Medical examination					

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						no	Yes to 1 or 2	170	No to 1 and 2 and 3	1920
ECRHS	Population based cohort study. Information provided is based on follow-up	1650	49.03%	42.8 (7.1)	1. Have you ever had an itchy rash that was coming and going for more than six months?		1. Yes			
					2. Have you had this itchy rash in the last 12 months?		2. Yes			
						no	Yes to 1 and 2	176	No to 1 or 2	1474
Generation R	Birth cohort	620	48%	1y, 2y, 3y, 4y	1. Has your child had eczema in the last 12 months for which he/she attended a general practitioner/hospital?					
				5y	2. Has your child ever had eczema?					
						no	Yes to 1 (at any timepoint) or 2	182	No to 1 and 2	438
Health2006	Population based cohort	3329	44.7%	49.4 years	The U.K. Working Party's diagnostic criteria for atopic dermatitis as a history of an itchy skin condition plus a minimum of two of four minor criteria were used. The major criteria was an itchy skin condition and the minor criteria were: 1) a history of involvement of the skin creases, 2) a personal history of asthma or hay fever, 3) a history of general dry skin in the last year, 4) onset under the age of 2 years.	no	AD cases according to U.K. Working Party's diagnostic criteria for atopic dermatitis	337	non-AD U.K. Working Party's diagnostic criteria for atopic dermatitis	2992
KORA F4	Population based controls	1100	49%	25.4	1. Did a physician ever diagnose you with atopic dermatitis/eczema?					
					2. Dermatologic examination, UK Working Party Criteria					
GENEVA	Tertiary care cases	1100	42%	49.3	1. Did a physician ever diagnose you with atopic dermatitis/eczema?					

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					2. Dermatologic examination, UK Working Party Criteria		Yes to 1 and actual dermatologist's diagnosis	1100	No to 1 and no actual dermatologist's diagnosis	1100
						yes	Yes to 1	1100	No to 2	1100
LISA/GINI	Birth cohort	665 (GINI: 499, LISA: 166)	51%	LISA: 6m, 12m, 18m, 24m	1. Did a physician diagnose your child having atopic dermatitis in the past 6 months?		1. Yes			
				LISA: 3y-6y, GINI: 1y-6y	2. Did a physician diagnose your child having atopic dermatitis in the past 12 months?		2. Yes			
						yes	Yes to 1 or 2 at any timepoint	231	No to all of the time points	434
MAAS	Unselected birth cohort	761	55%	1y, 3y, 5y, 8y	1. Has your child ever suffered from atopic dermatitis	no	'Yes' at any timepoint	435	No	326
MoBa	Pregnancy cohort	937	51%	6m, 18m, 36m	1. Does your child have or has he/she had any of the following health problems? (Enter a cross in a box for each item.) Atopic eczema (childhood eczema) - listed as an item		1. yes			
					2. If yes, has the mother and child health centre or someone else referred your child for further specialist investigation?		2. yes			
						yes	Yes to 1 and 2 at any timepoint	70	No to all of the time points	867
NFBC86	Birth cohort	4465	50%	15-16y	1. Have you ever had eczema which has been called infantile eczema, atopic eczema or allergic eczema?					
					2. Diagnosed or treated by a doctor?	yes	yes to 1+2	798	no to 1	3667
NTR	Population based cohort study	429	50.8%	5y	1. Did a physician since birth ever diagnosed your children with eczema? (Oldest/youngest answered seperately)					
					2. Did a physician since birth ever diagnosed your children with baby eczema? (Oldest/youngest answered seperately)					
						yes	Yes to 1 or 2	123	1. No to 1 and 2	306

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SAPALDIA	Population based cohort study.	976	50%	2 examinations: baseline in 1991, follow-up in 2002. Age (sd) in 2002: 53.2 (11.1)	Have you ever had atopic dermatitis or any other kind of skin allergy?	no	Yes. At either of the 2 examinations (1991 & 2002)	533	No' at both examinations 'No' at follow-up, if missing at baseline ('No' at baseline & missing at follow-up set to missing)	443

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Supplementary Table 2. Study genetic & analysis methods (a) discovery and (b) replication cohorts

(a)

	Genotyping		BEFORE IMPUTATION QUALITY CONTROL PER SUBJECT				BEFORE IMPUTATION QUALITY CONTROL PER SNP				IMPUTATION			DATA ANALYSIS	
Cohort	**Genotyping Platform**	**Genotype-Calling Algorithm**	**call rate threshold**	**heterozygosity thresholds**	**ethnicity exclusions**	**other exclusion criteria**	**SNP call rate**	**HWE p-value threshold**	**MAF threshold**	**other exclusion criteria**	**Imputation Software (Version)**	**HapMap CEU Release**	**NCBI Build**	**Association Software**	**GWAS Lambda**
ALSPAC	Illumina 317K or 610k		0.97	0.34 & 0.36	MDS - eigenstrat adjusted	sex discrpeancies, related individuals	0.97	5E-07	0.005	no	MACH 1.0	22	36	MACH2DAT	1.0068
B58C-WTCCC	Affymetrix 500	Chiamo	0.97	0.23 & 0.30	yes	external discordance, relatives,gender discrepancies	0.95	1E-04	0.01	no	IMPUTE	21	35	quicktest	1.0088
B58C-T1DGC	Illumina Infinium 550	Illuminus	0.98	no	yes	external discordance, relatives,gender discrepancies	no	no	no	multi-allelic SNPs, SNPs with mismatch in alleles between dbSNP and Illumina	MACH	21	35	probAbel	1.0125
CHOP	Illumina HH 550v1/v3 HH610	Illumina BeadStudio software	0.98	no	yes, non-cauc excluded	no	0.95	1E-04	0.01	no	Impute	22	36	snptest	1.05
COPSAC	Illumina 550K	BeadStudio v 3.3.4	0.98	no	yes	no	0.95	1E-04	0.01	no	IMPUTE v2	22	36	SNPTEST	1.0272
DNBC	Illumina Human 660w-quad	BeadStudio Genotyping Module, version 3.3.7	0.95	no	yes	no	0.98	0.001	0.01	SNPs where strand issues could not be resolved, e.g., A/T and C/G SNPs	MACH	22	36	MACH2DAT	1.0051
Generation R	Illumina 610K Quad	BeadStudio Genotyping Module, version 3.2.32	0.975	3 SD of the mean	Yes	yes (IBD - check: no family relations)	0.98	1E-06	0.01	no	MACH v1.0.16	22	36	Plink,MACH2DAT	1.0165
GOYA	Illumina 610k		0.95	0.3 & 0.35	MDS	sex discrepancies, related individuals	0.95	1E-07	0.01	NA	MACH	22	36	MACH2DAT	0.9949
KORA F3	Affymetrix 500K	BRLMM	0.93	no	german passport	gender discrepancies	no	no	no	no	IMPUTE		35	snptest	0.9648

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KORA F4	Affymetrix Genome-Wide Human SNP Array 6.0	Birdseed2	0.93	no	german passport	gender discrepancies	no	no	no	no	IMPUTE		36	snptest	0.9997
LISA	Affymetrix Genome-Wide Human SNP Array 5.0	BRLMM-P	0.95	no	no	no	0.95	0.01	0.01	no	IMPUTE v1.06	22	36	SNPTEST	1.0223
NFBC66	Illumina HumanCNV370DUO Analysis BeadChip	Beadstudio	0.95	no	no	no phenotype data, IBD, withdrew consent, gender discrepancies, contaminated or duplicate samples	0.95	1E-04	0.01	no	IMPUTE v1.0	21	35	quicktest	1.0097
PIAMA	Illumina Human610 quad array	GenomeStudio Software	0.95	no	no	inconsistent sex	0.95	1E-07	0.01	no	IMPUTE v2	22	36	snptest	1.053
QIMR	Illumina 610K or CNV370	Illumina BeadStudio software	0.95	no	yes	no	0.95	1E-06	0.01	yes (BeadStudio GenCall score <0.7; SNPs exclusive to 610K or CNV370)	MACH	21	35	Plink	0.9968
RAINE	Illumina 660K	Illumina's Bead Studio Genotyping Module software v.3.1	0.95	no	Yes	yes - IBD check and exclude family relations, congenital abnormalities	0.95	5.7E-07	0.01	no	MACH	22	36	MACH2DAT	0.9931
Twins UK	Illumina 317K (3/5) & 610K (2/5)	Illuminus	0.95	Yes	Yes, only caucasian	no	0.95	1E-04	0.01	no	IMPUTE	22	36	Plink	1.0846

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(b)

***In silico* replication**

	Genotyping		BEFORE IMPUTATION QUALITY CONTROL PER SUBJECT				BEFORE IMPUTATION QUALITY CONTROL PER SNP				IMPUTATION			DATA ANALYSIS
Cohort	**Genotyping Platform**	**Genotype-Calling Algorithm**	**call rate threshold**	**heterozygosity thresholds**	**ethnicity exclusions**	**other exclusion criteria**	**SNP call rate**	**HWE p-value threshold**	**MAF threshold**	**other exclusion criteria**	**Imputation Software (Version)**	**HapMap CEU Release**	**NCBI Build**	**Association Software**
AAGC	Illumina 610K or CNV370	Illumina BeadStudio software	0.95	no	yes	no	0.95	1E-06	0.01	yes (BeadStudio GenCall score <0.7; SNPs exclusive to 610K or CNV370)	Impute2	1000 Genomes Project (CEU Mar 2010) + HapMap3 (All 11 populations, Feb 2009)	36	Plink
ALSPAC	Illumina HumanHap550 quad		0.97	0.32 - 0.345 or 0.31 - 0.33	caucasians only	sex discrepancies, cryptic relatedness, replicates <80% IBD	0.95	5E-07	0.01	No	Mach 1.0.16	22	36	Mach2Dat
BAMSE*	illumina 610k	GenCall	no	no	caucasians only	sex discrepancies, related individuals	no	no	no	No	Mach	22	36	ProbAbel
B58C-REPL	Illumina 550k/610k	GenCall	0.98	none	yes	none	0.95	1E-04	0.01	inconsistency of allele frequency across multple deposits	Mach	21	35	ProbAbel
ECRHS*	illumina 610 quad	Gencall	no	no	caucasians only	sex discrepancies, cryptic relatedness	no	no	no	no	MACH	22	36	ProbABEL
Generation R	Illumina 610K quad	BeadStudio Genotyping Module, version 3.2.32	0.975	3 SD of the mean	Yes	yes (IBD - check: no family relations)	0.98	1E-06	0.01	no	MACH v1.0.16	22	36	Plink, MACH2DAT
LISA/GINI	Affymetrix 5.0 Affymetrix 6.0	BRLMM-P (5.0), BIRDSEED V2 (6.0)	0.95	Mean +/- 4 SD	caucasians only	sex discrepancies	0.95	1E-5	0.01	no	Impute2	22	36	SnpTest

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MAAS	illumina 610 quad	Illumina GenCall application	0.97	outliers	caucasians only	sex discrepancies, were non-concordant on sequenome genotyping	0.95	5.9E-07	0.01	No	Impute2	3 + 1000 genomes	36	SnpTest
MoBa	illumina 610 quad	Gen Call	0.97			sex discrepancies	0.95	1E-03	0.01		Plink	22	36	Plink
NTR	Affymetrix 6.0	Birdseed V2	no	no	no	Clear sample switches based on fingerprint data (64 SNPs)	0.95	1E-04	0.01	MI>35	Beagle	22	36	Plink
SAPALDIA*	illumina 610 quad	Gencall	no	no	caucasians only	sex discrepancies, cryptic relatedness	no	no	no	no	Mach	22	36	ProbABEL

***de novo* genotyping replication**

			QUALITY CONTROL PER SUBJECT			QUALITY CONTROL PER SNP		
Cohort	**Genotyping Method**	**Genotype-Calling Algorithm**	**call rate threshold**	**ethnicity exclusions**	**other exclusion criteria**	**lowest SNP call rate**	**SNPs with HWE p-values <0.05**	**Association Software**
Health2006	The PCR KASPar genotyping system (KBiosciences, Hoddesdon, UK).	Kraken (Kbioscience)	0.98	caucasians	Danish citizenship, born in Denmark	0.98	0	SPSS
KORA F4 / GENEVA	Sequenom MALDI-TOF MS 4.0	Sequenom Typer 4.0	0.97	caucasian	sex discrepancies	0.97	0	R
NFBC86	The PCR KASPar genotyping system (KBiosciences, Hoddesdon, UK).	Klustercaller (Kbioscience)	0.97			0.97	0	Quicktest

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*GABRIEL cohorts had QC applied only after the imputation step, see BAMSE methods for details.

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Supplementary Table 3. Discovery and replication results of the top 11 SNPs for atopic dermatitis. 1 SNP per region was followed up in the replication stage. Results are for the fixed effect inverse-variance meta-analysis, with genomic control applied to the individual studies in the discovery meta-analysis. The heterogeneity p-value (het p), testing for overall heterogeneity between all discovery and replication studies was generated using Cochran's Q-test for heterogeneity. All OR (odds ratios) are given with the minor allele representing the effect allele (Eff). CI denotes the confidence interval

				Alleles		Discovery				Replication			Combined			
chr	SNP	Position (bp)	Gene	Eff	Alt	Effect Allele Freq	N	OR (95% CI)	pvalue	N	OR (95% CI)	pvalue	N	OR(95%CI)	pvalue	het p
11	rs479844	65308533	OVOL1	A	G	0.44	26,151	0.89 (0.85, 0.93)	**7.8E-07**	25,098	0.87 (0.83,0.92)	**2.4E-08**	51,249	0.88 (0.85,0.91)	**1.1E-13**	0.23
19	rs2164983	8650381	ACTL9	A	C	0.15	17,403†	1.22 (1.13, 1.32)	**1.8E-07**	22,996	1.11 (1.04,1.19)	**0.002**	40,399	1.16 (1.10,1.22)	**7.1E-09**	0.004
1	rs9050*	150345938	TCHH	A	C	0.06	25,788	1.33 (1.20, 1.47)	**1.9E-08**	-	-	-	-	-	-	0.95
5	rs2897442	132076926	KIF3A	C	T	0.29	26,164	1.12 (1.07, 1.18)	**7.8E-06**	25,064	1.09 (1.04,1.15)	**0.001**	51,228	1.11 (1.07,1.15)	**3.8E-08**	0.52
8	rs7000782	81470705	ZBTB10	A	T	0.43	26,077	1.14 (1.09, 1.20)	**1.6E-08**	20,873	1.03 (0.98,1.08)	**0.296**	46,950	1.09 (1.05,1.13)	**1.1E-06**	0.24
22	rs4821544	35588449	NCF4	C	T	0.29	24,770	1.13 (1.07, 1.19)	**3.5E-06**	25,103	1.05 (0.99,1.10)	**0.077**	49,873	1.09 (1.05,1.13)	**5.5E-06**	0.53
6	rs3853601	31607582	BAT1	G	C	0.12	25,528	1.17 (1.09, 1.26)	**7.6E-06**	21,964	1.09 (1.01,1.17)	**0.031**	47,492	1.13 (1.08,1.19)	**1.9E-06**	0.04
10	rs10994675	51233999	MSMB	A	G	0.42	24,787	1.12 (1.07, 1.17)	**3.1E-06**	22,903	1.00 (0.95,1.05)	**0.929**	47,690	1.06 (1.03,1.10)	**0.001**	0.39
13	rs1327914	95891570	HS6ST3	C	T	0.17	26,168	1.16 (1.10, 1.24)	**8.9E-07**	25,088	0.98 (0.92,1.04)	**0.434**	51,256	1.07 (1.02,1.12)	**0.003**	0.005
10	rs4520482	67139368	CTNNA3	A	G	0.43	26,031	0.90 (0.86, 0.94)	**8.7E-06**	25,109	1.02 (0.97,1.07)	**0.457**	51,140	0.96 (0.92,0.99)	**0.008**	0.32
9	rs10983837	119738636	TLR4	A	C	0.03	26,101	1.35 (1.18, 1.54)	**6.8E-06**	24,168	0.92 (0.80,1.05)	**0.229**	50,269	1.12 (1.02,1.24)	**0.015**	0.002

*rs9050 (and other associated SNP rs11205006 in the same region) were excluded from the replication phase after they were found to not be independent from the association with the *FLG* mutations in the same region.

†rs2164983 was not included in the HapMap release 21 and so was missing for some discovery cohorts.

The SNP rs1327914 was replaced by the SNP rs927709 (r^2=1.00) in the B58C-WTCCC, B58C-T1DGC, KORA-F3, NFBC66 and the B58C replication cohort

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Supplementary Table 4. AD association results from the discovery meta-analysis for the 15 loci associated with asthma or total serum IgE levels in a recent GWAS. Moffat MF, Gut IG, Demenais F, et al. A large-scale consortium-based genomewide association study of asthma. N Engl J Med 2010;125:328-35.

					Moffat et al. (2010) asthma association results		AD association results from current meta-analysis	
SNP	**Gene**	**Position**	**effect allele**	**other allele**	**OR (95% CI)**	**pvalue**	**OR (95% CI)**	**pvalue**
rs3771166	*IL18R1*	2q12.1	a	g	0.87 (0.83-0.91)	3.4E-09	1.00 (0.95-1.05)	0.9791
rs9273349	*HLA-DQB1*	6p21.32	g	a	1.18 (1.13-1.24)	7.0E-14	0.95 (0.86-1.04)	0.2647
rs1342326	*IL33*	9p24.1	c	a	1.20 (1.13-1.28)	9.2E-10	0.99 (0.93-1.05)	0.7789
rs744910	*SMAD3*	15q22.33	a	g	0.89 (0.86-0.92)	3.9E-09	0.98 (0.94-1.03)	0.3987
rs2305480	*GSDMB*	17q12	a	g	0.85 (0.81-0.90)	9.6E-08	1.00 (0.96-1.05)	0.8723
rs3894194	*GSDM1*	17q21.1	a	g	1.17 (1.11-1.23)	4.6E-09	1.00 (0.95-1.04)	0.8893
rs2284033	*IL2RB*	22q12.3	a	g	0.89 (0.86-0.93)	1.1E-08	1.03 (0.98-1.07)	0.2845
rs1295686	*IL13*	5q31.1	c	t	0.85 (0.79-0.90)	1.4E-07	0.91 (0.86-0.96)	0.0008
rs2073643	*SLC22A5*	5q31.1	c	t	0.89 (0.84-0.93)	2.2E-07	0.96 (0.92-1.00)	0.0771
rs11071559	*RORA*	15q22.2	t	c	0.88 (0.81-0.95)	1.1E-07	1.01 (0.95-1.08)	0.7475

					Moffat et al. (2010) total serum IgE association results		AD association results from current meta-analysis	
SNP	**Gene**	**Position**	**effect allele**	**other allele**	**beta**	**pvalue**	**OR (95% CI)**	**pvalue**
rs2252226	*FCER1A*	1q23.2	t	c	NA	6.6E-05	0.96 (0.92-1.01)	0.0817
rs20541	*IL13*	5q31.1	a	g	NA	1.0E-06	1.10 (1.04-1.16)	0.0007
rs9271300	*HLA-DRB1*	6p21.32	c	g	NA	8.3E-15	0.99 (0.90-1.09)	0.9013
rs167769	*STAT6*	12q13.3	t	c	NA	8.5E-07	1.05 (1.00-1.10)	0.0379
rs1859308	*IL4-R/IL21R*	16p12.1	a	g	NA	8.2E-06	0.97 (0.91-1.04)	0.4477

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Supplementary Table 5. Meta-analysis results for interactions between the three identified loci. Results are based on the discovery cohorts. Betas are the ln(odds) of AD per 1 unit change in the interaction variable (SNP1*SNP2, SNPs coded as 0,1,2 with the minor allele as the increasing allele).

Interaction	beta	95% CI	p-value
rs2897442*rs479844	-0.020	-0.090 to 0.050	0.578
rs2897442*rs2164983	0.038	-0.081 to 0.157	0.535
rs2164983*rs479844	0.019	-0.086 to 0.124	0.722

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Supplementary Table 6. Immunochip association results on region 5q31.1. Conditional association analysis for markers of the cytokine cluster on 5q31.1 including *IL13* polymorphisms previously shown to be associated with asthma and psoriasis risk, as well as the GWAS *KIF3A* polymorphism showing the strongest association in the meta-analysis and the lead SNP of the corresponding putative LD-block from the finemapping approach.

		Marker 1				
		rs1800925 (IL13)	rs20541 (IL13)	rs848 (IL13)	rs66913936 (IL4)	rs2897442 (KIF3A)
Marker 2	rs1800925 (IL13)	***1.32 (1.20-1.46)*** ***P=1.74 x 10^{-8}***	1.26 (1.11-1.41) P=0.0002	1.27 (1.12-1.42) P=0.0001	1.23 (1.11-1.35) P=5.36 x 10^{-5}	1.19 (1.08-1.31) P=0.0005
	rs20541 (IL13)	1.17 (1.04-1.32) P=0.0085	***1.37 (1.24-1.52)*** ***P=4.07 x 10^{-10}***	1.42 (0.72-2.81) P=0.3090	1.18 (1.06-1.31) P=0.0018	1.14 (1.03-1.26) P=0.0096
	rs848 (IL13)	1.17 (1.04-1.31) P=0.0107	0.97 (0.49-1.92) P=0.9249	***1.38 (1.25-1.52)*** ***P=1.93 x 10^{-10}***	1.18 (1.06-1.31) P=0.0022	1.14 (1.03-1.26) P=0.0113
	rs66913936 (IL4)	1.24 (1.12-1.37) P=3.53 x 10^{-5}	1.27 (1.13-1.42) P=2.86 x 10	1.28 (1.14-1.42) P=1.74 x 10^{-5}	***1.31 (1.19-1.43)*** ***P=2.58 x 10^{-8}***	0.91 (0.71-1.17) P=0.4742
	rs2897442 (KIF3A)	1.26 (1.14-1.39) P=1.01 x 10^{-5}	1.29 (1.16-1.44) P=3.42 x 10^{-6}	1.30 (1.17-1.45) P=2.00 x 10^{-6}	1.43 (1.10-1.84) P=0.0069	***1.26 (1.15-1.38)*** ***P=8.84 x 10^{-7}***

Conditional analysis of Marker 1 conditioned on Marker 2 using the logistic regression framework. The diagonal elements shows results of the unconditional analysis. Displayed are odds ratios with corresponding 95% confidence intervals in brackets and P-values

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Supplementary Table 7. Immunochip linkage disequilibrium (LD) in region 5q31.1. Pair-wise LD measures between markers of the cytokine cluster on 5q31.1 including *IL13* polymorphisms previously shown to be associated with asthma and psoriasis risk, as well as the GWAS KIF3A polymorphism showing the strongest association in the meta-analysis and the lead SNP of the corresponding putative LD-block from the finemapping approach.

				Marker 1		
Marker 2		rs1800925 (IL13)	rs20541 (IL13)	rs848 (IL13)	rs66913936 (IL4)	rs2897442 (KIF3A)
	rs1800925 (IL13)	1	0.301	0.298	0.090	0.078
	rs20541 (IL13)	0.564	1	0.979	0.192	0.164
	rs848 (IL13)	0.558	0.995	1	0.194	0.166
	rs66913936 (IL4)	0.328	0.493	0.492	1	0.858
	rs2897442 (KIF3A)	0.323	0.483	0.483	0.982	1

The upper triangular matrix shows r^2 values, whereas the lower triangular matrix displays D' values. The color coding refers to the strength of LD.

Values	R^2	D'
<0.2		
0.2-0.4		
0.4-0.6		
0.6-0.8		
>0.8		

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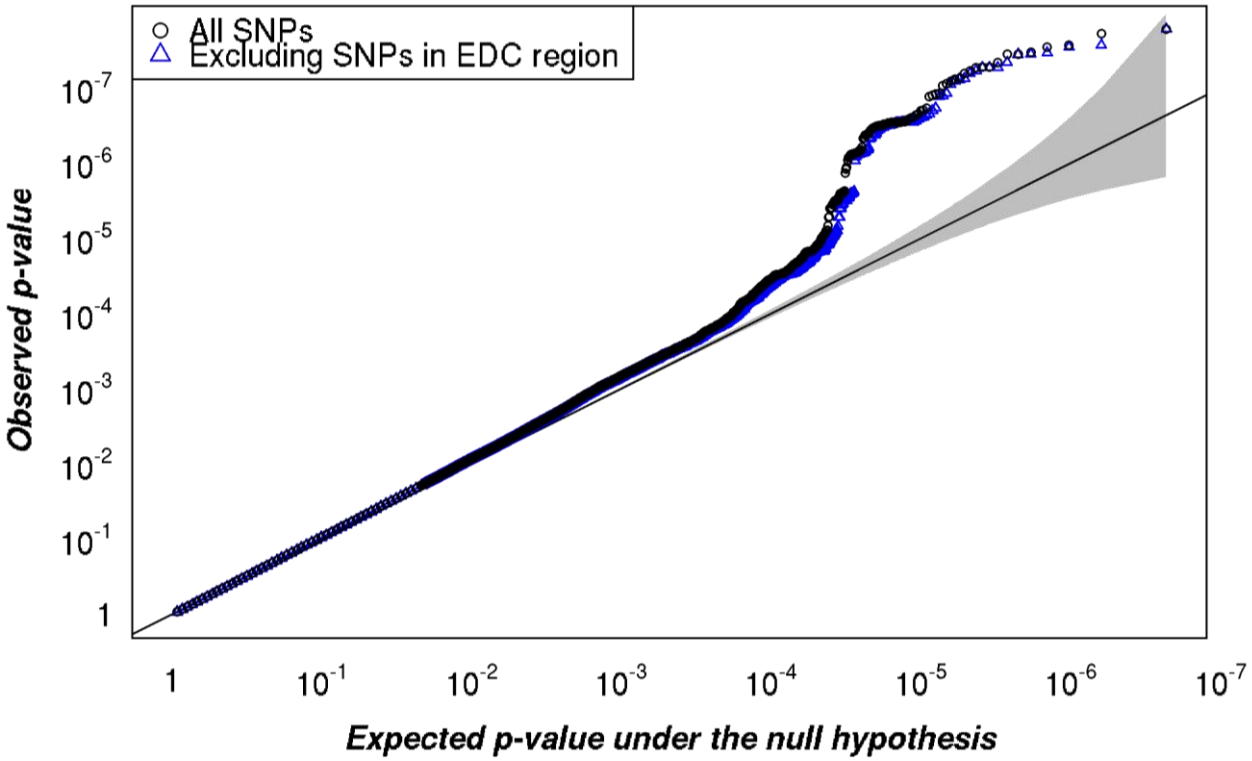
Supplementary Figure 1. QQ plot for the discovery genome-wide association meta-analysis, after excluding all SNPs MAF<1% and Rsqr<0.3 or proper_info<0.4. □=1.017.EDC=epidermal differentiation complex region (which contains FLG) defined as Chr 1:150.2-151.9Mb.



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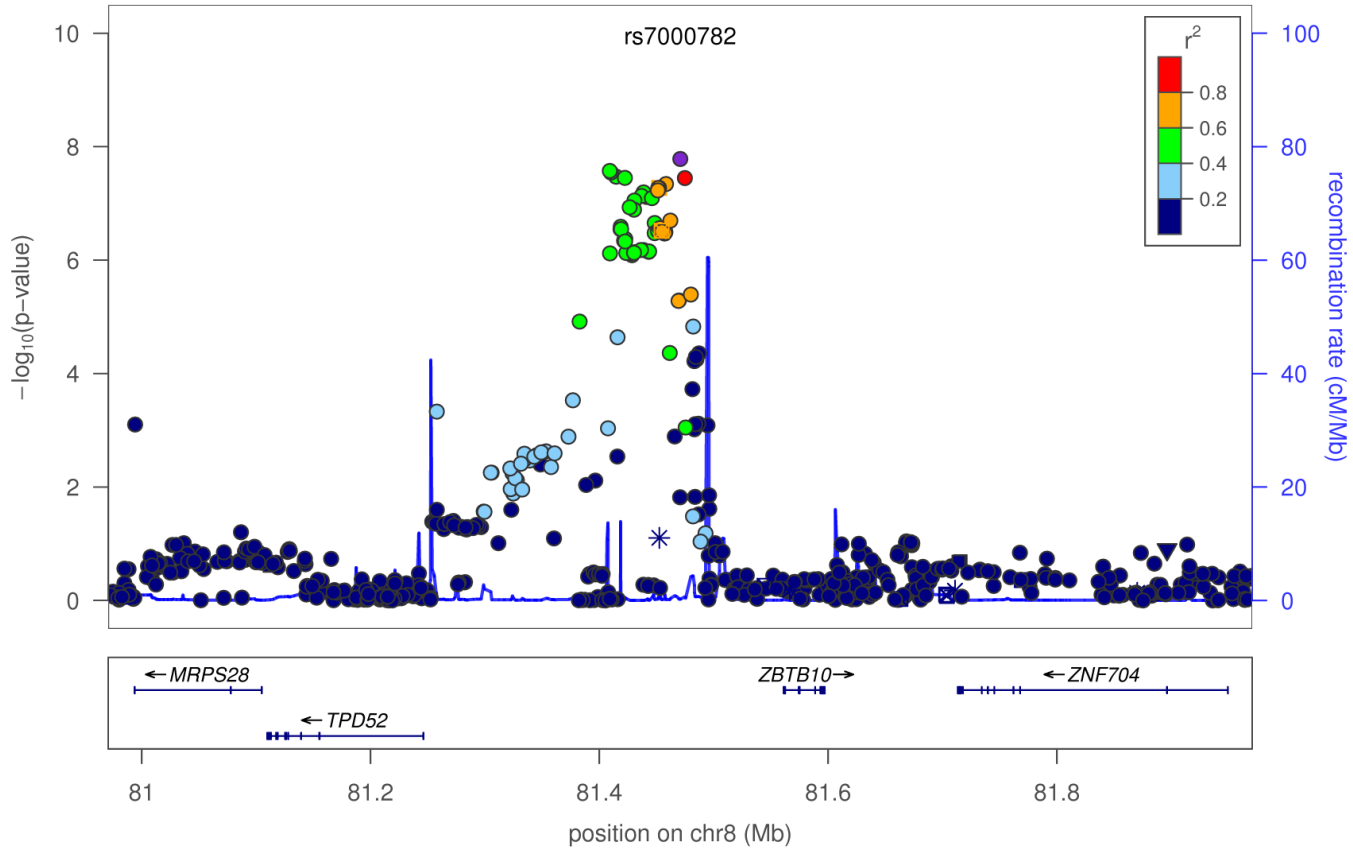
Supplementary Figure 2. Regional association plots for the top 11 regions. Ordered by significance in the discovery analysis.



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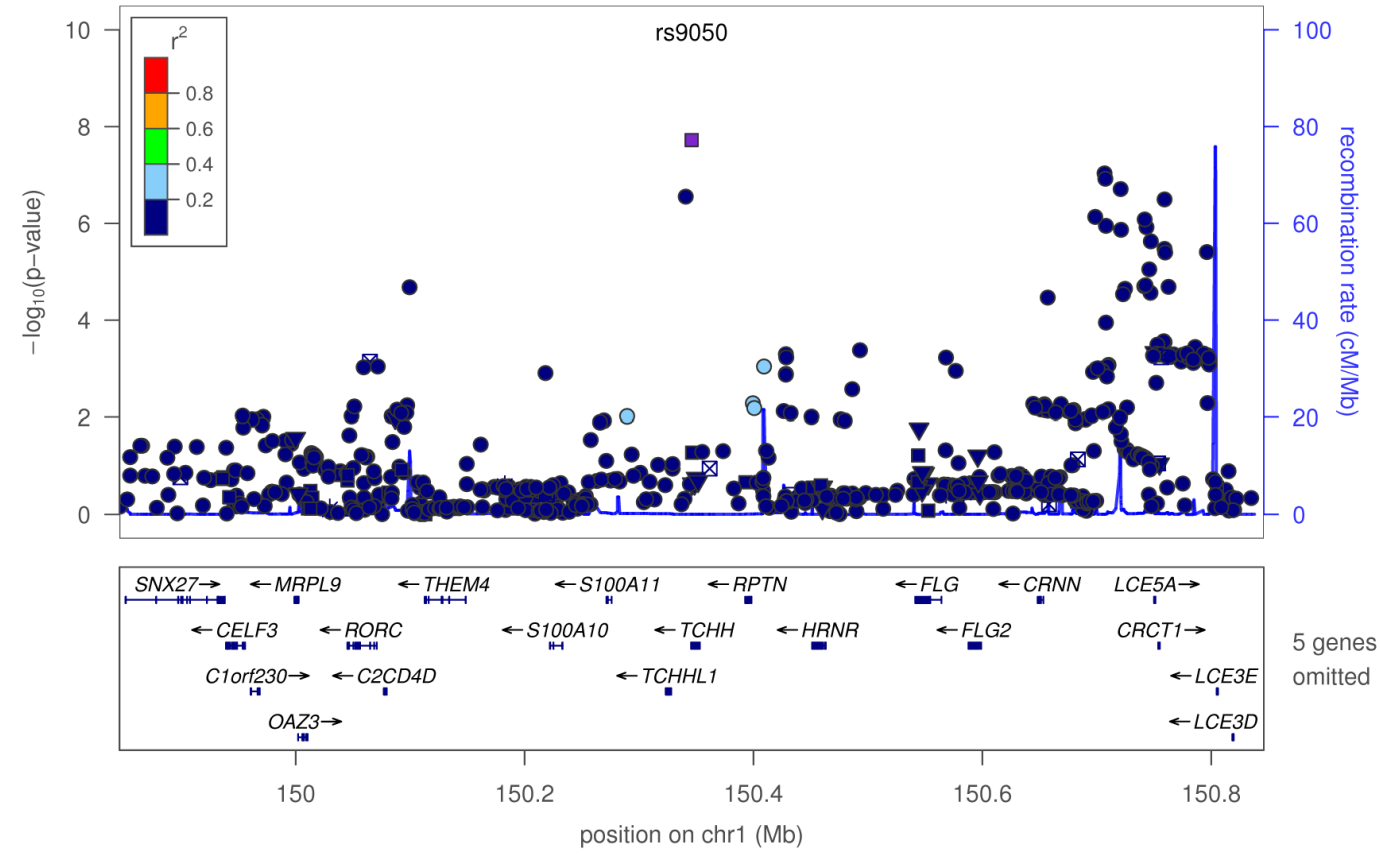

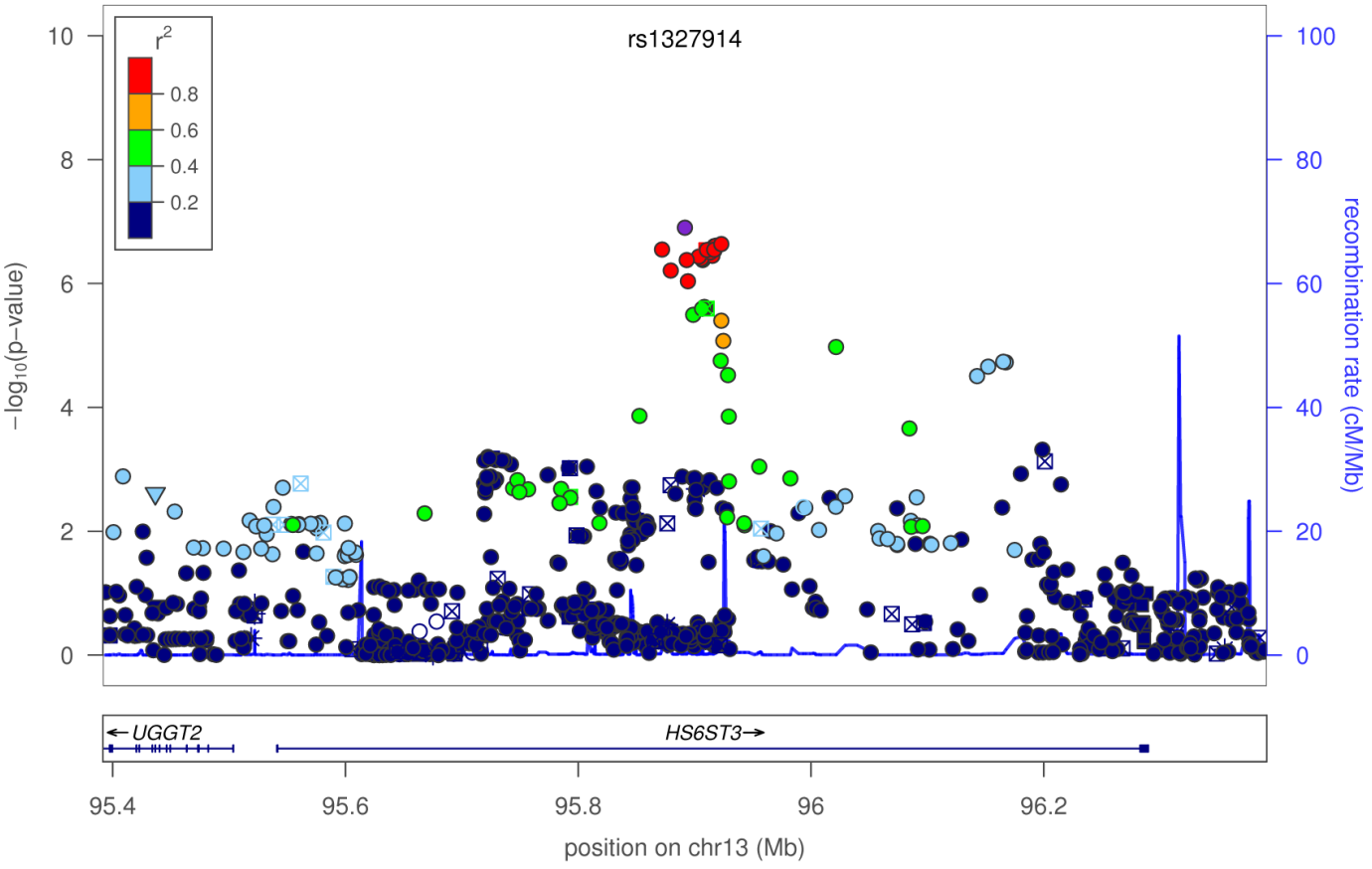

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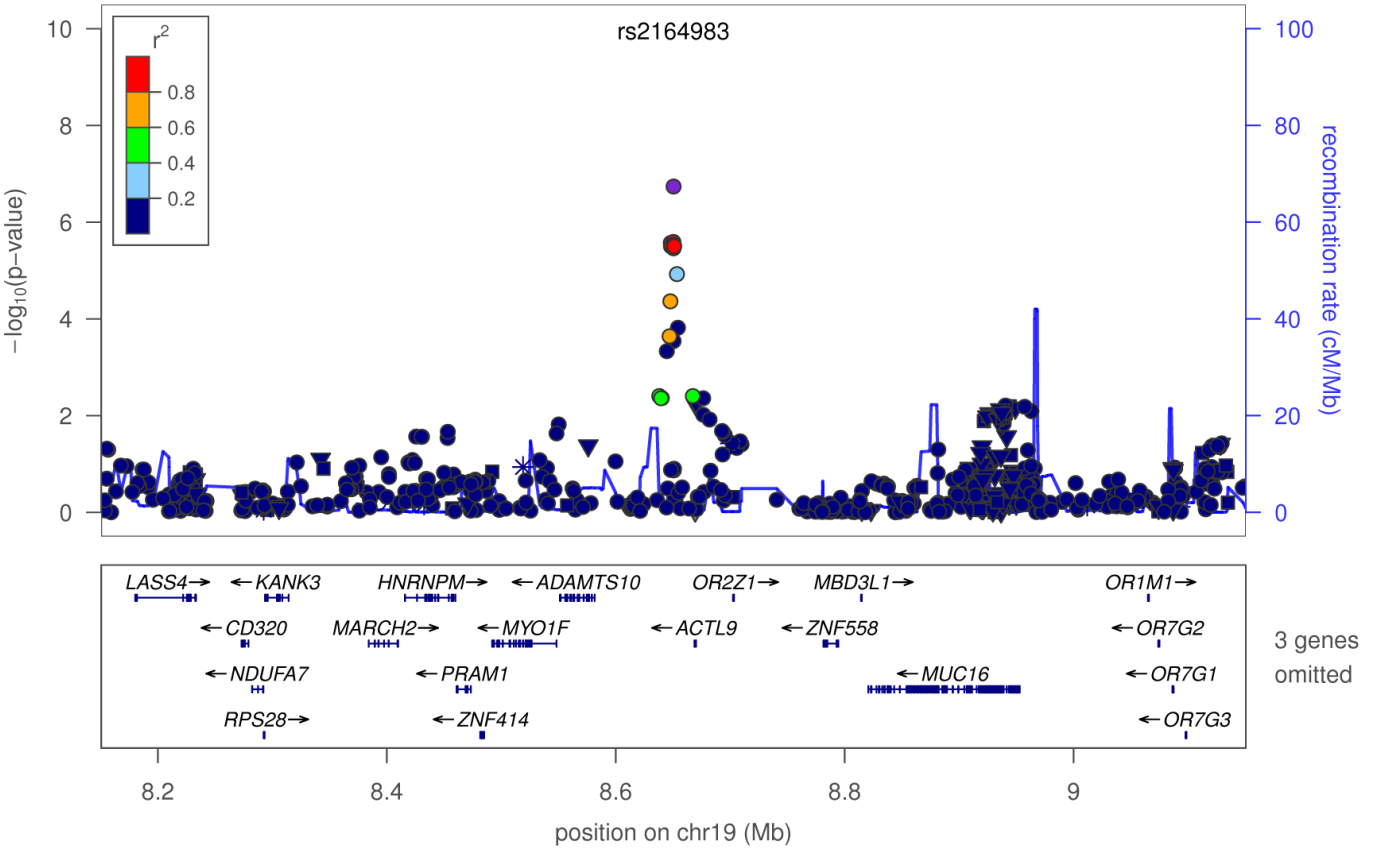

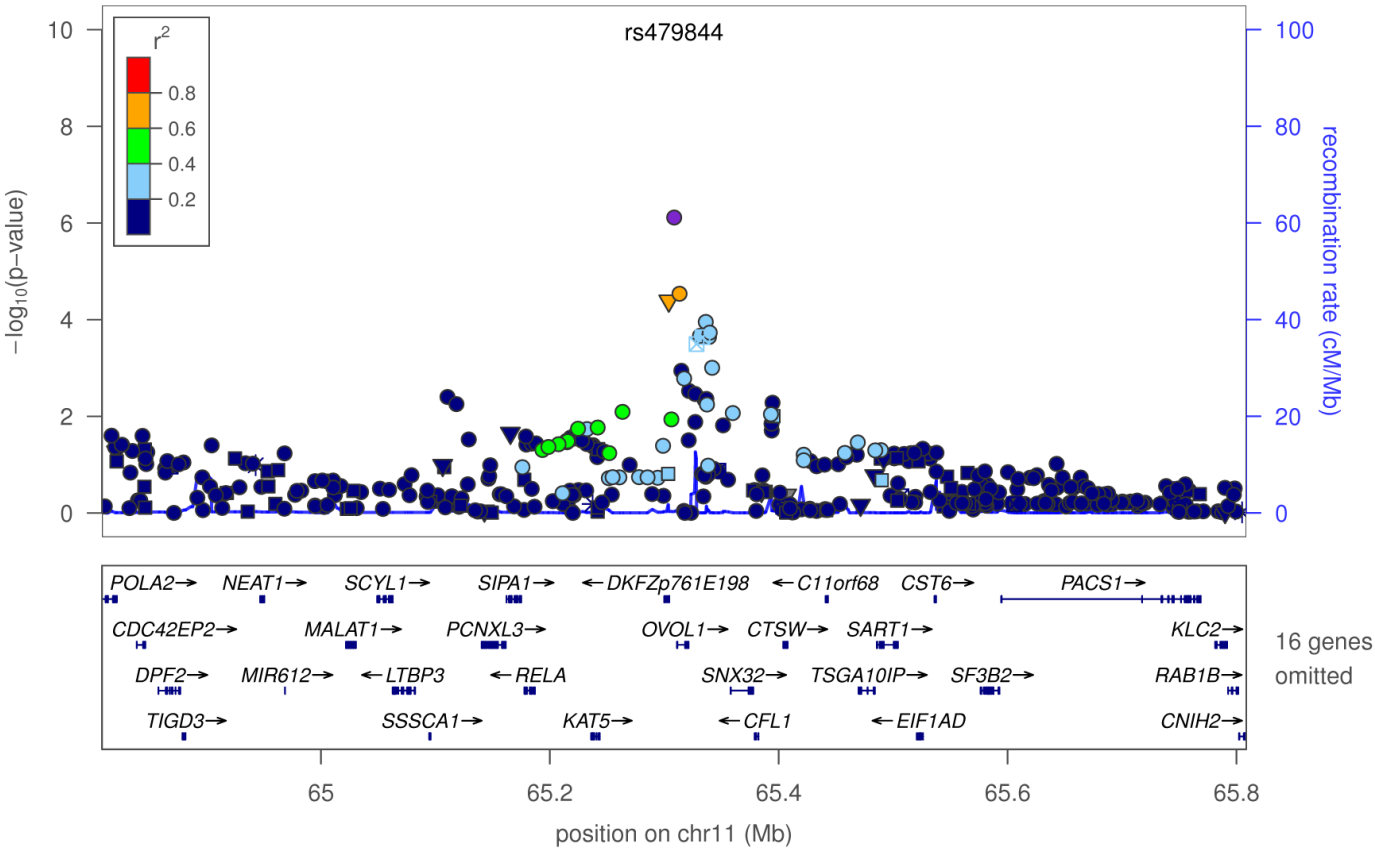

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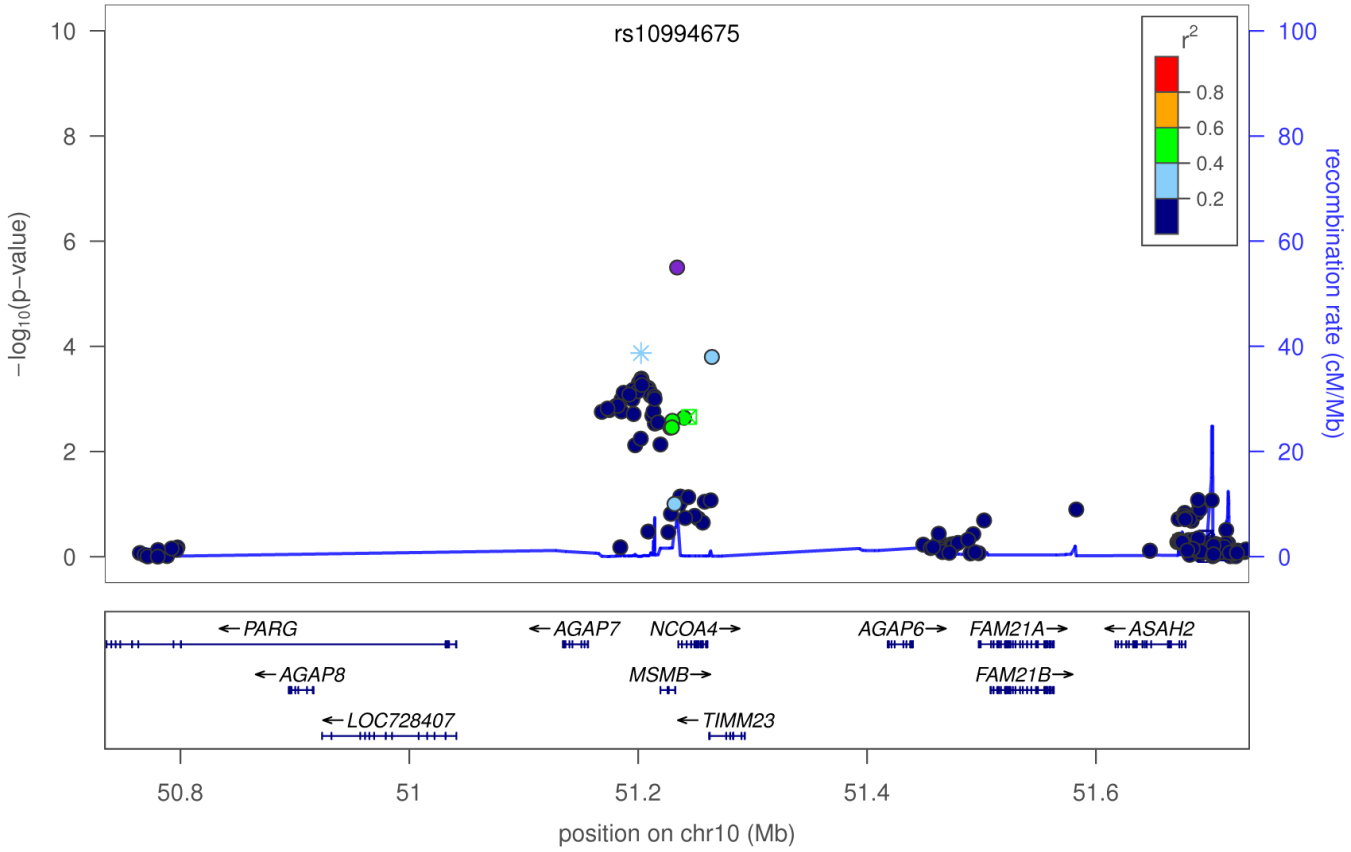

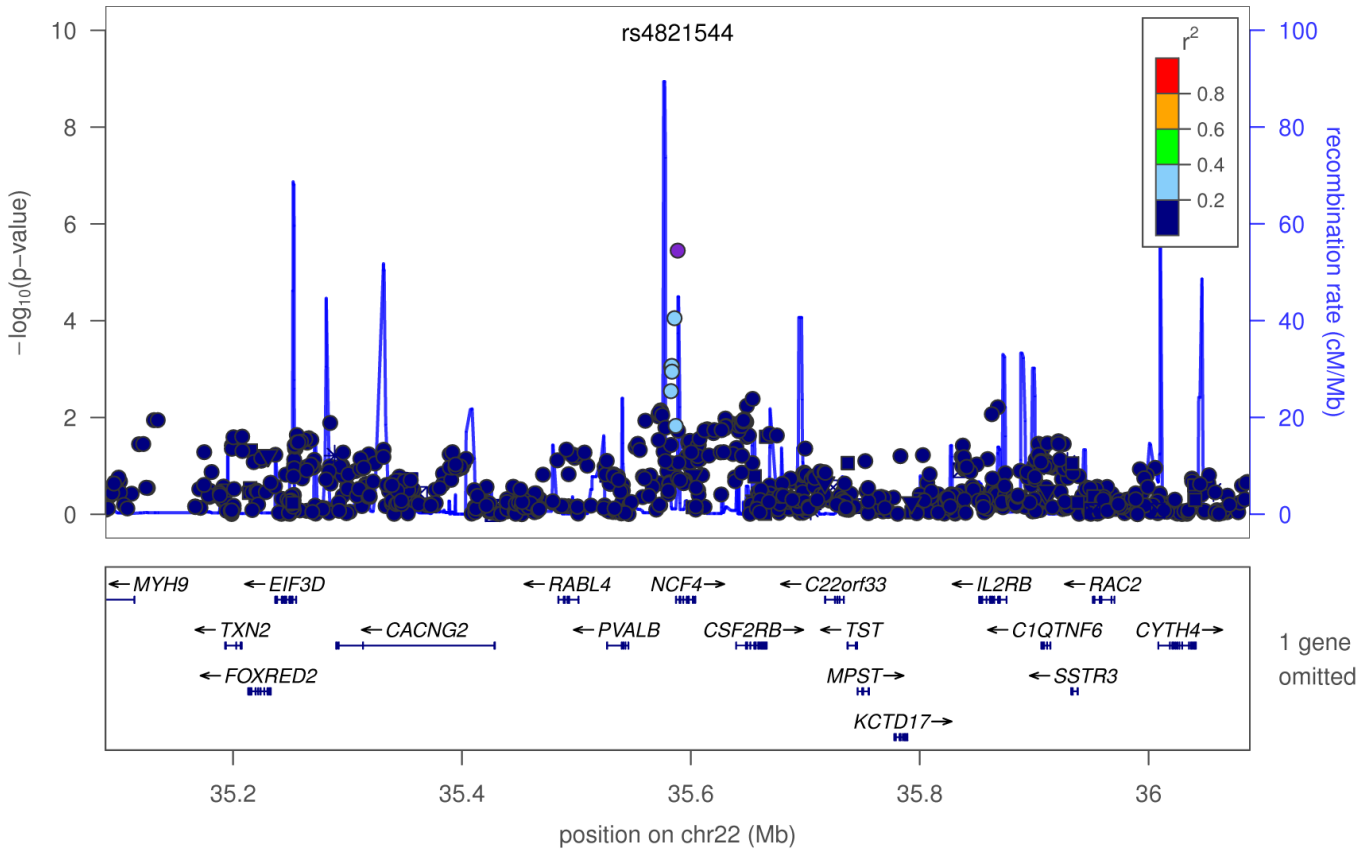

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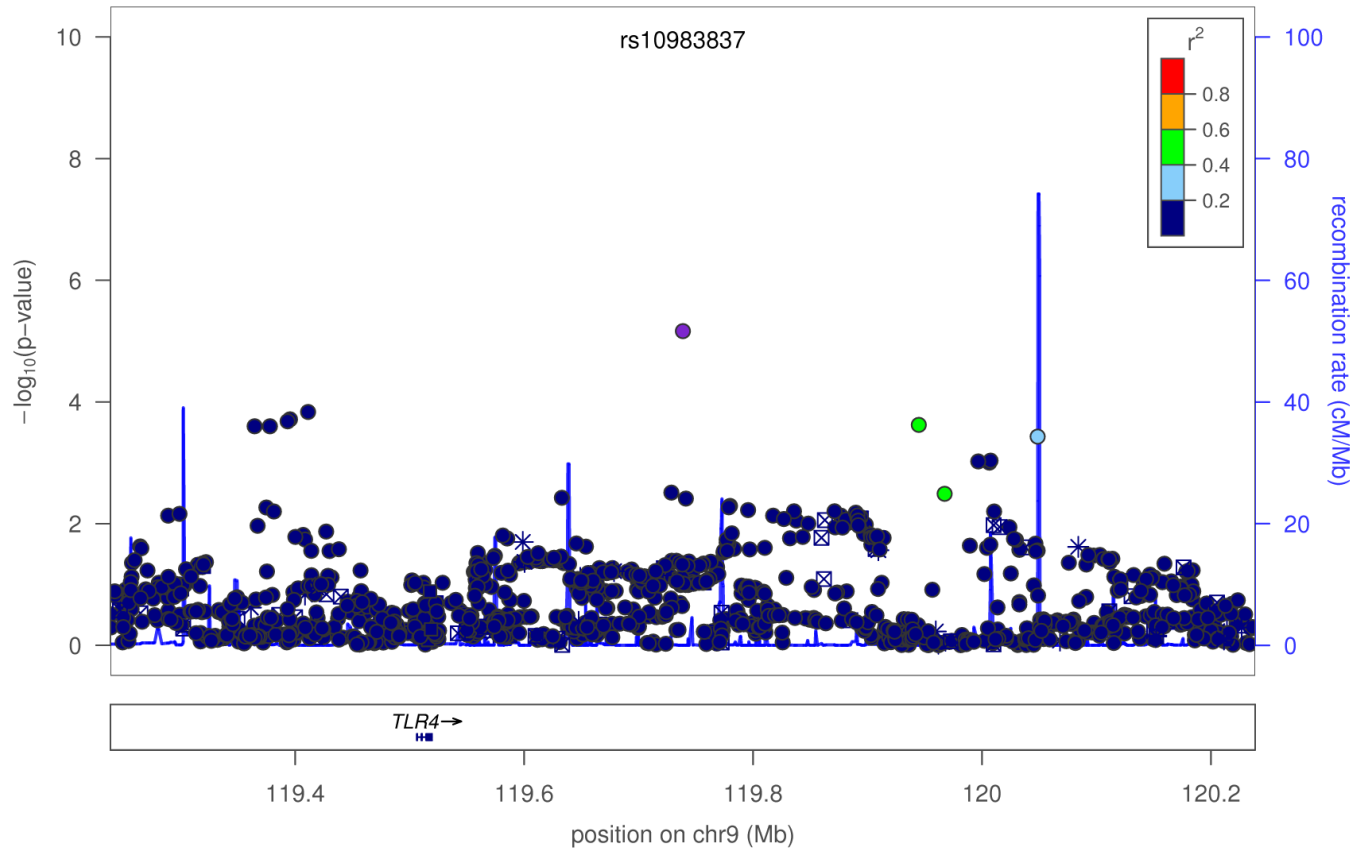

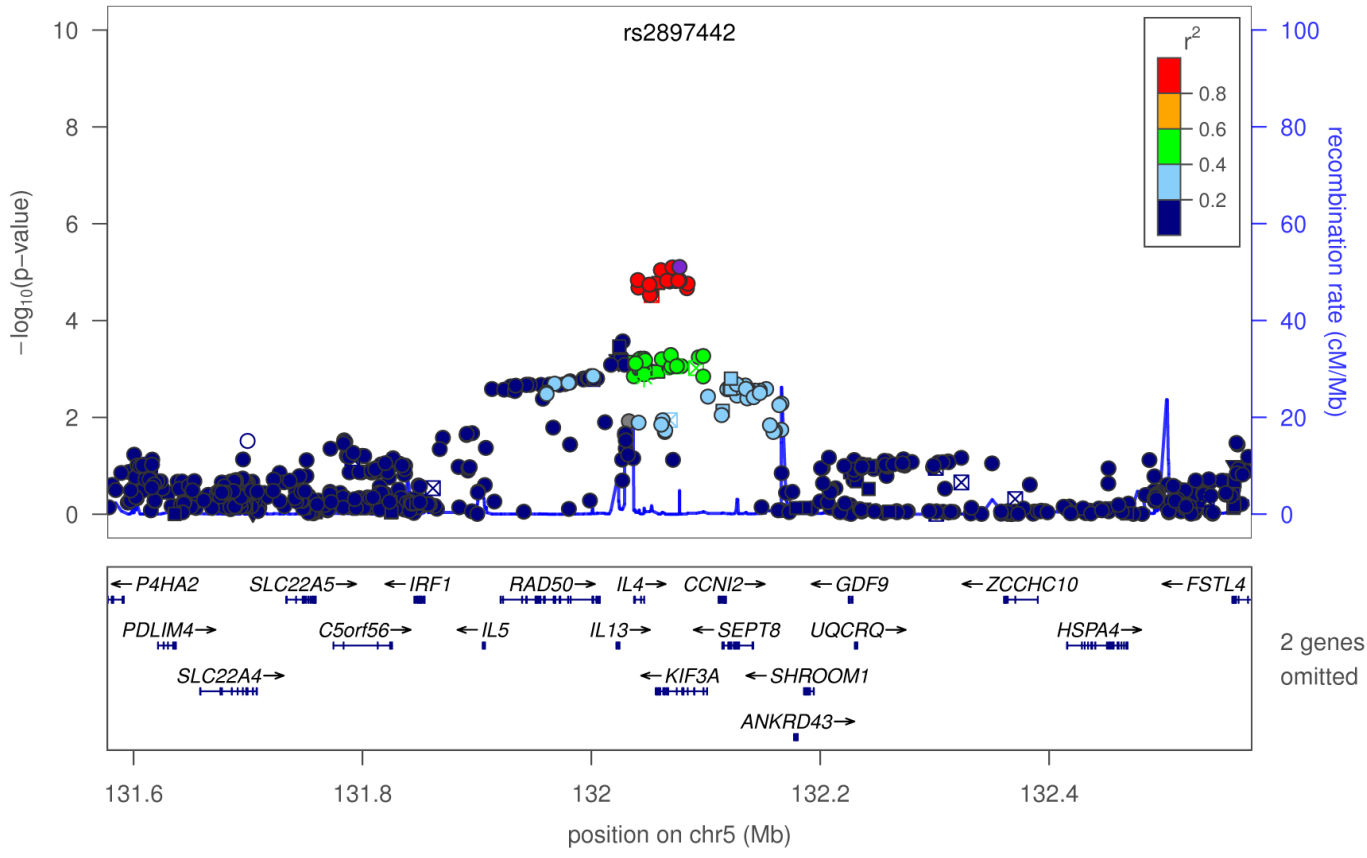

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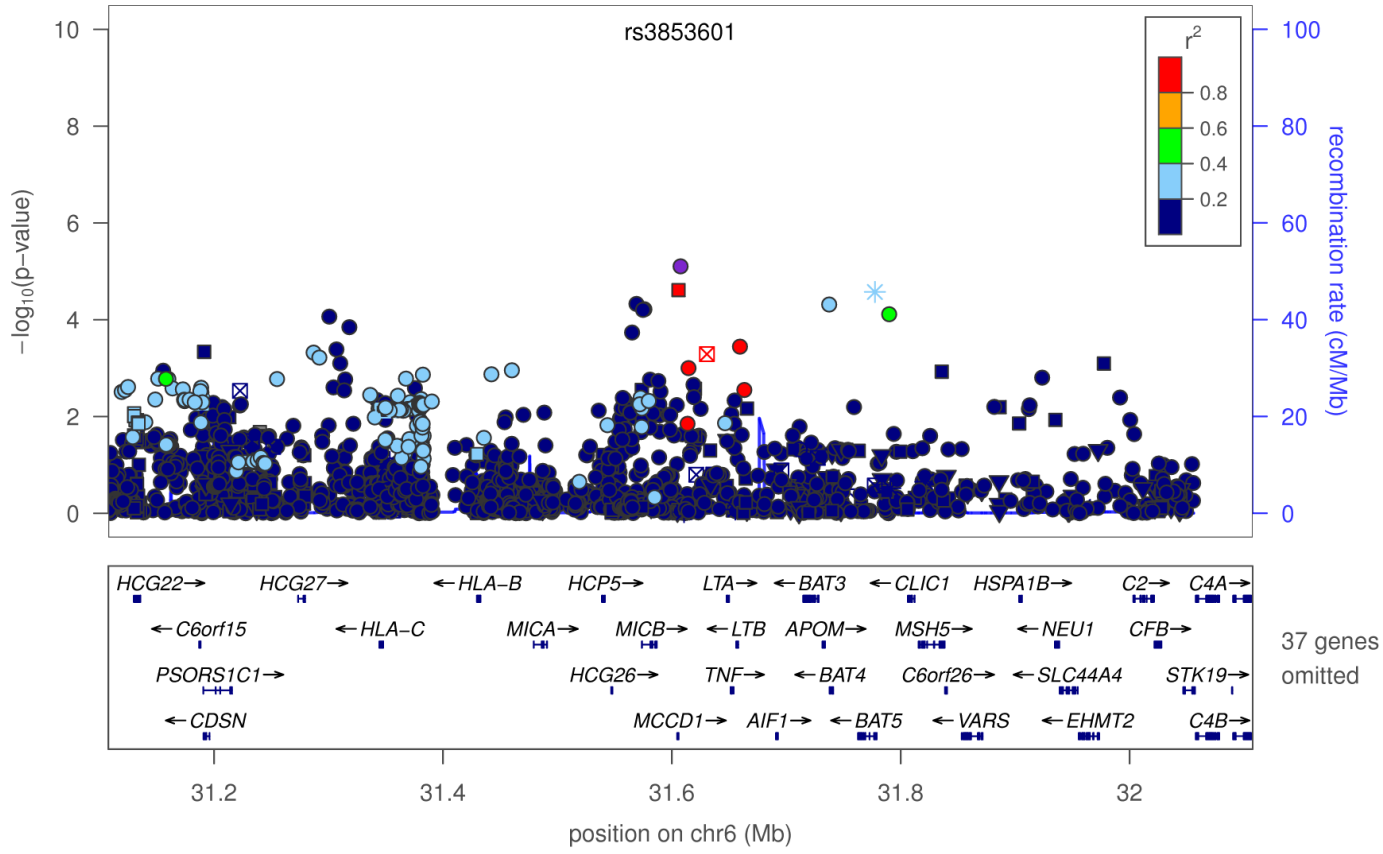
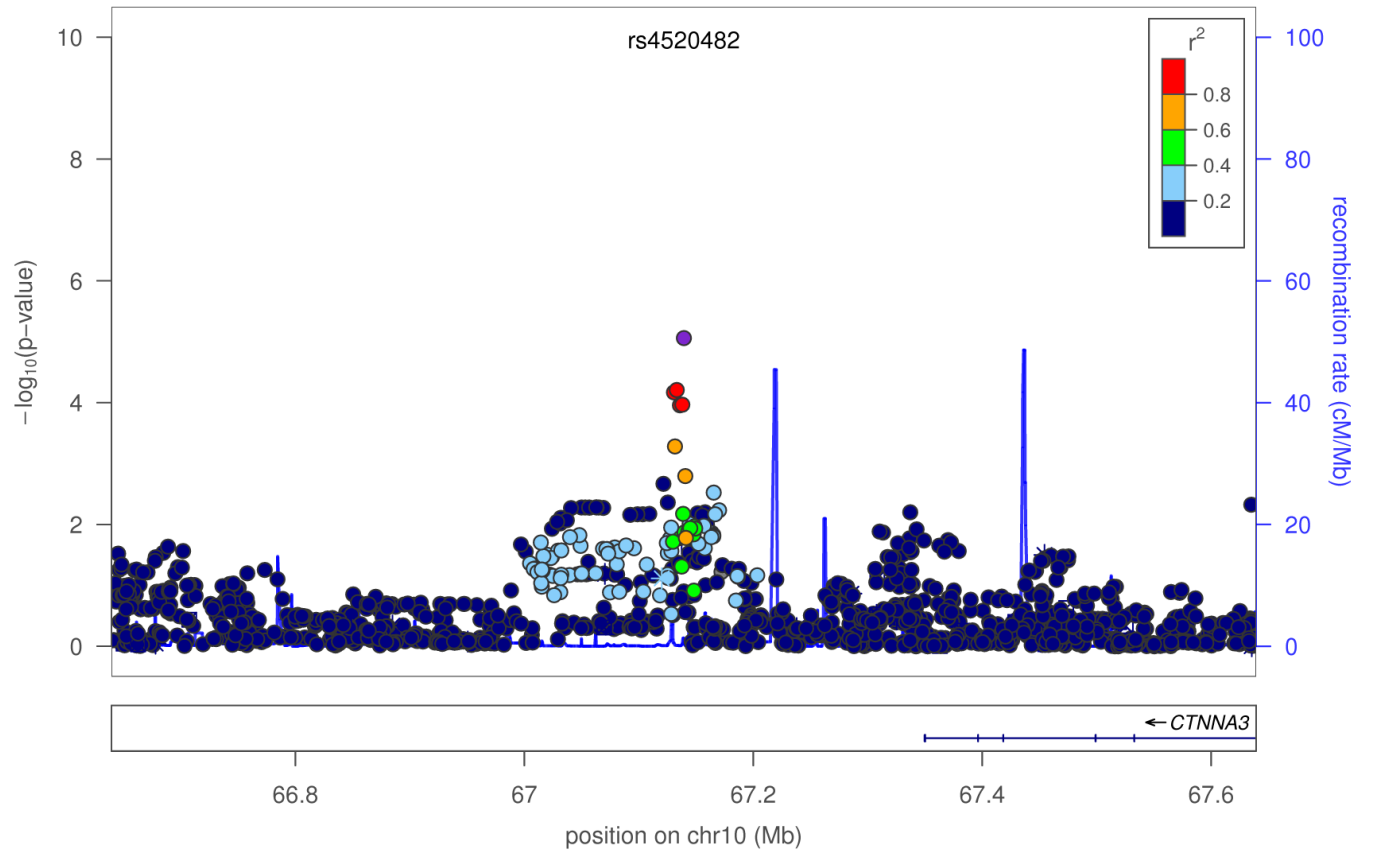
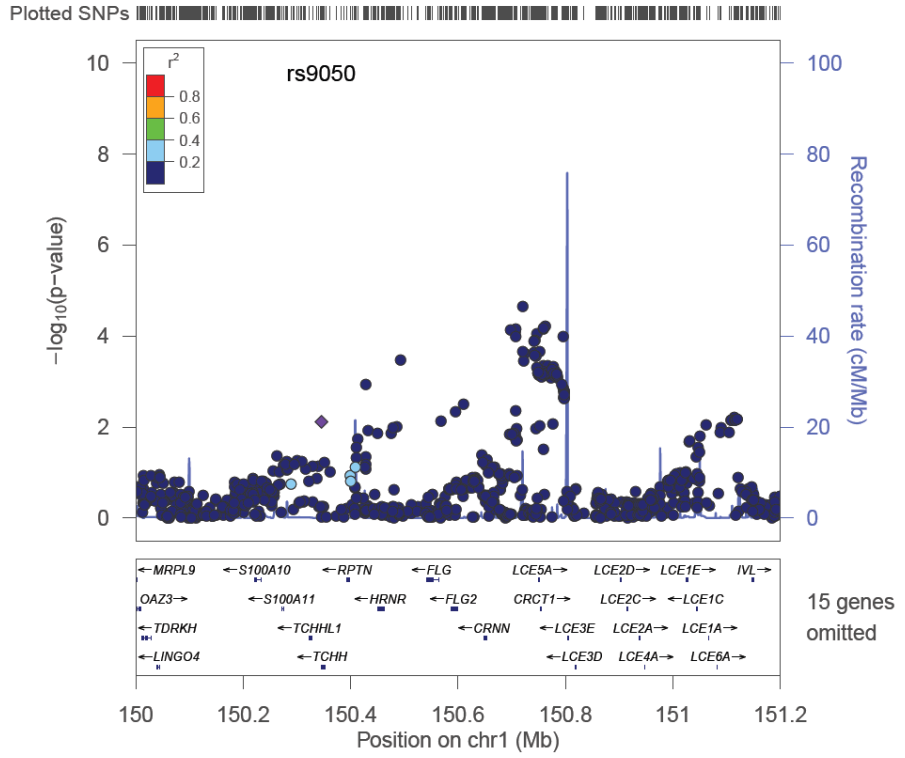




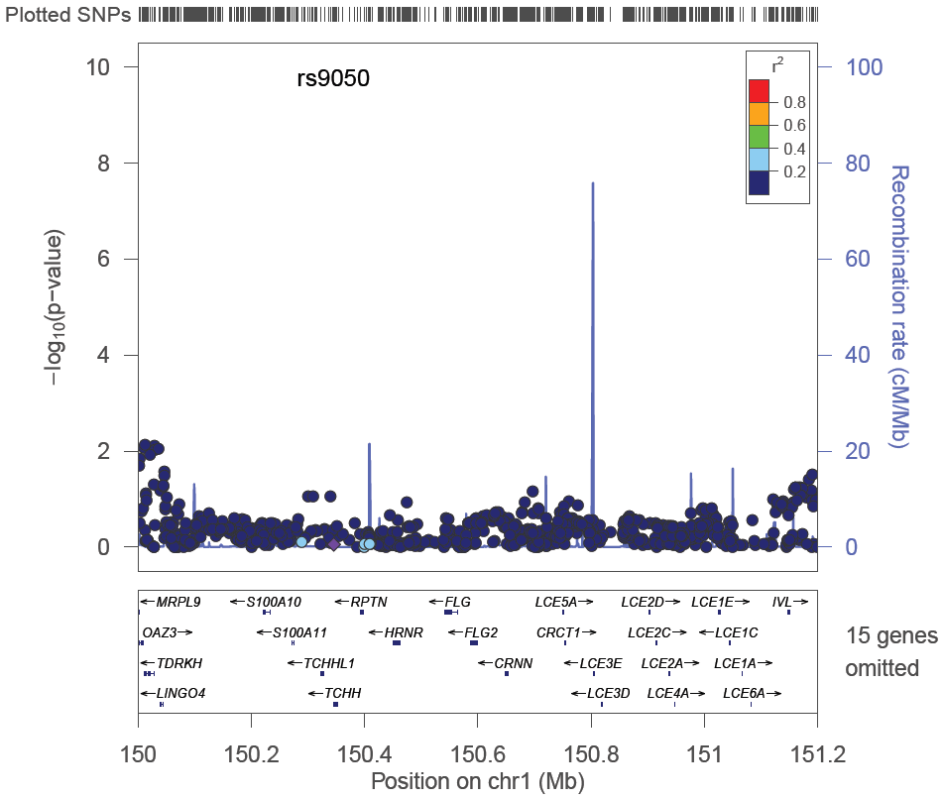
Nature Genetics: doi:10.1038/ng.1017

Supplementary Figure 3. Meta-analysis of 8 studies with no adjustment for FLG mutations (a) and with adjustment for FLG R501X and 2282del4 mutations (b). rs9050 (purple diamond) OR=1.28, p-value=0.008 in (a) and OR=0.98, p-value=0.88 in (b). A second SNP in the region (rs11205006 at ~150.7Mb) OR=1.21, p-value=$8x10^{-5}$ in (a) and OR=1.09, p-value=0.15 in (b). Plotted using LocusZoom (csg.sph.umich.edu/locuszoom/). Data from ALSPAC, BAMSE, COPSAC, KORA F3, KORA F4, LISA, MAAS and PIAMA studies contributed to these analyses.

a.



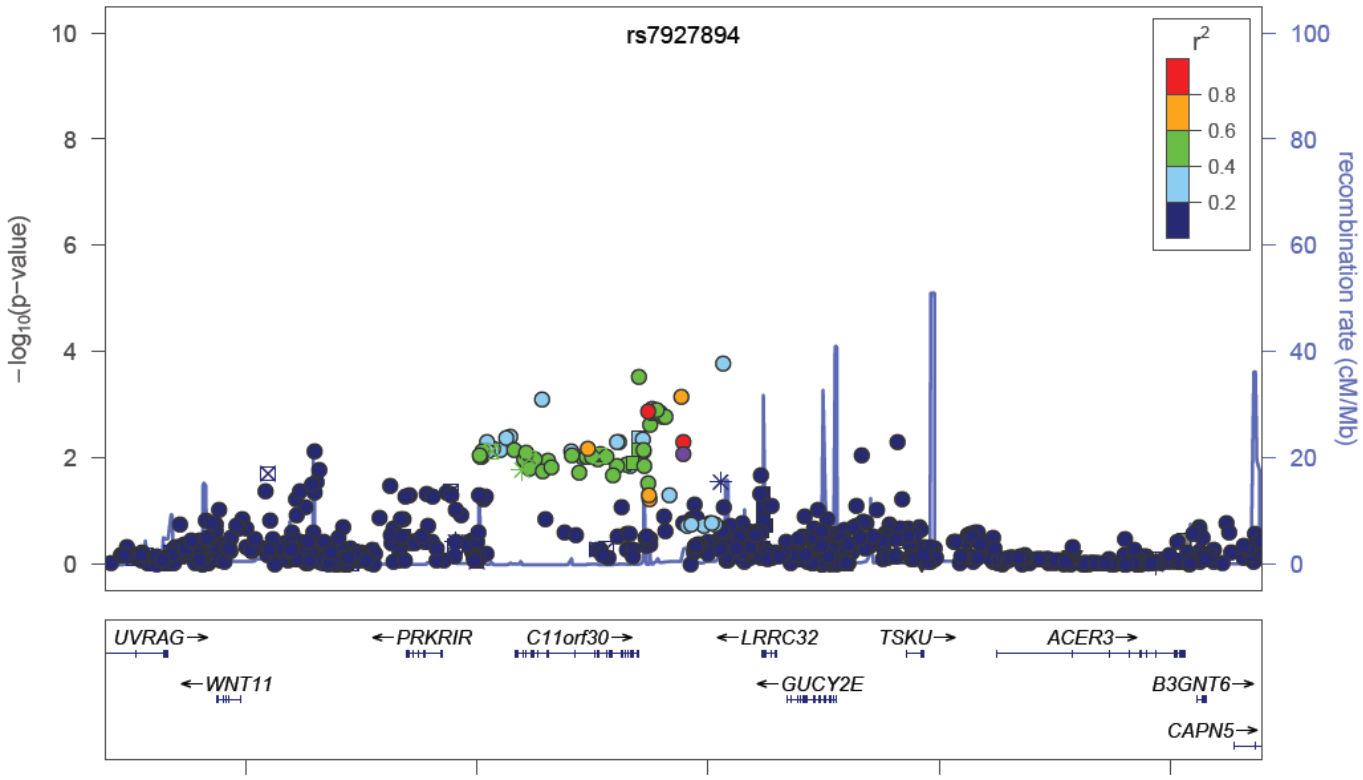
b.



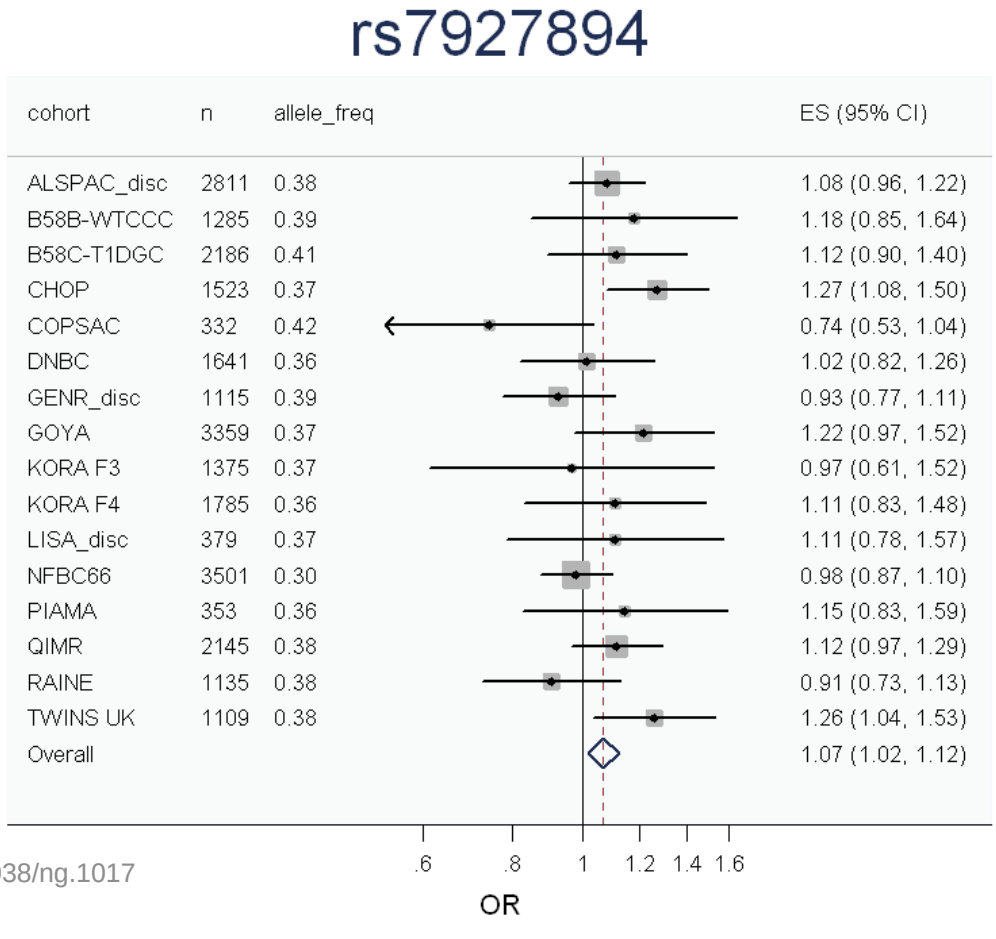
Nature Genetics: doi:10.1038/ng.1017

Supplementary Figure 4. Previously known 11q13 (rs7927894) association in our study. (a) The regional association plot for the discovery meta-analysis (top SNP, rs11236810 p=0.0002**),** (b) The forest plot for the association in each of the discovery cohorts for rs7927894 with T as the risk allele (het p=0.127). GENR= Generation R.

a.

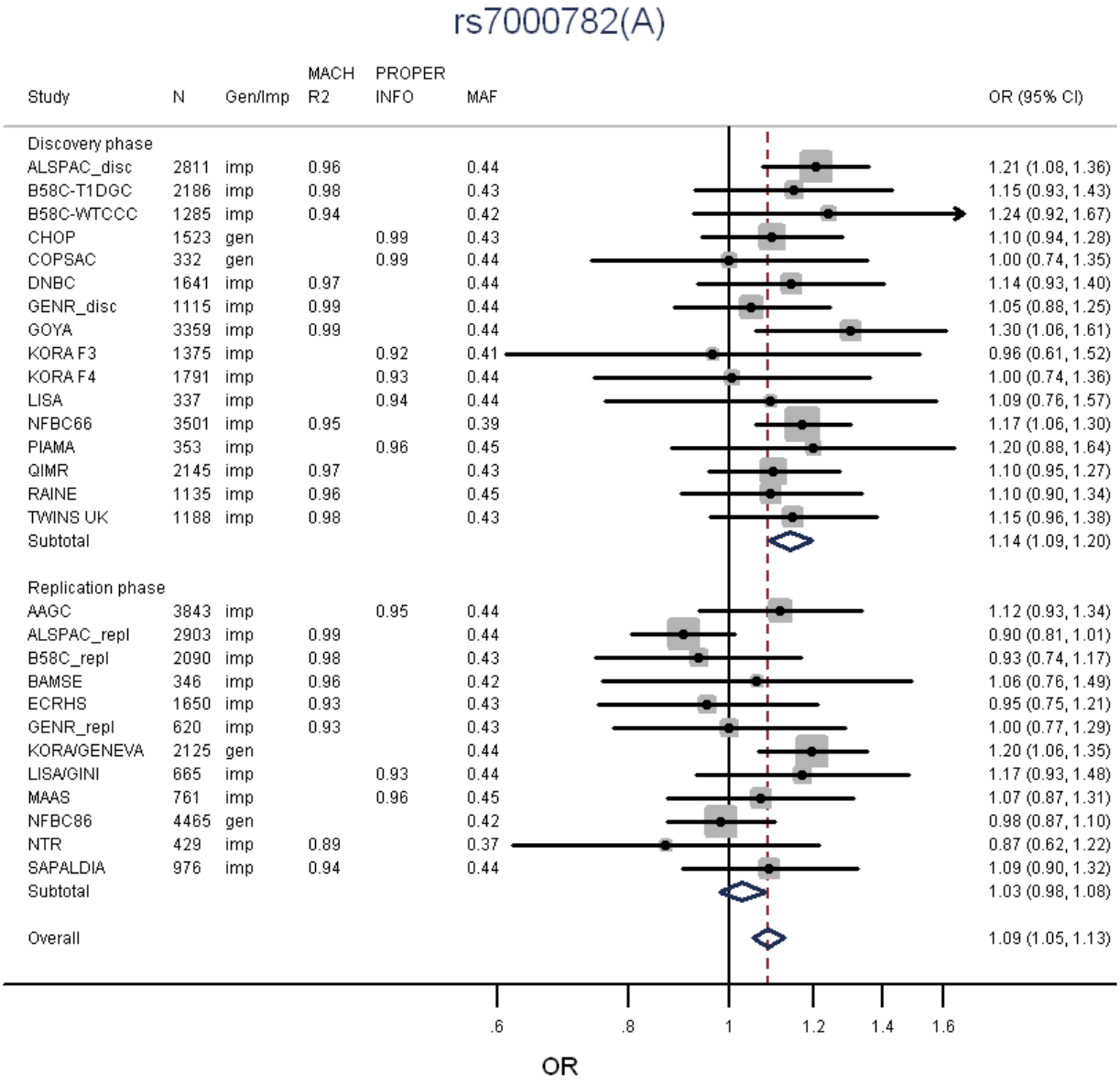


b.

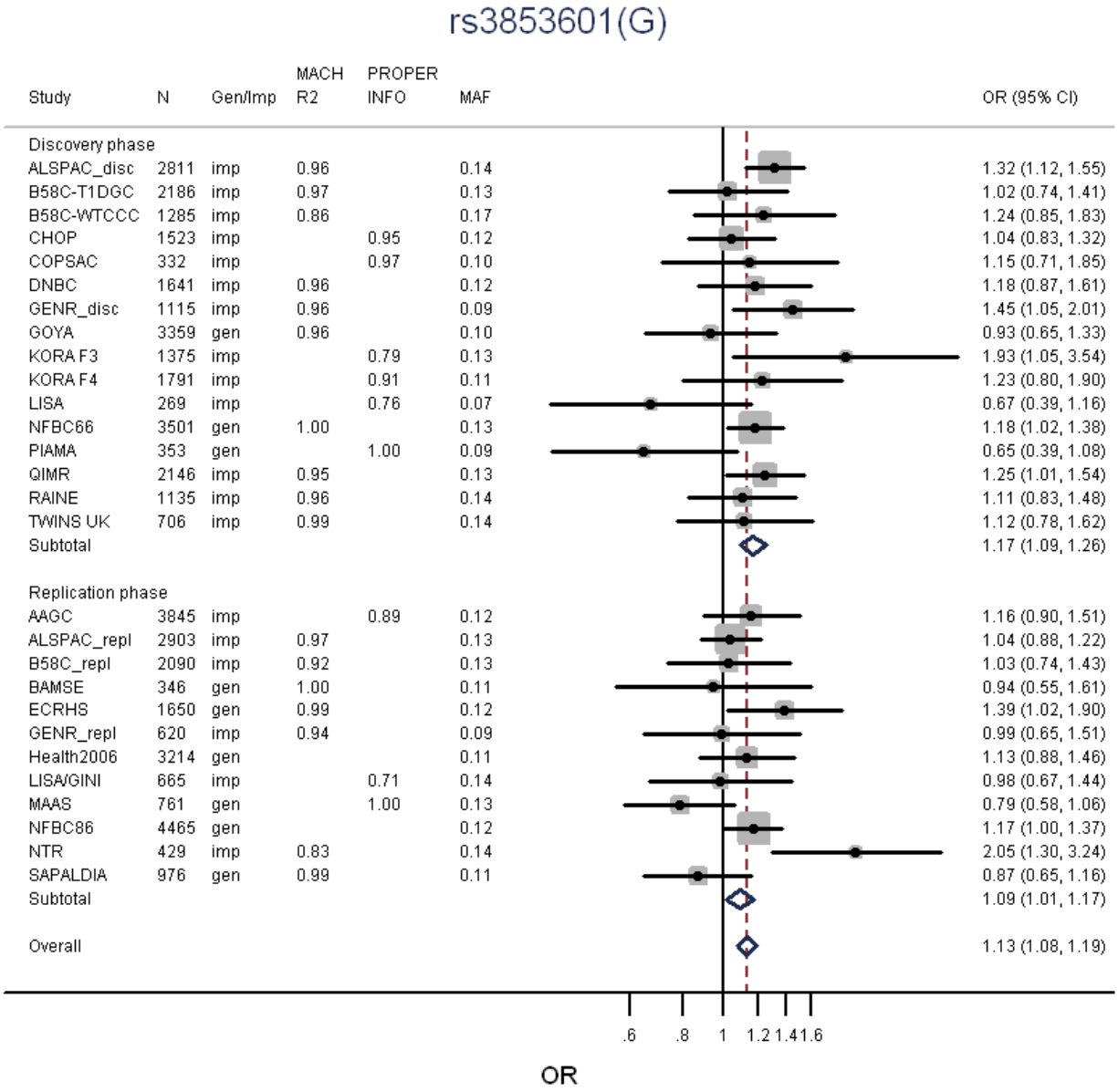


Nature Genetics: doi:10.1038/ng.1017

Supplementary Figure 5. Forest plots of the association of the 7 SNPs which did not meet genome-wide significance with atopic dermatitis for the discovery and replication studies.
All ORs are reported with the minor allele (shown in brackets) as the effect allele. *MoBa imputation quality score was 'info' from PLINK. GENR= Generation R. 'gen' in the imputation (Gen/Imp) column refers to SNPs that were on the genome-wide genotyping chip for the discovery samples and were either on the genome-wide genotyping chip or were individually genotyped for the replications samples. Only Health2006, KORA/GENEVA and NFBC86` underwent individual SNP genotyping.



Nature Genetics: doi:10.1038/ng.1017



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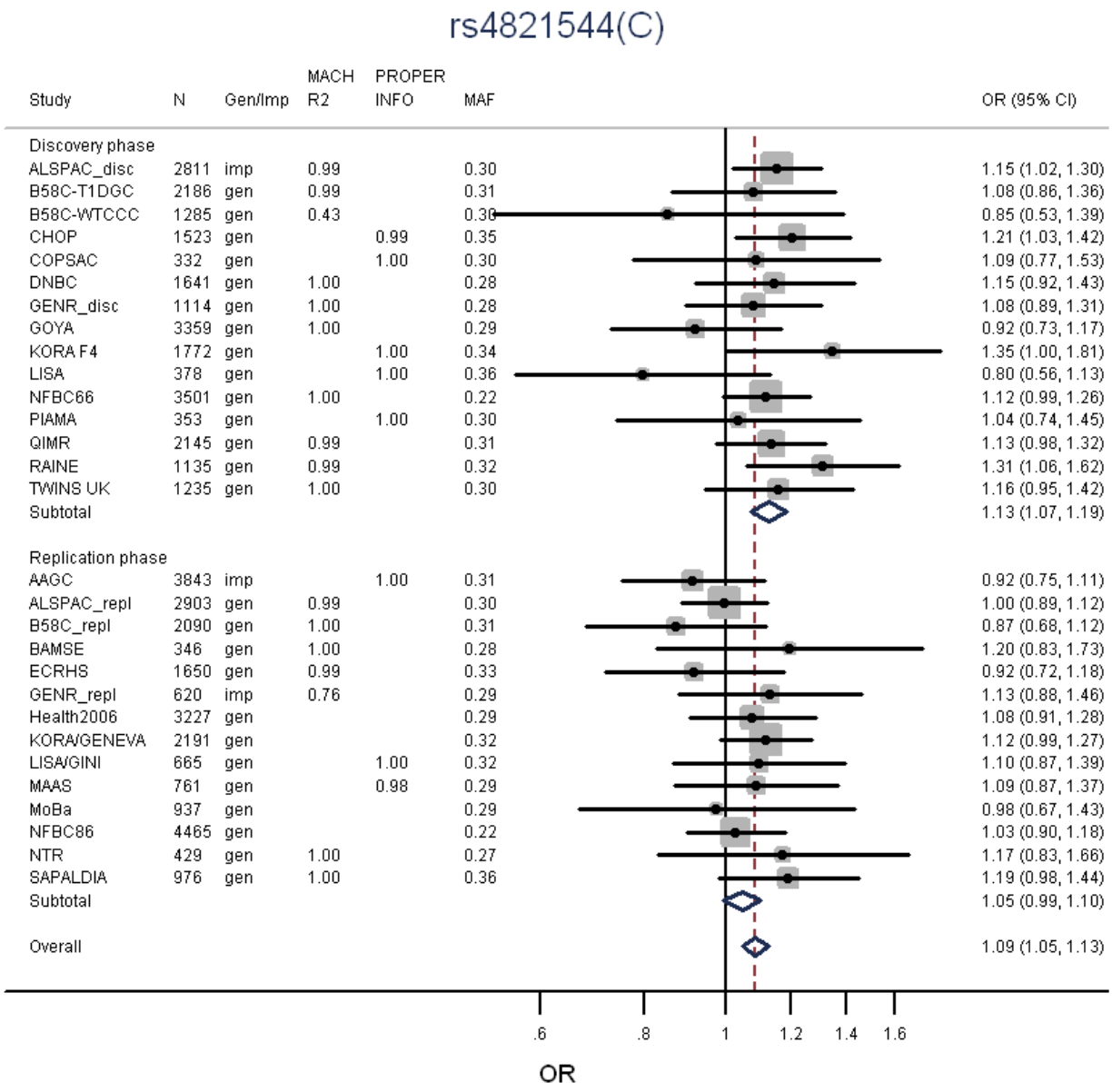

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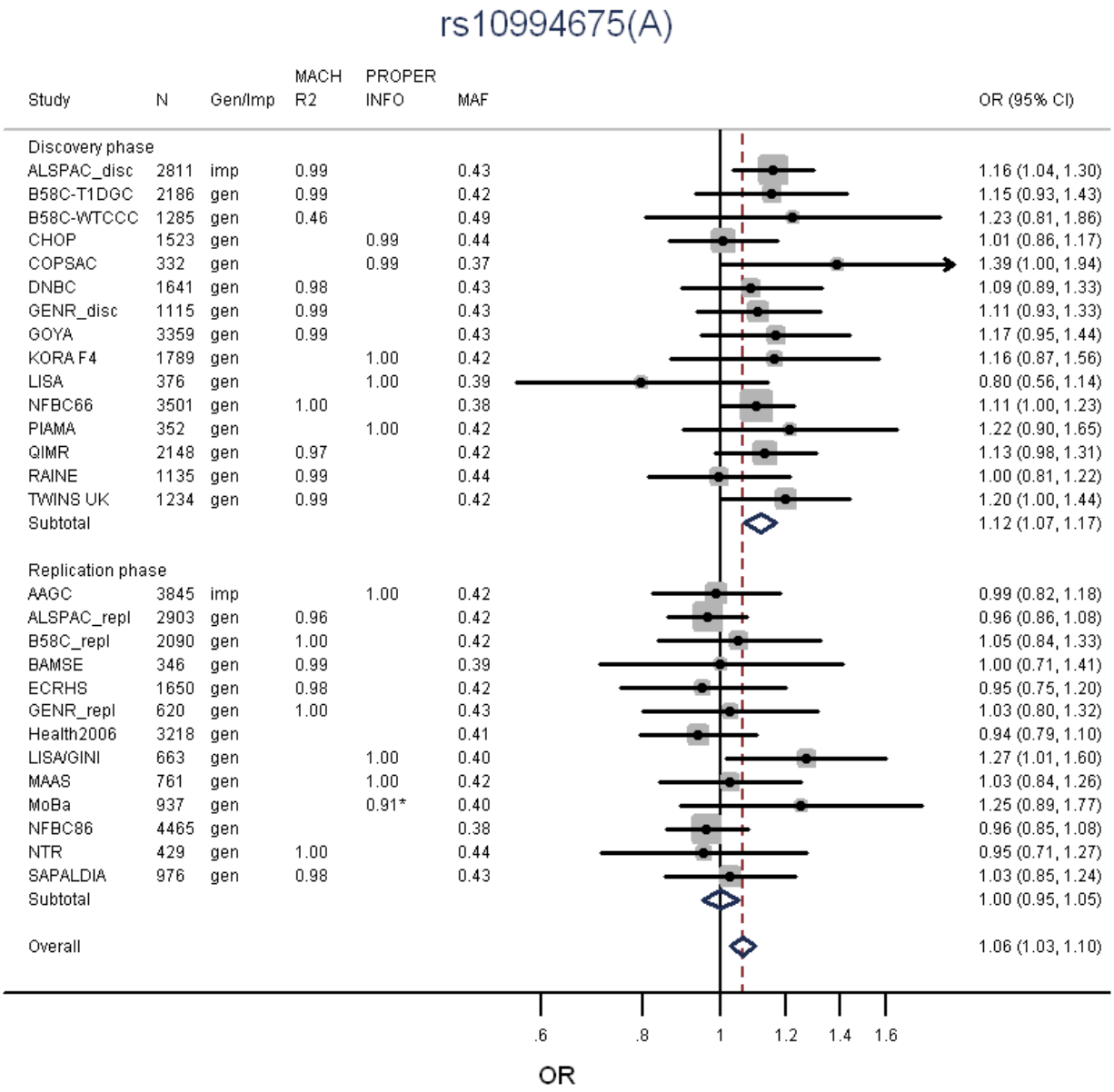

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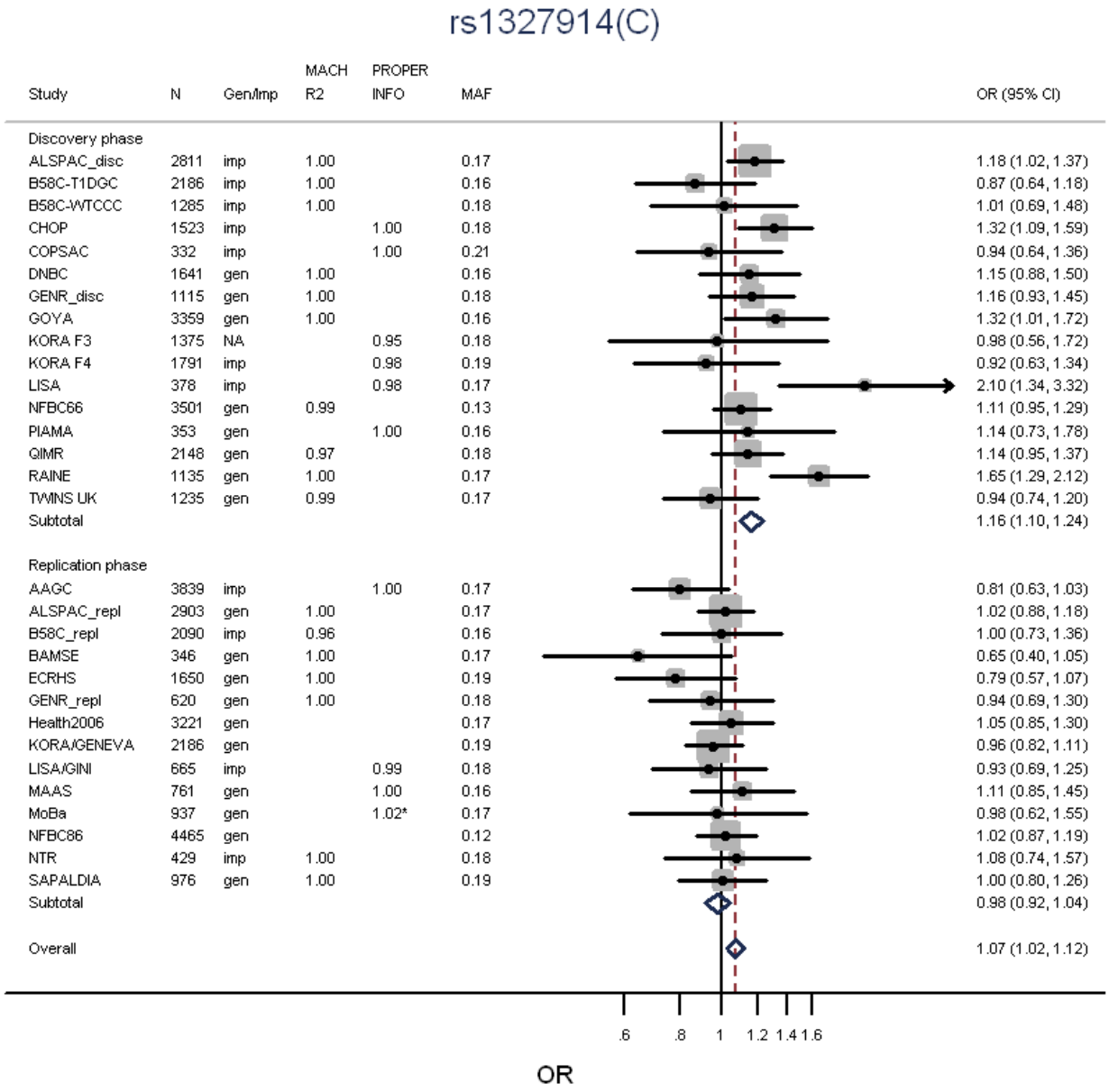

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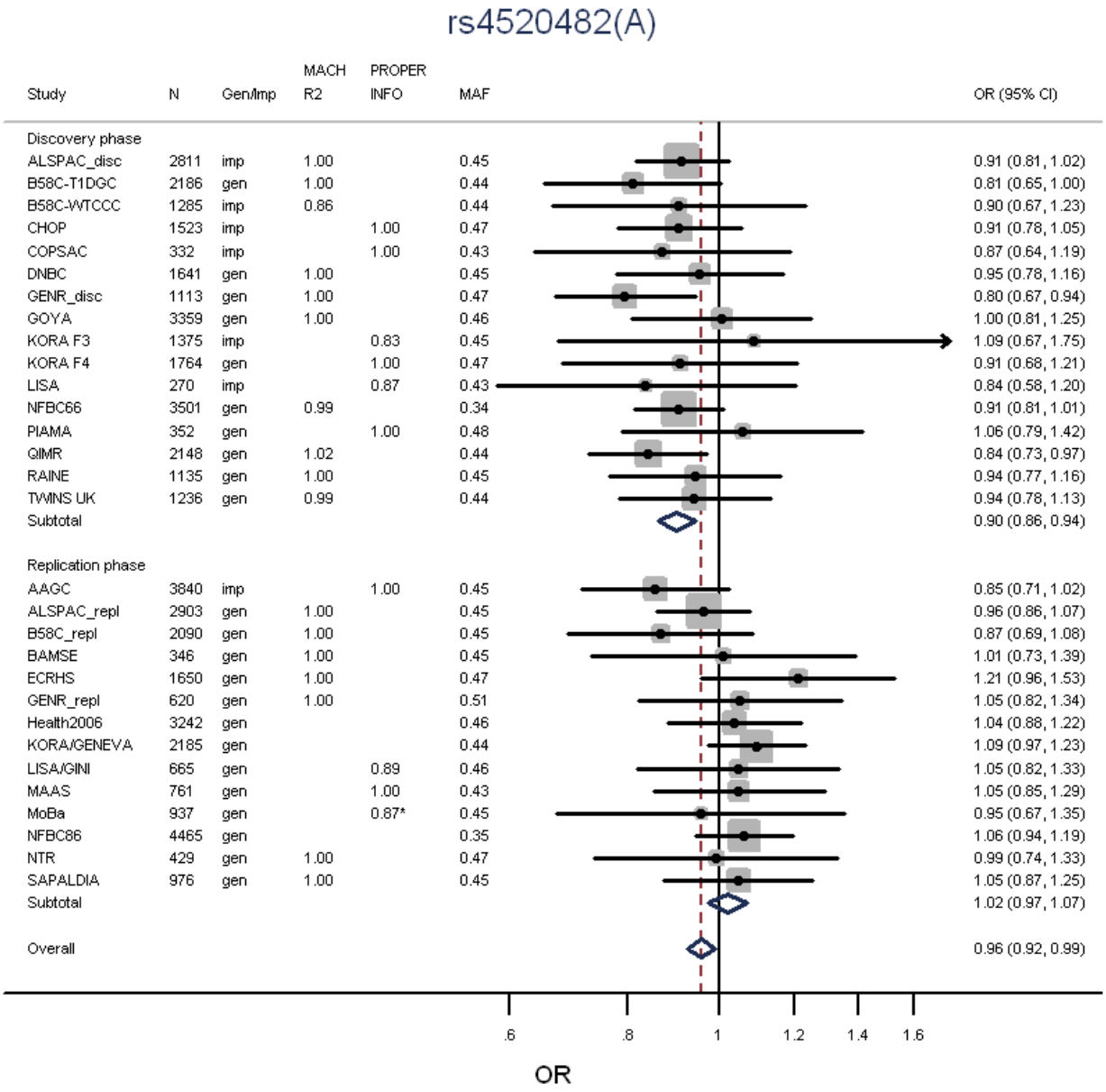

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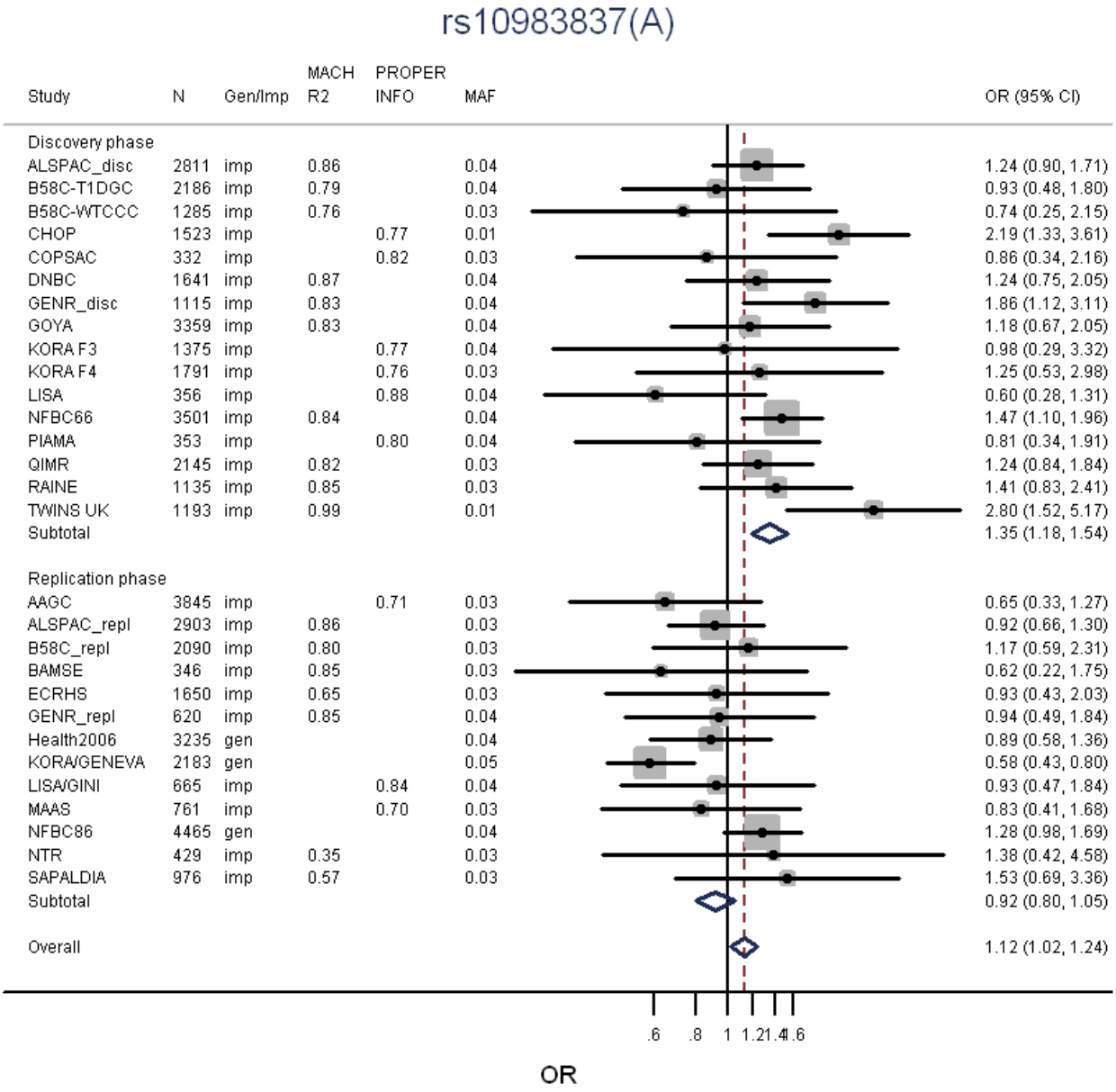
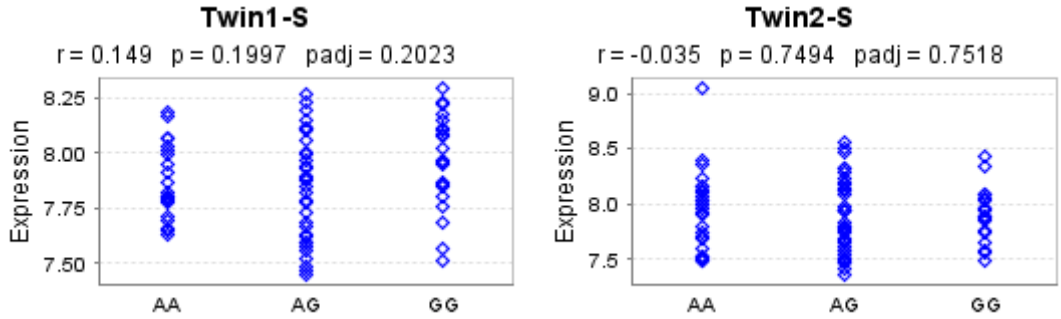


Nature Genetics: doi:10.1038/ng.1017

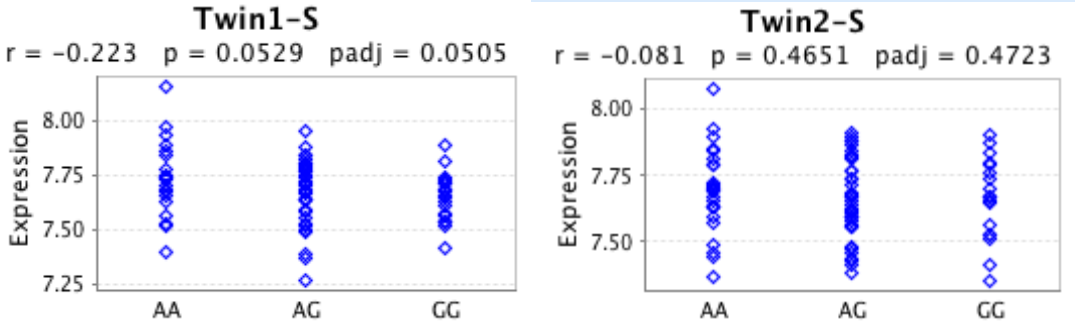
Supplementary Figure 6. MuTHER pilot eQTL skin data for probes within 1Mb of the SNP (a) rs479844, (b) rs2164983 and (c) rs2897442 for 160 female twins. Data is split into two sets (with one of each twin pair in each). Results are shown for the candidate genes near to the SNP of interest (*OVOL1/*, *ACTL9/ADAMTS10*, *KIF3A/IL4/IL13*) and for any gene with p<0.01 (within 1Mb of the SNP) in either twin set. r=regression coefficient, p= unadjusted p-value, padj=adjusted p-value, 10,000 permutations.

a. rs479884

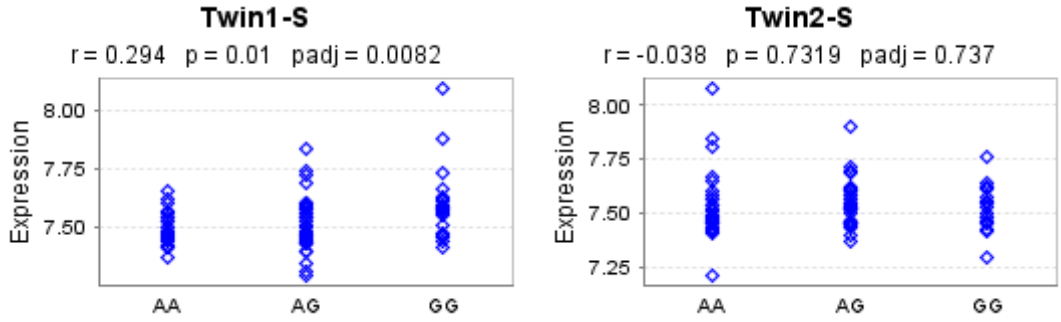
i. *OVOL1* – closest gene. Probe=ILMN_1692936



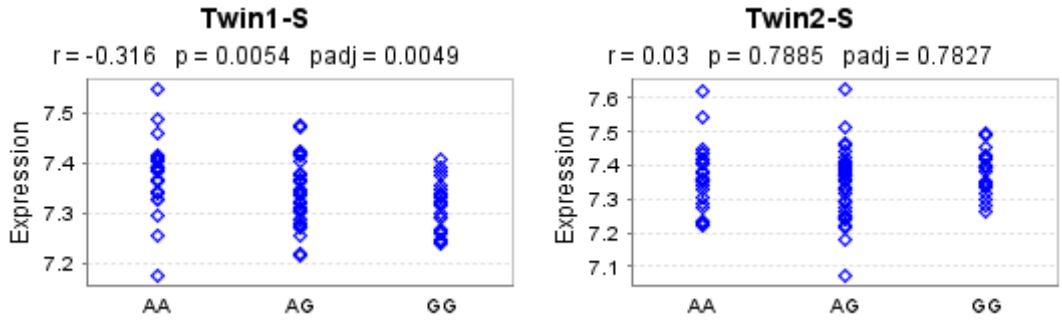
ii. DKFZp761E198 - close gene. Probe=ILMN_1717594



iii *KLC2* – p<0.01 in Twin1. Not confirmed in Twin2. Probe=ILMN_1653470

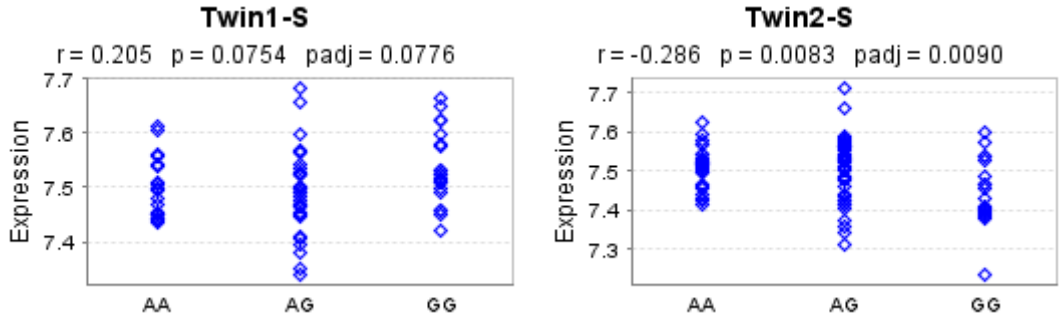


iv. *LTBP3* – p<0.01 in Twin1. Not confirmed in Twin2. Probe=ILMN_1777121



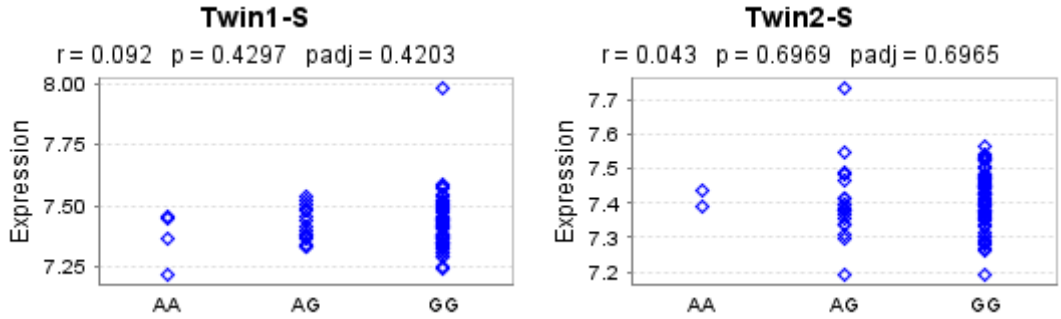
Nature Genetics: doi:10.1038/ng.1017

v. *SLC25A45* – p<0.01 in Twin2. Not confirmed in Twin1. Probe=ILMN_1810727



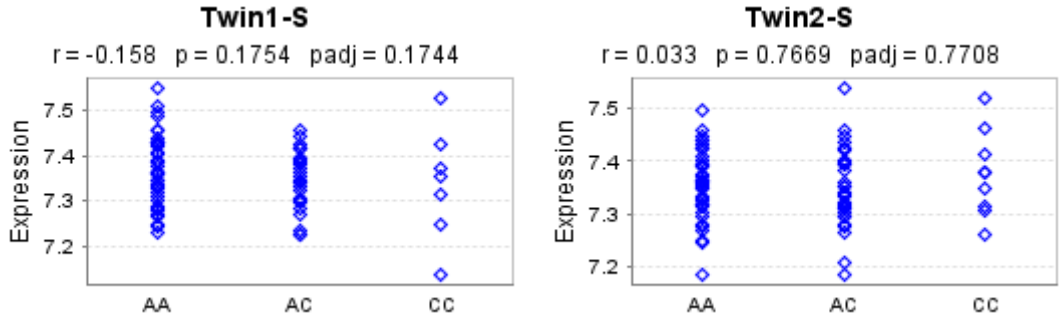
b. rs2967675 – best available proxy for rs2164983 (r^2=0.94)

i. *ACTL9* – closest gene. Probe=ILMN_1656193



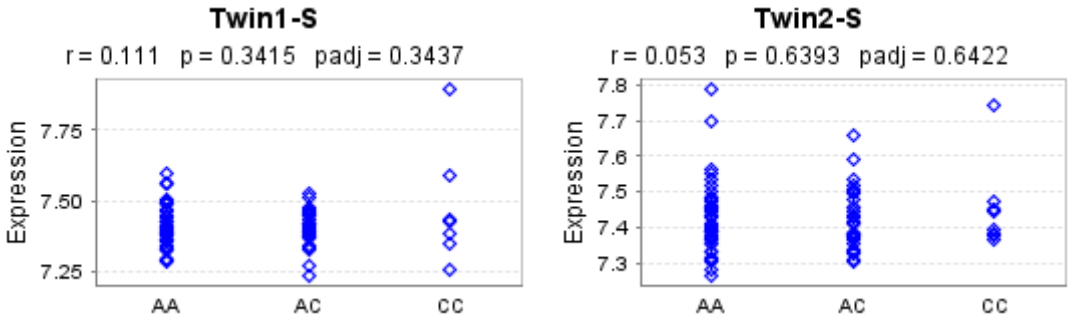
c. rs2299009 – best available proxy for rs2897442 (r^2=1.0)

i. *KIF3A* – close gene. Probe=ILMN_1653385

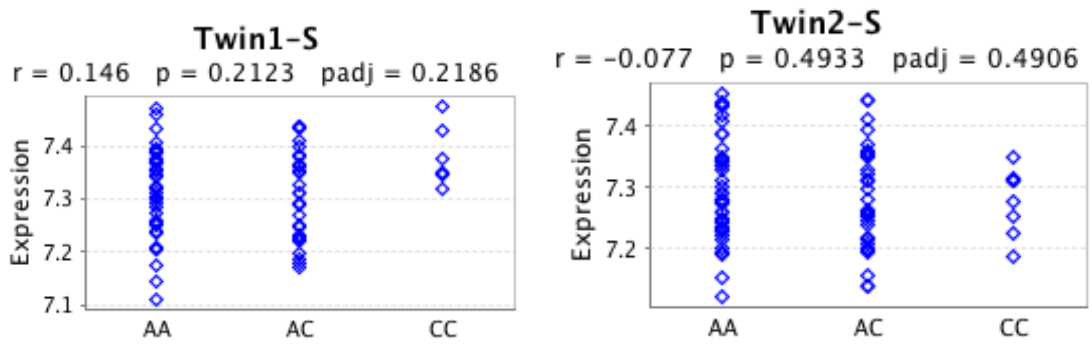


Nature Genetics: doi:10.1038/ng.1017

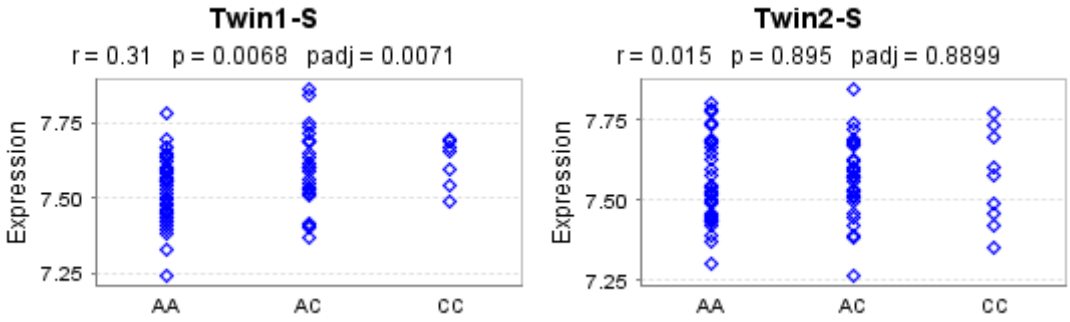
iii.*IL4* -close gene. Probe=ILMN_1669174



iv. IL13 – close gene. Probe=ILMN_2052511



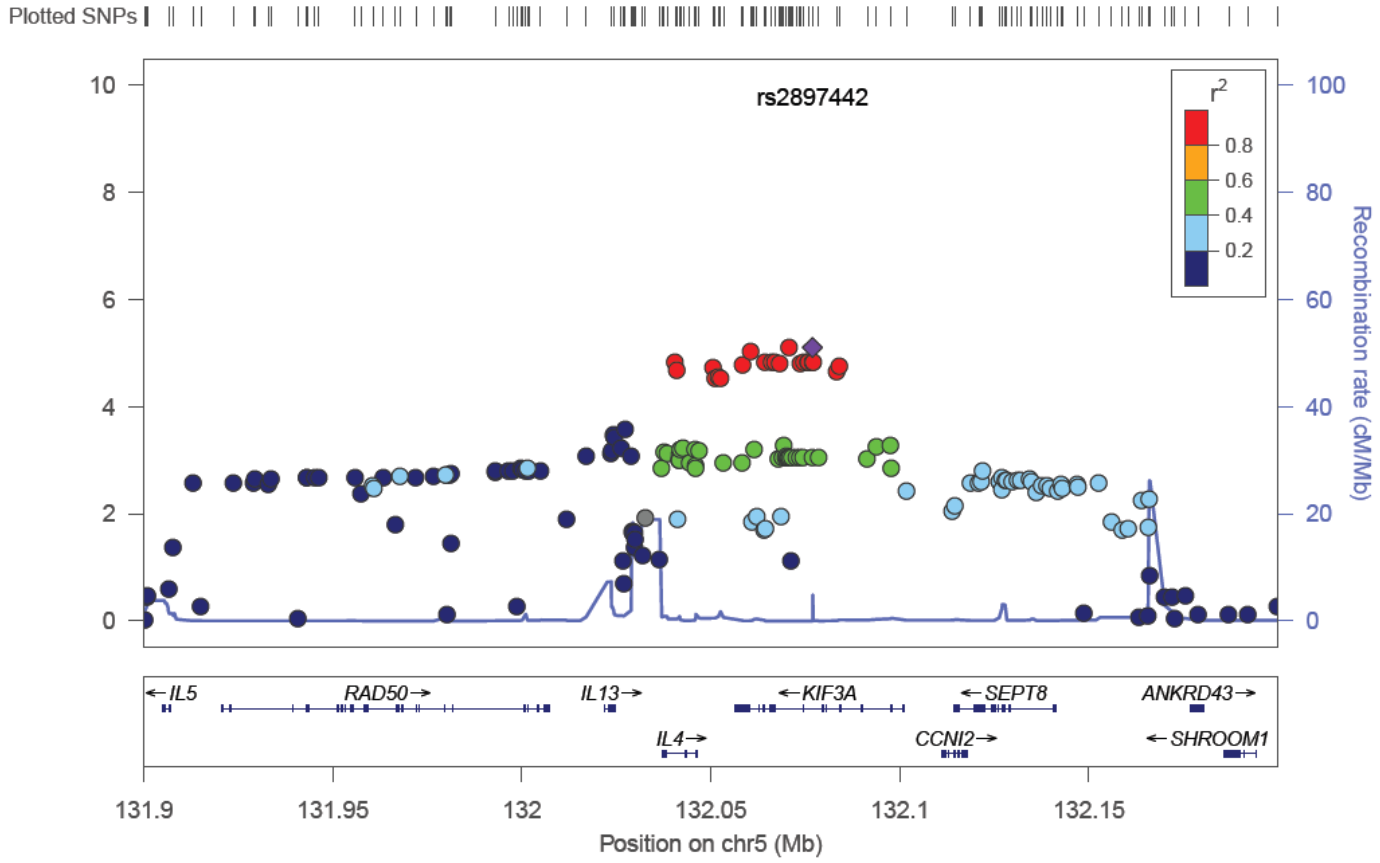
iv. *HSPA4* – p<0.01 in Twin1. Not confirmed in Twin2. Probe=ILMN_175513



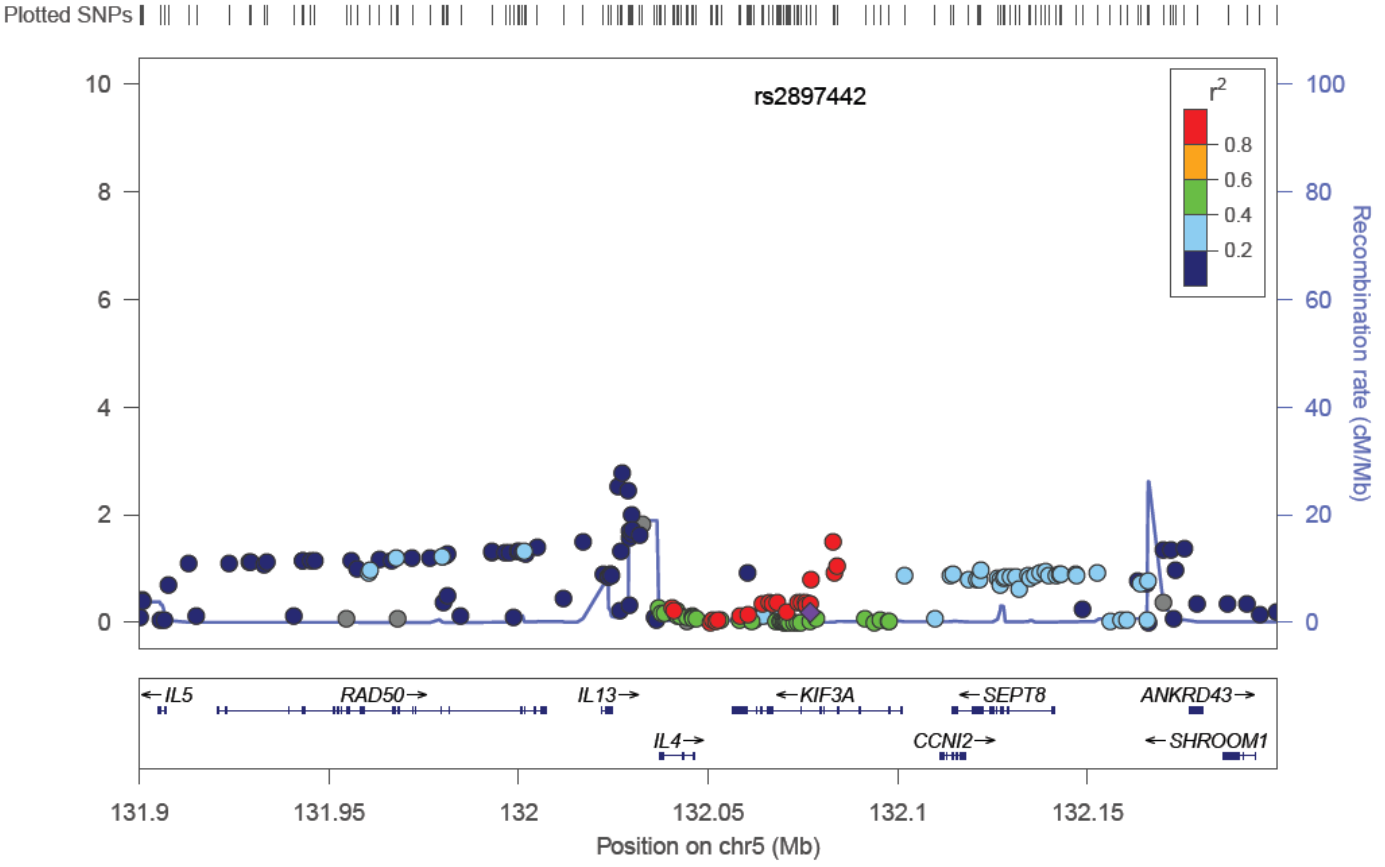
Nature Genetics: doi:10.1038/ng.1017

Supplementary Figure 7. Regional Association Plots for 5q31.1 in the discovery cohorts (a) no conditional SNPs and (b) conditional on rs2897442. N=26,164.

a.

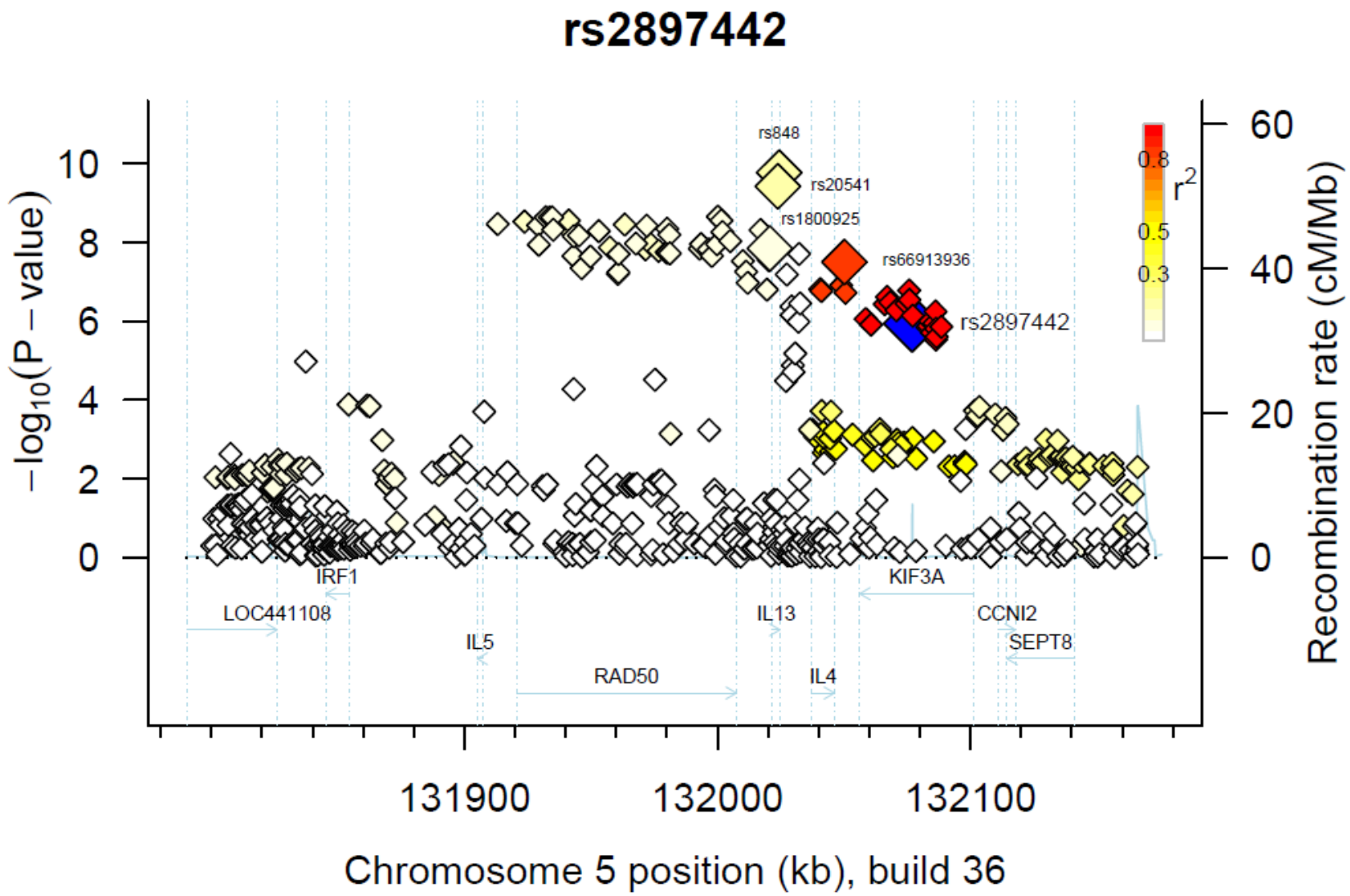


b.



Nature Genetics: doi:10.1038/ng.1017

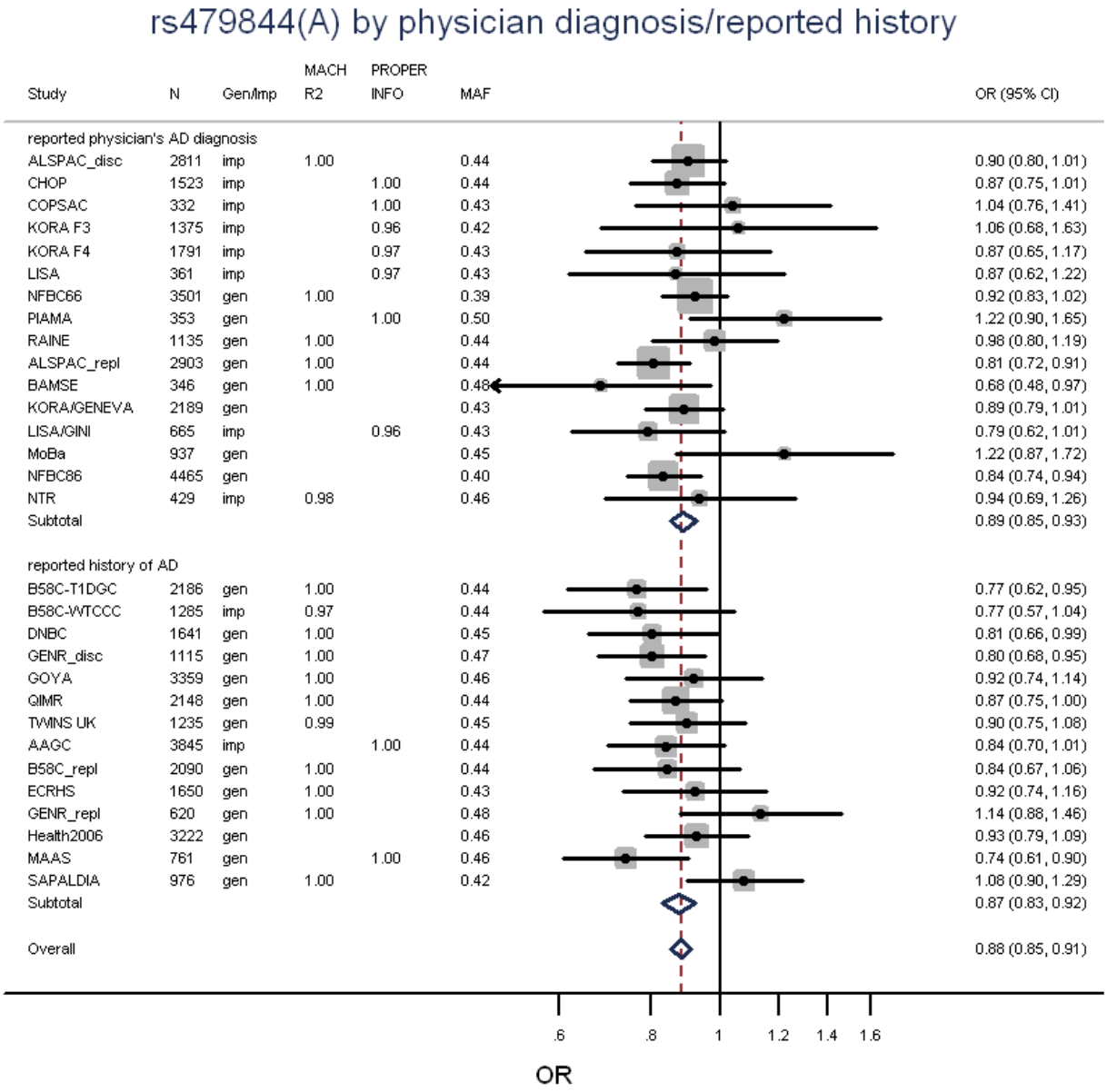
Supplementary Figure 8. Regional association plot of markers within the cytokine cluster on 5q31.1. Results from the Immunochip (custom genotyping SNP- chip designed for immunogenetic studies) including *IL13* polymorphisms previously shown to be associated with asthma and psoriasis risk, as well as the GWAS *KIF3A* polymorphism showing the strongest association in the meta-analysis and the lead SNP of the corresponding putative LD-block from the finemapping approach.



Nature Genetics: doi:10.1038/ng.1017

Supplementary Figure 9. Stratified forest plots for SNPs associated with AD (rs479844, rs2164983, rs2897442) or with evidence of heterogeneity (rs2164983, rs1327914, rs10983837). Stratified by (a) reported physician AD diagnosis versus reported history of AD, (b) diagnosis before the age of 15 (child) versus up to and including adults. GENR = Generation R.

a. **reported physician AD diagnosis versus reported history of AD.** Difference between subgroup p-values: rs479844 p=0.653; rs2164983 p=0.134; rs2897442 p=0.023; rs1327914 p=0.191; rs10983837 p=0.568.



*CHOP used sub-optimal ICD9 diagnosis in medical record to identify cases. Reported physician's AD diagnosis subgroup result with CHOP excluded: OR=0.89 (95%CI 0.85 - 0.93).

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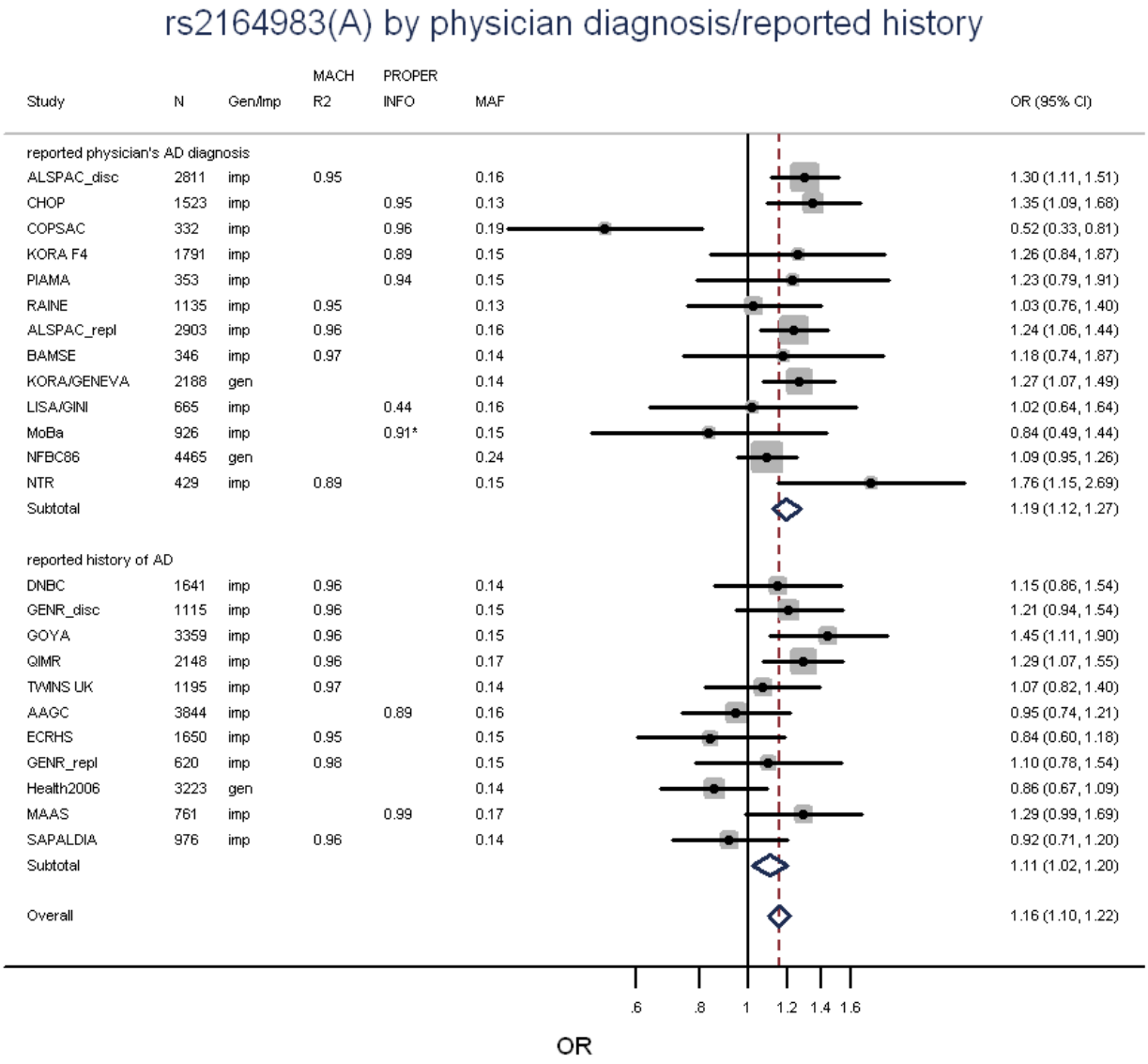
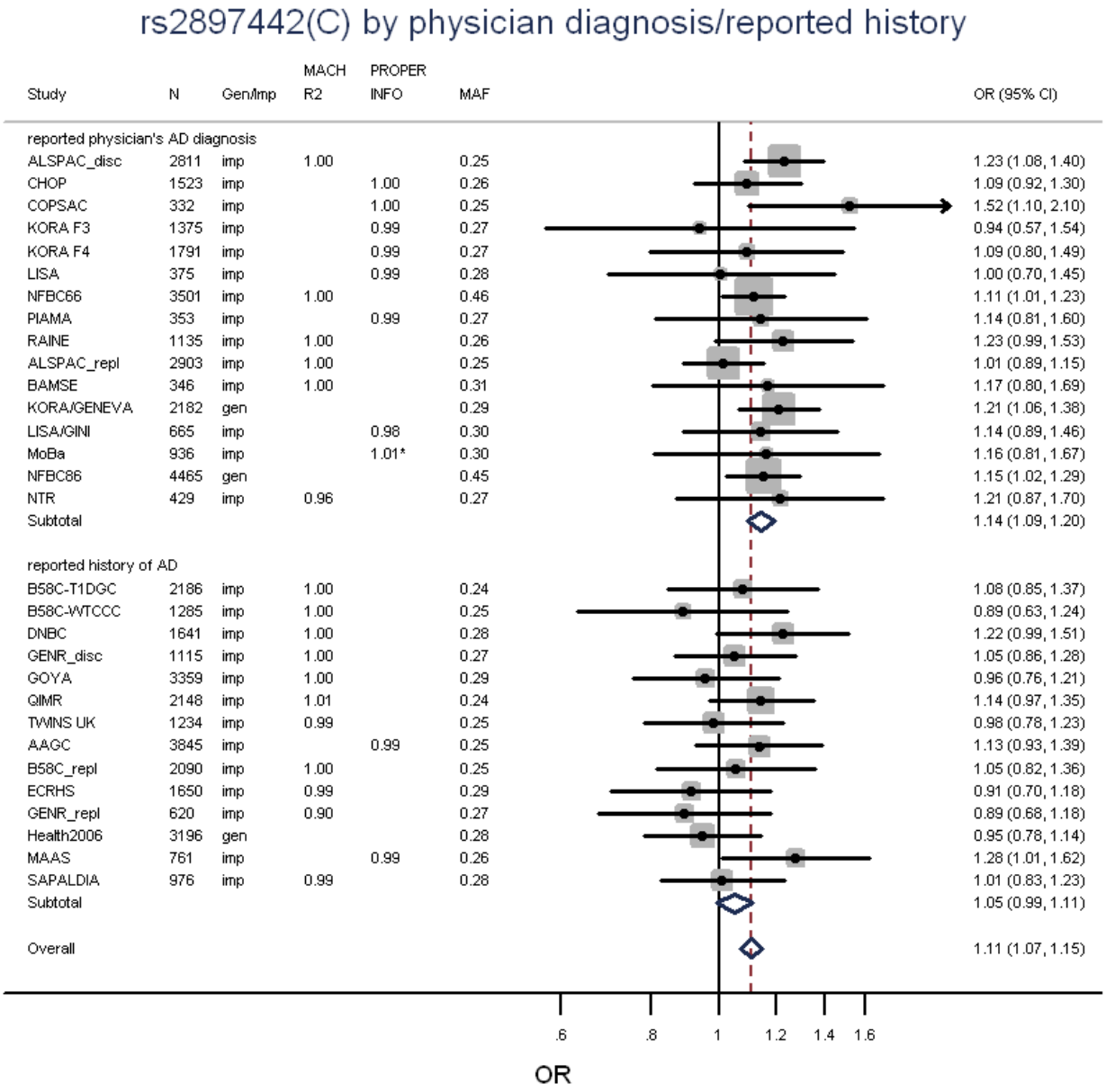


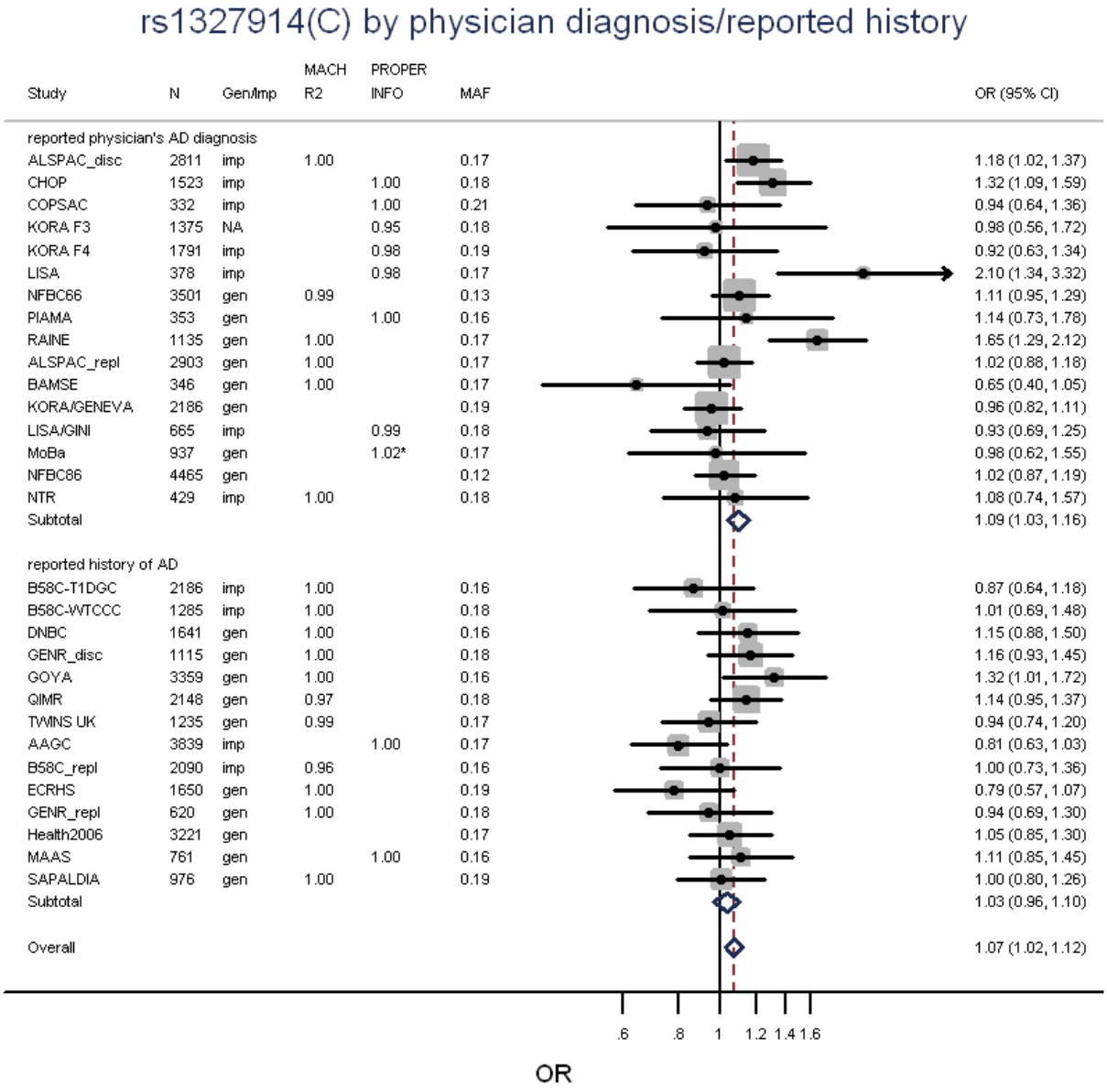
*CHOP used sub-optimal ICD9 diagnosis in medical record to identify cases. Reported physician's AD diagnosis subgroup result with CHOP excluded: OR=1.18 (95%CI 1.10 - 1.26).

Nature Genetics: doi:10.1038/ng.1017



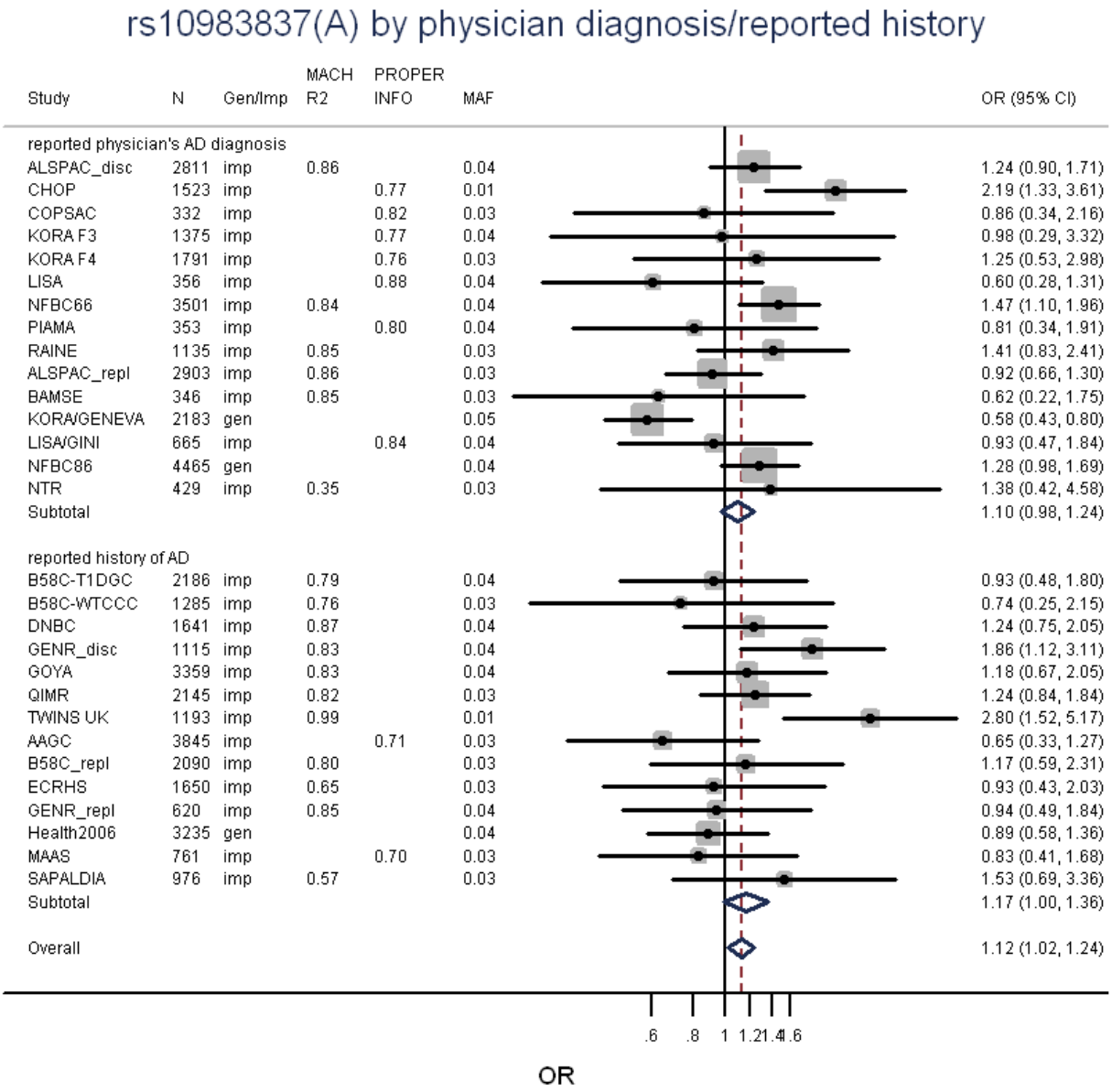
*CHOP used sub-optimal ICD9 diagnosis in medical record to identify cases. Reported physician's AD diagnosis subgroup result with CHOP excluded: OR=1.15 (95%CI 1.09 - 1.20).

Nature Genetics: doi:10.1038/ng.1017



*CHOP used sub-optimal ICD9 diagnosis in medical record to identify cases. Reported physician's AD diagnosis subgroup result with CHOP excluded: OR=1.07 (95%CI 1.01 - 1.14).

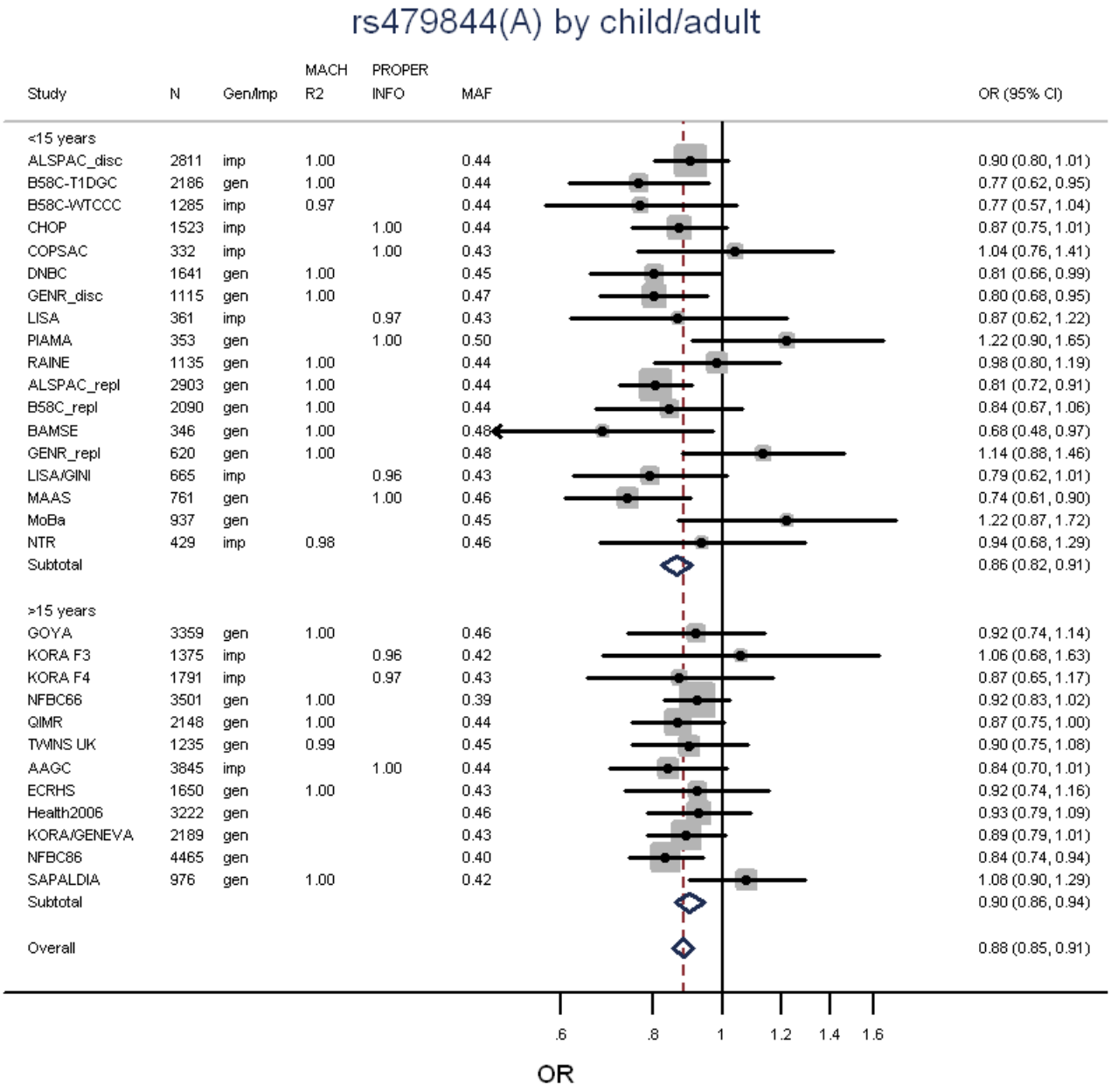
Nature Genetics: doi:10.1038/ng.1017



*CHOP used sub-optimal ICD9 diagnosis in medical record to identify cases. Reported physician's AD diagnosis subgroup result with CHOP excluded: OR=1.06 (95%CI 0.94 - 1.19).

Nature Genetics: doi:10.1038/ng.1017

b. diagnosis before the age of 15 (child) versus up to and including adults. Difference between subgroup p-values: rs479844 p=0.224; rs2164983 p=0.037; rs2897442 p=0.465; rs1327914 p=0.028; rs10983837 p=0.773.



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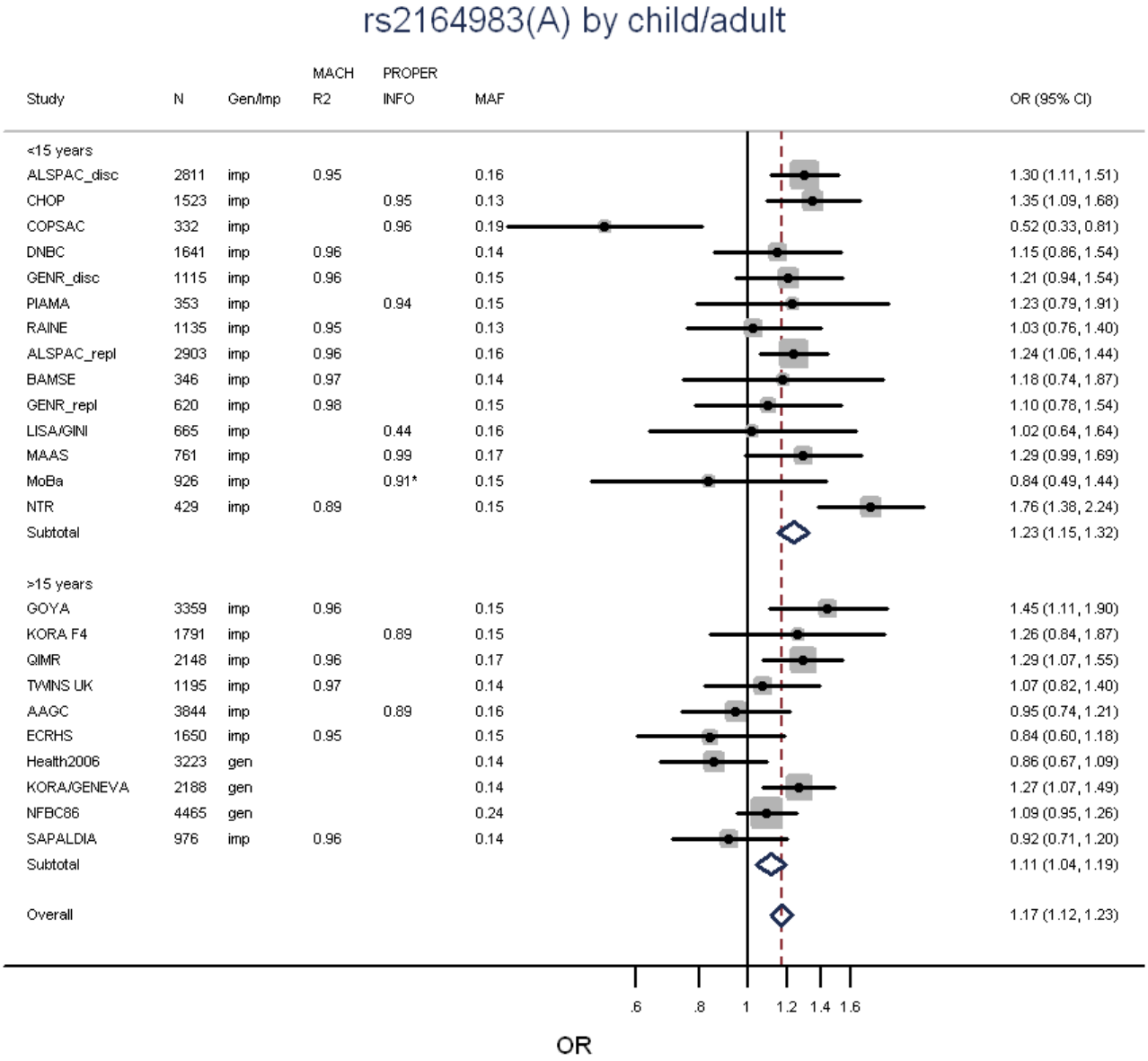

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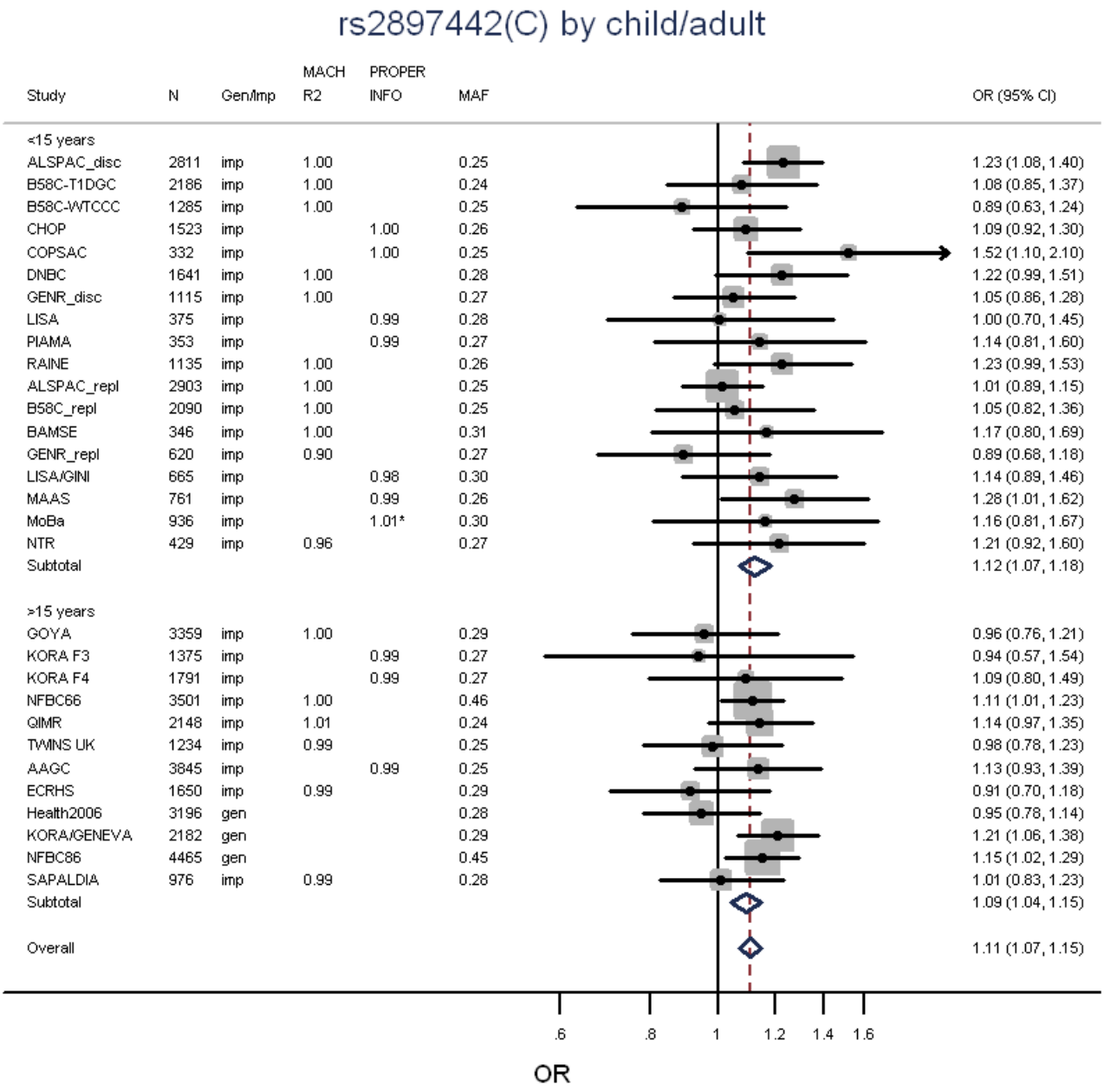

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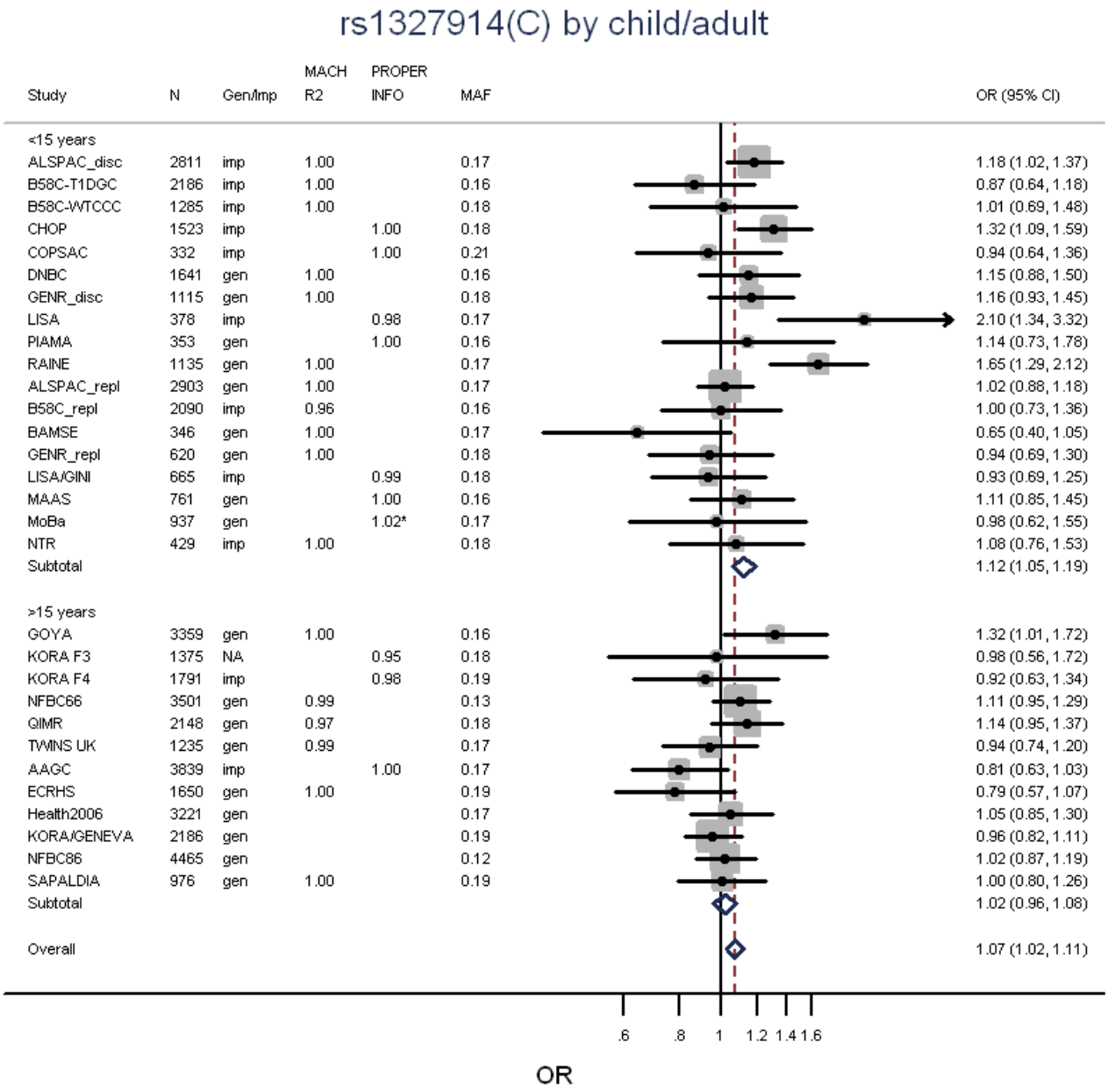

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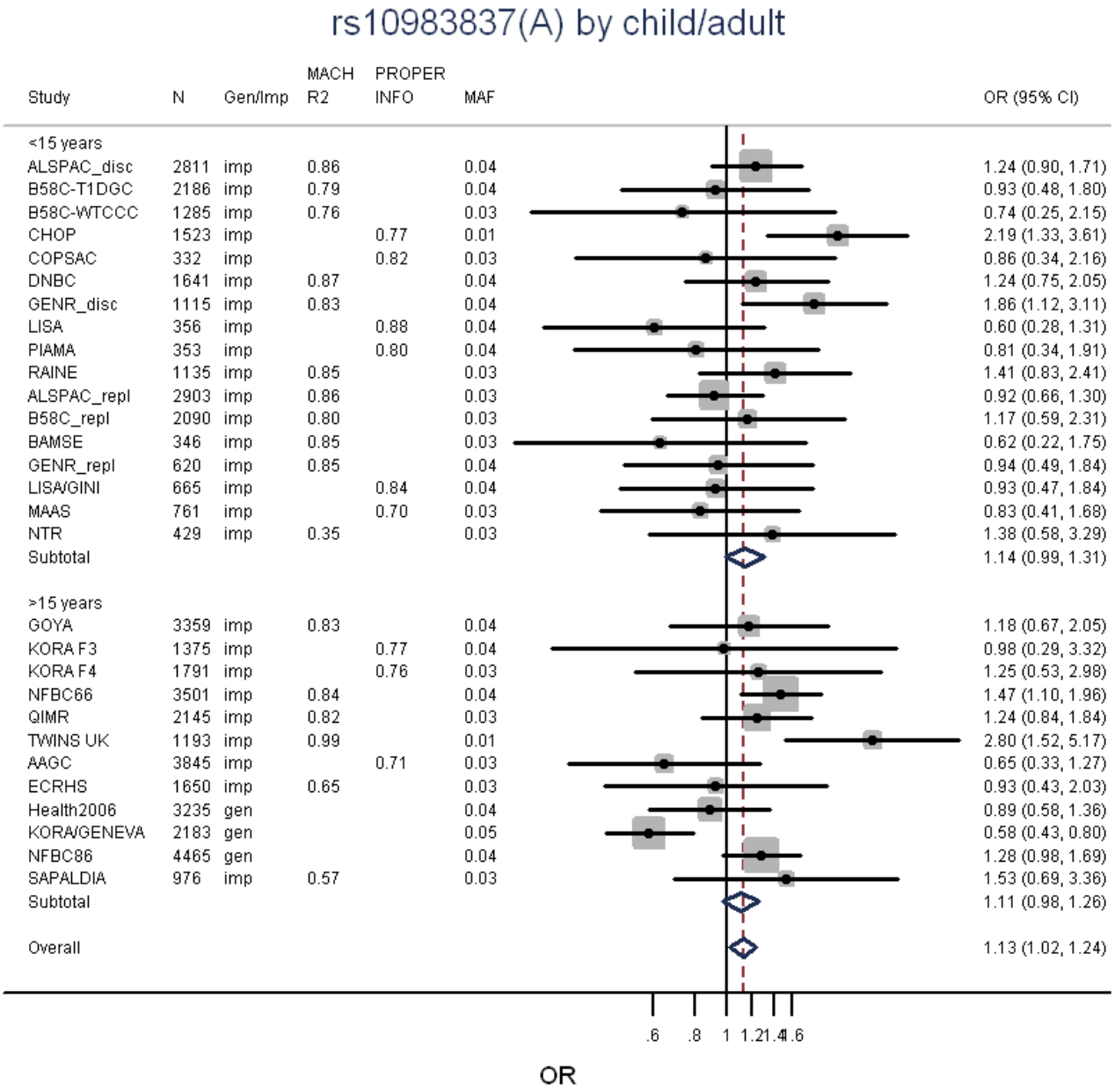

Nature Genetics: doi:10.1038/ng.1017

Supplementary References

1 Johansson SGO *et al*. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol*. **113**, 832–836 (2004).

2 Golding J, Pembrey M, Jones R, Team ALSPACS. ALSPAC–the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatr Perinat Epidemiol*. **15**, 74–87 (2001).

3 Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. *Annu Rev Genomics Hum Genet*. **10**, 387–406 (2009).

4 Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol*. **34**, 816–834 (2010).

5 Henderson J *et al*. The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. *J Allergy Clin Immunol*. **121**, 872–7.e9 (2008).

6 Kull I *et al*. Breast-feeding in relation to asthma, lung function, and sensitization in young schoolchildren. *J Allergy Clin Immunol*. **125**, 1013–1019 (2010).

7 Moffatt MF *et al*. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med*. **363**, 1211–1221 (2010).

8 Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*. **11**, 134 (2010).

9 Williams HC, Strachan DP, Hay RJ. Childhood eczema: disease of the advantaged? *BMJ*. **308**, 1132–1135 (1994).

10 Strachan DP *et al*. Lifecourse influences on health among British adults: effects of region of residence in childhood and adulthood. *Int J Epidemiol*. **36**, 522–531 (2007).

11 Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. **447**, 661–678 (2007).

12 Barrett JC *et al*. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet*. **41**, 703–707 (2009).

13 Bisgaard H. The Copenhagen Prospective Study on Asthma in Childhood (COPSAC): design, rationale, and baseline data from a longitudinal birth cohort study. *Ann Allergy Asthma Immunol*. **93**, 381–389 (2004).

14 Bisgaard H, Hermansen MN, Loland L, Halkjaer LB, Buchvald F. Intermittent inhaled corticosteroids in infants with episodic wheezing. *N Engl J Med*. **354**, 1998–2005 (2006).

15 Bisgaard H *et al*. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med*. **357**, 1487–1495 (2007).

16 Bisgaard H *et al*. Gene-environment interaction in the onset of eczema in infancy: filaggrin loss-of-function mutations enhanced by neonatal cat exposure. *PLoS Med*. **5**, e131 (2008).

17 Halkjaer LB *et al*. Development of atopic dermatitis during the first 3 years of life: the Copenhagen prospective study on asthma in childhood cohort study in high-risk children. *Arch Dermatol*. **142**, 561–566 (2006).

Nature Genetics: doi:10.1038/ng.1017

18 Palmer CNA *et al*. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet*. **38**, 441–446 (2006).

19 Hakonarson H *et al*. A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene. *Nature*. **448**, 591–594 (2007).

20 Olsen J *et al*. The Danish National Birth Cohort–its background, structure and aim. *Scand J Public Health*. **29**, 300–307 (2001).

21 Benn CS *et al*. Atopic dermatitis in young children: diagnostic criteria for use in epidemiological studies based on telephone interviews. *Acta Derm Venereol*. **83**, 347–350 (2003).

22 Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J*. **7**, 954–960 (1994).

23 European Community Respiratory Health Survey II Steering Committee. The European Community Respiratory Health Survey II. *Eur Respir J*. **20**, 1071–1079 (2002).

24 Harrop J *et al*. Eczema, atopy and allergen exposure in adults: a population-based study. *Clin Exp Allergy*. **37**, 526–535 (2007).

25 Jaddoe VWV *et al*. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. *Eur J Epidemiol*. **22**, 917–923 (2007).

26 Jaddoe VWV *et al*. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol*. **25**, 823–841 (2010).

27 Estrada K *et al*. GRIMP: a web- and grid-based tool for high-speed analysis of large-scale genome-wide association using imputed data. *Bioinformatics*. **25**, 2750–2752 (2009).

28 Nohr EA *et al*. Severe obesity in young women and reproductive health: the Danish National Birth Cohort. *PLoS ONE*. **4**, e8444 (2009).

29 Paternoster L *et al*. Genome-Wide Population-Based Association Study of Extremely Overweight Young Adults – The GOYA Study. *PLoS ONE*. **6**, e24303 (2011).

30 Thyssen JP, Linneberg A, Menné T, Nielsen NH, Johansen JD. The prevalence and morbidity of sensitization to fragrance mix I in the general population. *Br J Dermatol*. **161**, 95–101 (2009).

31 Thyssen JP, Linneberg A, Menné T, Nielsen NH, Johansen JD. The effect of tobacco smoking and alcohol consumption on the prevalence of self-reported hand eczema: a cross-sectional population-based study. *Br J Dermatol*. **162**, 619–626 (2010).

32 Holle R, Happich M, Löwel H, Wichmann HE, MONICA/KORA Study Group. KORA–a research platform for population based health research. *Gesundheitswesen*. **67** Suppl 1, S19–S25 (2005).

33 Wichmann HE, Gieger C, Illig T, MONICA/KORA Study Group. KORA-gen–resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen*. **67** Suppl 1, S26–S30 (2005).

34 Esparza-Gordillo J *et al*. A common variant on chromosome 11q13 is associated with atopic dermatitis. *Nat Genet*. **41**, 596–601 (2009).

35 Williams HC *et al*. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol*. **131**, 383–396 (1994).

Nature Genetics: doi:10.1038/ng.1017

36 Heinrich J *et al*. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur Respir J*. **20**, 617–623 (2002).

37 von Berg A *et al*. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol*. **111**, 533–540 (2003).

38 Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet*. **39**, 906–913 (2007).

39 Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet*. **5**, e1000529 (2009).

40 Custovic A, Simpson BM, Murray CS, Lowe L, Woodcock A, N A C Manchester Asthma and Allergy Study Group. The National Asthma Campaign Manchester Asthma and Allergy Study. *Pediatr Allergy Immunol*. **13** Suppl 15, 32–37 (2002).

41 Lowe LA *et al*. Wheeze phenotypes and lung function in preschool children. *Am J Respir Crit Care Med*. **171**, 231–237 (2005).

42 Murray CS *et al*. Lung function at one month of age as a risk factor for infant respiratory symptoms in a high risk population. *Thorax*. **57**, 388–392 (2002).

43 Nicolaou NC *et al*. Exhaled breath condensate pH and childhood asthma: unselected birth cohort study. *Am J Respir Crit Care Med*. **174**, 254–259 (2006).

44 Nicolaou NC *et al*. Day-care attendance, position in sibship, and early childhood wheezing: a population-based birth cohort study. *J Allergy Clin Immunol*. **122**, 500–6.e5 (2008).

45 Magnus P *et al*. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol*. **35**, 1146–1150 (2006).

46 Rønningen KS *et al*. The biobank of the Norwegian Mother and Child Cohort Study: a resource for the next 100 years. *Eur J Epidemiol*. **21**, 619–625 (2006).

47 Rantakallio P. The longitudinal study of the northern Finland birth cohort of 1966. *Paediatr Perinat Epidemiol*. **2**, 59–88 (1988).

48 Sovio U *et al*. Genetic determinants of height growth assessed longitudinally from infancy to adulthood in the northern Finland birth cohort 1966. *PLoS Genet*. **5**, e1000409 (2009).

49 Frayling TM *et al*. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*. **316**, 889–894 (2007).

50 Sabatti C *et al*. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. *Nat Genet*. **41**, 35–46 (2009).

51 Järvelin MR, Hartikainen-Sorri AL, Rantakallio P. Labour induction policy in hospitals of different levels of specialisation. *Br J Obstet Gynaecol*. **100**, 310–315 (1993).

52 Boomsma DI *et al*. Netherlands Twin Register: from twins to twin families. *Twin Res Hum Genet*. **9**, 849–857 (2006).

53 van Beijsterveldt CEM, Boomsma DI. Genetics of parentally reported asthma, eczema and rhinitis in 5-yr-old twins. *Eur Respir J*. **29**, 516–521 (2007).

Nature Genetics: doi:10.1038/ng.1017

54 Brunekreef B *et al*. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol*. **13** Suppl 15, 55–60 (2002).

55 Ferreira MAR *et al*. Association between ORMDL3, IL1RL1 and a deletion on chromosome 17q21 with asthma risk in Australia. *Eur J Hum Genet*. **19**, 458–464 (2011).

56 Evans S, Newnham J, MacDonald W, Hall C. Characterisation of the possible effect on birthweight following frequent prenatal ultrasound examinations. *Early Hum Dev*. **45**, 203–214 (1996).

57 Newnham JP, Evans SF, Michael CA, Stanley FJ, Landau LI. Effects of frequent ultrasound during pregnancy: a randomised controlled trial. *Lancet*. **342**, 887–891 (1993).

58 Williams LA, Evans SF, Newnham JP. Prospective cohort study of factors influencing the relative weights of the placenta and the newborn infant. *BMJ*. **314**, 1864–1868 (1997).

59 Ackermann-Liebrich U *et al*. Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991-2003: methods and characterization of participants. *Soz Praventivmed*. **50**, 245–263 (2005).

60 Downs SH *et al*. Reduced exposure to PM10 and attenuated age-related decline in lung function. *N Engl J Med*. **357**, 2338–2347 (2007).

61 Spector TD, Williams FMK. The UK Adult Twin Registry (TwinsUK). *Twin Res Hum Genet*. **9**, 899–906 (2006).

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7 Paper 4: Regional and Socioeconomic Differences in Dietary Intake in Children

(Sausenthaler* & Standl* et al. Public Health Nutrition, 2011)

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Regional and socio-economic differences in food, nutrient and supplement intake in school-age children in Germany: results from the GINIplus and the LISAplus studies

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Abstract

Objective: To describe regional differences between eastern and western Germany with regard to food, nutrient and supplement intake in 9–12-year-old children, and analyse its association with parental education and equivalent income.

Design: Data were obtained from the 10-year follow-up of the two prospective birth cohort studies – GINIplus and LISAplus. Data on food consumption and supplement intake were collected using an FFQ, which had been designed for the specific study population. Information on parental educational level and equivalent income was derived from questionnaires. Logistic regression modelling was used to analyse the effect of parental education, equivalent income and region on food intake, after adjusting for potential confounders.

Setting: Germany.

Subjects: A total of 3435 children aged 9–12 years.

Results: Substantial regional differences in food intake were observed between eastern and western Germany. Intakes of bread, butter, eggs, pasta, vegetables/salad and fruit showed a significant direct relationship with the level of parental education after adjusting for potential confounders, whereas intakes of margarine, meat products, pizza, desserts and soft drinks were inversely associated with parental education. Equivalent income had a weaker influence on the child's food intake.

Conclusions: Nutritional education programmes for school-age children should therefore account for regional differences and parental education.

Keywords
Diet
Children
Germany
Regional differences
Socio-economic determinants

In Germany, in the early 1990s, shortly after reunification, substantial differences in diet between the eastern and western parts were reported in adults[1]. Although food consumption patterns have since largely converged, differences still existed in 1998[2] and hence they probably do even today.

Furthermore, socio-economic inequalities in food choice and dietary intakes have been reported constantly in the past[3–6]. These studies supported the hypothesis that people from higher socio-economic classes have higher

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intakes of healthy foods, such as whole grains, fish, low-fat dairy products, fresh fruit and vegetables, and lower intakes of unfavourable foods, such as meat and added fat, and were thus more likely meet dietary recommendations than people from lower socio-economic classes. Although these associations have been studied mainly in adults, as reviewed recently[7], it is less clear how parental socio-economic position influences food intake in school-age children. In this context, it would be worth knowing whether and how dietary supplement use in children depends on parental education and household income, as this has become common in German children and adolescents over the past years[8]. According to the German Health Interview and Examination Survey for Children and Adolescents (KiGGS), 15 % of 12–13-year-old boys and 23 % of 12–13-year-old girls were taking dietary supplements in 2006[9]. Educational inequalities were found; however, as most dietary supplements are quite expensive, household income is likely to further limit supplement intake in children.

Detailed knowledge of dietary differences associated with socio-economic inequalities or region of living has important implications, both for the analysis of epidemiological studies and for improving interventions to promote healthy eating. Therefore, the aim of the present study was to describe potential differences between eastern and western Germany and the effect of parental education and equivalent income on food, nutrient and supplement intake in our study population of 9–12-year-old children living in Germany.

We hypothesise that the intake of favourable foods in children, such as fruit and vegetables, as well as the intake of clearly unfavourable foods such as sweets and cakes, shows a social gradient, which is probably mainly determined by the level of parental education and equivalent income. We further hypothesise that, in addition to socio-economic differences, regional differences between eastern and western Germany exist for particular foods and food groups.

Methods

Study population

Data from two ongoing German birth cohort studies were combined for the present analysis.

The GINIplus (German Infant Nutritional Intervention) study is a prospective birth cohort study that comprises the GINI intervention study (I) and the GINI non-intervention study (NI) and was initiated to investigate the influence of nutritional intervention during infancy, as well as that of air pollution and genetics on allergy development. Details on study design, recruitment and exclusion criteria have been described elsewhere[10,11]. In brief, between September 1995 and June 1998, a total of 5991 healthy full-term newborns were recruited from obstetric clinics in two regions of Germany (urban Munich and rural Wesel). Group I (n 2252) included infants with a family history of allergy. In this prospective, double-blind intervention trial, newborns were randomized at birth to one of three hydrolysed formulae or to a conventional cow's milk formula. The formulae were provided only if the recommended exclusive breast-feeding for the first 4 months was not feasible or wanted. Infants with no family history of allergy (n 2507) or with a positive family history but from parents who refused to participate in the intervention trial (n 1232) were allocated to the NI group. This group was sent only the yearly questionnaires and did not receive any of the additional intervention procedures.

All children (I and NI groups) were followed up with identical questionnaires at 1, 2, 3, 4, 6 and 10 years of age to collect information on health outcomes and covariates, such as children's nutrition and other lifestyle factors. A total of 3317 children (55·4 %) of the original study population participated in the 10-year follow-up. Loss to follow-up was associated with a lower level of parental education, a negative history of parental atopy, the absence of atopic diseases in the child during the first 2 years of life and residency in Wesel; the presence of older siblings was not associated with discontinuation of the study.

The LISAplus study is a population-based birth cohort investigating 'influences of lifestyle-related factors on the immune system and the development of allergies in childhood'. Details on study design are described elsewhere[12,13]. In brief, between November 1997 and January 1999, a total of 3097 newborns were initially recruited from four German cities: Munich, Leipzig, Wesel and Bad Honnef. Questionnaires on family history of atopy, parental education and other lifestyle factors were completed by parents shortly after delivery. Data on the child's health were collected by repeated parental-completed questionnaires at regular time intervals during the first 10 years (0·5, 1, 1·5, 2, 4, 6 and 10 years of age). Since the 6-year follow-up, the influences of traffic emission and genetics on allergy development have become the focus of the study. A total of 1761 children (56·9 %) of the original study population participated in the 10-year follow-up. Loss to follow-up was strongly associated with a lower level of parental education and residency in Wesel or Leipzig, whereas the absence of older siblings, a negative history of parental atopy and absence of atopic diseases in the child during the first 2 years of life showed a weak association with discontinuation of the study.

In the 10-year follow-up of both studies, an FFQ was sent to all parents of children who completed the main questionnaire in advance and who indicated a willingness to participate in the dietary assessment. Since the FFQ was provided subsequent to the main questionnaire and only one written reminder was sent, the participation rate in the FFQ was overall 67·9 %, based on participation in the 10-year main questionnaire. Subjects were excluded because of excessive missing information in the FFQ (n 6) or because of implausible energy intake (n 6). Thus, the

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present analysis is based on 3435 subjects from GINIplus (*n* 2190) and LISAplus (*n* 1245).

For both studies, approval from the local ethics committees (Bavarian General Medical Council, University of Leipzig, Medical Council of North-Rhine-Westphalia) and written consent from participant's families were obtained.

FFQ

The FFQ administered to parents was designed to measure children's usual food and nutrient intake over the past year, and more specifically to estimate energy, fatty acid and antioxidant intake at 10 years of age. The development including the selection process of the food item list and validation of the FFQ has been described previously[14].

The FFQ comprised a list of eighty-two food items accompanied by several questions about the preferred fat and energy content of products, preparation methods, diets and food preferences, buying habits and dietary supplement use. To estimate how often food was consumed by their child on average over the previous year, parents were asked to choose one of nine frequency categories, ranging from 'never' to 'four times a day or more'. In addition, common portion sizes were assigned for each food item to enable an estimation of quantities. For food items that are difficult to describe in common household measures, coloured photographs from the EPIC (European Prospective Investigation into Cancer and Nutrition) study showing three different portion sizes were included[15]. The consumption frequencies and portion size estimates were converted into average consumption in g/d and this information was linked to the German Food Code and Nutrient Database (BLS) version II·3·1[16]. Where food portions were not illustrated by pictures, portion size data were obtained from a German list of portion sizes, and weighting of single foods of one FFQ item was carried out according to information obtained from the DONALD (Dortmund Nutritional and Anthropometric Longitudinally Designed) Study[17] in order to represent the common consumption frequencies among the children's age group. This has been described in more detail by Stiegler *et al.*[14]. Missing categorical and continuous variables were replaced by median and mean frequencies of consumption, respectively, obtained from the remaining study population.

For food items rich in fat, such as butter, margarine, sausages and dairy products, additional questions on the fat content that subjects usually choose were asked and answers were applied to the relevant items on the list. Likewise, the energy content of beverages such as soft drinks and fruit drinks was considered.

Summation questions as introduced by Block *et al.*[18] about the average consumption frequencies (per day or per week) of the food groups bread, fruit, vegetables and beverages were included at the end of the FFQ as general consumption frequencies have been assumed to be more valid than the sum of single frequencies[19]. They permit the adjustment of food consumption to reduce the overestimation resulting from the use of a long food item list while retaining the reported distribution of these foods. If the sum of the single food items was different from the overall estimate, individual correction factors were calculated by dividing the food group intake obtained by the summation question by the sum of intakes from the single food items belonging to this food group. Daily food intake was then multiplied by individual correction factors.

Questions on regular dietary vitamin and mineral supplement use were included at the end of the FFQ. The list of possible answers included 'multivitamins', 'vitamin C', 'vitamin E', 'vitamin B', 'β-carotene', 'Ca', 'Mg', 'fluorine' and 'others', allowing to specify other supplements regularly used by the participant. For the purpose of nutrient calculation, this information was linked to the supplement database of the DONALD Study, which contains detailed data on all supplement products ever recorded in the study's 3 d weighted dietary records[17]. However, as the contribution of vitamin intake by supplements was not significant and as we had only rough information about the dosage and frequency of supplement use, we abstained from including vitamin intake from supplements for our analyses. Fatty acid intake by supplementation such as fish oil could anyway not be considered because this was not recorded in the DONALD supplement database.

Socio-economic status: parental education and household income

Parental education was determined on the basis of questionnaire-derived information about school education according to the German educational system, and was defined by the highest grade completed by either the mother or the father. Thus, children were assigned to the group of low (less than tenth grade), medium (tenth grade) or high (more than tenth grade) parental education.

Net household income per month was reported in the 10-year questionnaire using a 9-point scale ranging from <€500 to >€3500. The calculation of equivalent income according to the OECD (Organisation for Economic Cooperation and Development) guidelines[20] was carried out by dividing the net household income by an equivalence factor, which gives a weight of 1·0 to the first adult, 0·5 to all other adults and children >14 years, and 0·3 to all children up to 14 years. As income was measured categorically, we took the mid-point of each income class to calculate the income level. For the lowest income level (<€500) we calculated two-thirds (€333) of this limit, and for the highest income level (>€3500) four-thirds (€4667), as described before[21]. Finally, the new variable was collapsed into three groups of low, medium and high household income. To enable comparing parental education with household income, a similar class size for both variables was needed. Therefore, the classification was based on quintiles so that the percentage of subjects in each group of household income equals the percentage of children in the respective

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group of parental education. Owing to the different income structure in the four study areas, classes were calculated separately for each study centre.

Statistical analysis

Nutrient and food intake data are presented by median and the 25th and 75th percentiles, stratified by region and gender. Food intake data are presented in g/d (Table 2) and as mean contribution to total energy intake (Table 4). For all nutrients, intake data are given in U/MJ and for macronutrients the percentage contribution to total daily energy intake (%E) is also shown (Table 3). Statistically significant differences between study centres and gender in food and nutrient intake were tested by the non-parametric Kruskal–Wallis test.

Logistic regression modelling was used to analyse the effect of parental education, equivalent income and region on food intake adjusting for potential confounders. Food intake data were dichotomised and the upper 25 % of intake for each gender- and centre-specific subgroup was used as outcome variable. Adjusted OR and the corresponding 95 % CI were computed. First, we examined the association between parental education and food intake adjusted for the covariates age and parental atopy. We then calculated a model that included equivalent income, age and parental atopy. Owing to the correlation between parental education and household income ($r = 0{\cdot}28$; $P < 0{\cdot}0001$), mutual adjustment for these two influencing variables was not allowed. Furthermore, a model for the effect of living in Leipzig compared with Munich on food intake was analysed. For this purpose, gender-specific cut-off points for defining high food intake were used.

Supplement use is described as a percentage of the study population for boys and girls. In addition, logistic regression models were calculated to estimate the probability of regular supplement intake dependent on parental education and equivalent income, adjusted for gender, study centre and age. All computations were carried out using the statistical software R, version 2·9·1 (R Foundation for Statistical Computing, Vienna, Austria)[22].

Results

The study population consisted of 3435 children (1683 girls and 1752 boys) aged 9–12 years (median: 10·7). Altogether, 50·3 % and 7·8 % of all children lived in the urban areas of Munich (western Germany) and Leipzig (eastern Germany), respectively, whereas 37·4 % and 4·5 % lived in the more rural areas of Wesel and Bad Honnef (both in western Germany). Table 1 shows the basic characteristics of the study population stratified for the four study centres. Children in Munich and Bad Honnef had a higher parental education than children in Leipzig and Wesel. Similarly, the highest equivalent income was reported in Munich, followed by Bad Honnef, Leipzig and Wesel. The mean body weight and height were 38·0 (SD 7·5) kg and 147·8 (SD 7·4) cm, respectively. The median (50th percentile) BMI in the whole study population was 16·8 kg/m^2, 90 % of all children showed a BMI $<20{\cdot}7$ kg/m^2 and 97 % $<23{\cdot}2$ kg/m^2. Minor differences in the distribution of these anthropometric measures among the study centres could be explained by age differences. An FFQ was mostly completed in autumn (63·9 %) compared with winter (15·5 %), spring (13·3 %) and summer (7·3 %).

The food group intakes in g/d are presented in Table 2 according to study centre and gender. Children in Munich consumed less bread, margarine, dairy products and potatoes and more breakfast cereals (only boys), vegetable oil and fruit/vegetable juices than did children in the other study centres. The intakes of cheese, fruit, cake/pastry and tea were highest in children from Leipzig, whereas they had the lowest intakes of rice, pizza and vegetables/salad compared with children from the other study centres. The highest intake for meat and the lowest intake for butter were observed in Wesel.

The median nutrient intakes per unit energy are presented in Table 3 according to study centre and gender. For boys, total energy intake was lowest in Munich, and for girls in Bad Honnef, whereas Wesel showed the highest energy intake for boys and Leipzig for girls. The macronutrient profiles, including energy derived from protein, carbohydrates and fat, were similar between the four study centres. Relying on *P* values, statistically significant differences between the four study centres were observed for all nutrients, except vitamin C.

The two main sources of total daily energy intake were dairy products and bread, followed by meat products, breakfast cereals, pasta and fruit/vegetable juices (Table 4). The food groups with the smallest contribution to total energy intake (<1 %) were ice cream and fruit gums, eggs and nuts and seeds. The food group ranking in terms of %E contribution was similar although not identical between boys and girls. Regional differences between the study centres were small and derived from the differences in daily food intake described in Table 2.

Parental education, household income, region and food intake

The impact of parental education, equivalent income and regional effects on children's food intake was investigated by logistic regression models (Table 5), where the upper 25 % of food intake for each gender- and centre-specific subgroup was the dependent variable. Food groups that showed a significant direct relationship with the level of parental education after adjusting for age and parental atopy were butter, eggs, vegetables/salad, fruit and vegetable oil. In turn, the intake of meat products, desserts, snacks and soft drinks was inversely associated with parental education.

In contrast, equivalent income showed a weaker influence on the child's food intake. Children with a comparatively high equivalent income had lower intakes

Table 1 Basic characteristics of the study population: German children aged 9–12 years, GINIplus and LISAplus studies

	Total (*n* 3435)		Munich (*n* 1730)		Leipzig (*n* 267)		Wesel (*n* 1284)		Bad Honnef (*n* 154)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Boys (%)	51·0		51·0		51·7		51·2		48·1	
Age (years)										
Median	10·72		10·7		10·3		11·0		10·6	
P5	10·13		10·2		9·9		10·3		10·0	
P95	11·77		11·7		10·9		11·8		11·1	
Level of parental education (%)										
Low	5·9		3·5		1·1		10·5		3·3	
Medium	26·7		16·9		39·7		37·6		22·2	
High	67·4		79·6		59·2		52·0		74·5	
Equivalent income										
Median	1513		1729		1317		1250		1498	
P25	1071		1310		972		913		1071	
P75	1945		2222		1765		1528		1945	
Body height (cm)	147·8	7·4	147·1	7·2	144·5	7	149·5	7·5	146·4	6·2
Body weight (kg)	38	7·5	37·2	7	35·6	6·4	39·6	8·2	37·6	7·4
BMI (kg/m^2)										
P3	13·8		13·8		14·1		13·8		13·7	
P50 (median)	16·8		16·7		16·6		17·1		16·9	
P90	20·7		20·1		19·5		21·3		20·9	
P97	23·2		22·5		21·8		23·9		22·4	

P5, 5th percentile; P95, 95th percentile; P25, 25th percentile; P75, 75th percentile; P3, 3rd percentile; P50, 50th percentile; P90, 90th percentile; P97, 97th percentile.

of meat products and soft drinks and a higher pasta and fruit intake than those in the lowest income category. A model including age, parental atopy, parental education and equivalent income did not indicate that mutual adjustment would have any effect on the magnitude of OR (data not shown).

Table 5 also shows the effect estimates for the association between region (Leipzig, eastern Germany) compared with Munich (western Germany) and gender-specific high food intake controlling for parental education, age and parental atopy. Compared with Munich, children from Leipzig had statistically significant higher intakes of margarine, dairy products, eggs, potatoes, fruit, cake/pastry, soft drinks and tea and lower intakes of breakfast cereals, pasta, rice, pizza and vegetable oil.

Supplement use

In total, the percentage of regular supplement users was 6·9 among boys and 6·6 among girls. About 9·2 % of both sexes reported adding supplements irregularly to their diet (Table 6). Multivitamins were the most common supplements, used by 57·0 % (boys) and 50·5 % (girls) of all regular supplement users. Among the single vitamin or mineral products, vitamins C and E, Ca and Mg were the most important ones. Fluorine was used by 9·0 % of the boys and 17·1 % of the girls who were taking supplements regularly. A high proportion of children (28·9 % of all boys and 27·0 % of all girls) indicated taking dietary supplements other than the ones presented here. Due to the large variety of different supplements, they have been summarised into one heterogeneous group labelled 'other supplements'. The majority of supplement users (5·8 % and 4·8 %) reported the use of only one type of supplement; <2 % took two or more.

Regular supplement use was more common among children in Munich (7·9 %) and Bad Honnef (8·4 %) than in Leipzig (4·5 %) and Wesel (5·5 %; data not shown). The proportion of supplement types varied between study centres, but because of the small numbers significance testing is limited. In Leipzig (66·7 %) and Bad Honnef (69·2 %), the use of multivitamins was more often reported than in Munich (54·0 %) and Wesel (48·6 %; data not shown).

Supplement users were compared with non-users with respect to parental education and equivalent income in a multiple logistic regression model (Table 7). Neither parental education nor equivalent income showed a statistically significant relationship with regular supplement use in children, although more regular supplement users were found among children with medium (7·4 %) or high (6·8 %) parental education compared with children with low parental education (4·7 %).

Discussion

The results of the present study suggest an association of parental education and region with food intake in 9–12-year-old children, whereas association with the level of equivalent income was weak. No statistically significant associations were observed between parental education or equivalent income and supplement intake in children.

The descriptive food and nutrient intake data of the present study were partly in very good agreement with data from the nationally representative eating study (EsKiMo)[23] as part of the KiGGS study. The absolute intakes (in g/d) for 10–11-year-old boys in the EsKiMo study and for 9–12-year-old boys in the present study

Table 2 Median intake of food groups per day in the study population by gender and region

		Total (*n* 3435)			Munich (*n* 1730)			Leipzig (*n* 267)			Wesel (*n* 1284)			Bad Honnef (*n* 154)			
Food group (g/d)	Gender	Mean	P25	P75	Mean	P25	P75	Mean	P25	P75	Mean	P25	P75	Mean	P25	P75	*P* value*
Bread	Boys	115	82	158	104	77	146	124	93	180	134	92	216	124	92	205	<0·0001
	Girls	103	76	141	98	72	137	127	96	170	118	76	176	125	82	162	<0·0001
Butter	Boys	2	0	8	4	1	10	2	1	6	0	0	3	3	0	9	<0·0001
	Girls	2	0	6	4	1	10	4	1	10	0	0	2	2	0	5	<0·0001
Margarine	Boys	1	0	5	0	0	2	3	0	9	4	1	10	1	0	4	<0·0001
	Girls	1	0	5	0	0	1	3	0	10	3	1	9	3	1	6	<0·0001
Cheese	Boys	15	6	35	17	7	36	21	8	38	13	4	33	12	4	23	<0·0001
	Girls	16	6	33	17	7	34	23	12	40	13	5	29	22	8	40	<0·0001
Meat	Boys	52	33	81	45	30	70	46	33	70	64	43	91	47	32	79	<0·0001
	Girls	43	28	64	35	24	55	44	28	61	54	35	79	45	32	65	<0·0001
Meat products	Boys	49	29	75	47	29	73	49	30	73	51	30	82	46	27	74	0·0492
	Girls	38	22	59	38	20	57	44	26	60	38	22	63	33	25	52	0·0841
Breakfast cereals	Boys	25	8	54	36	11	54	27	9	50	18	7	49	19	9	51	<0·0001
	Girls	19	7	40	21	8	50	20	8	36	18	6	37	22	7	43	0·0074
Dairy products	Boys	341	210	580	294	190	464	358	219	571	449	243	740	335	175	577	<0·0001
	Girls	263	153	427	246	134	369	302	176	471	307	166	521	272	147	397	<0·0001
Eggs	Boys	8	5	16	8	5	16	8	8	16	8	5	16	8	5	19	0·0150
	Girls	8	5	9	8	5	8	8	5	14	8	5	10	8	5	8	0·5300
Potatoes	Boys	51	29	83	38	23	65	64	41	87	73	46	110	65	43	83	<0·0001
	Girls	46	28	73	32	21	63	52	41	77	63	43	98	63	37	83	<0·0001
Pasta	Boys	38	18	61	38	24	61	38	18	38	38	15	38	38	18	55	<0·0001
	Girls	38	18	61	38	24	61	38	15	38	38	15	38	38	18	61	<0·0001
Rice	Boys	13	7	19	13	7	22	8	7	13	13	7	18	10	6	13	0·0123
	Girls	13	7	13	13	7	18	8	4	13	13	7	13	13	7	13	0·0060
Pizza	Boys	28	13	33	28	16	47	13	11	28	28	13	33	28	11	33	<0·0001
	Girls	28	11	28	28	13	33	11	6	28	28	11	28	28	11	28	<0·0001
Fish	Boys	17	10	28	17	10	28	19	11	28	17	10	28	18	10	31	0·2765
	Girls	14	7	24	16	7	25	17	10	26	12	7	22	12	7	23	0·0214
Vegetables and salad	Boys	104	65	157	111	69	167	54	24	91	100	62	145	119	79	171	<0·0001
	Girls	115	72	171	123	78	177	54	34	102	106	70	160	109	63	161	<0·0001
Fruit	Boys	110	66	159	110	65	156	131	77	232	108	66	154	110	69	161	<0·0001
	Girls	114	79	162	115	83	162	137	93	234	111	67	157	113	67	156	<0·0001
Cake and pastry	Boys	24	15	38	23	15	37	30	19	49	24	15	38	22	13	34	0·0005
	Girls	23	15	37	23	14	35	28	19	53	23	15	38	23	15	32	0·0010
Desserts	Boys	10	3	18	10	3	17	10	8	18	10	4	18	8	3	18	<0·0001
	Girls	8	3	14	8	3	14	8	4	14	8	3	16	8	4	14	0·0296
Chocolate	Boys	11	6	23	10	5	23	12	7	19	12	7	23	10	4	23	0·0653
	Girls	10	5	19	9	4	18	9	5	19	10	5	19	10	6	18	0·0871
Ice cream and fruit gums	Boys	7	4	12	7	4	12	7	3	16	7	4	12	8	4	15	0·5502
	Girls	6	4	11	6	3	11	8	4	14	6	4	11	7	4	12	0·1968
Snacks	Boys	4	1	7	2	1	4	4	2	6	4	2	7	2	1	7	<0·0001
	Girls	2	1	6	2	1	4	2	1	4	4	2	7	2	1	4	<0·0001
Nuts and seeds	Boys	1	0	3	1	0	3	1	0	2	1	0	2	1	0	3	0·4357
	Girls	1	0	2	1	0	2	1	0	2	1	0	2	1	0	4	0·0670
Vegetable oil	Boys	2	1	5	3	2	6	1	1	2	2	1	4	2	1	4	<0·0001
	Girls	2	1	4	3	2	5	1	1	3	2	1	3	2	1	5	<0·0001
Fruit/vegetable juice	Boys	368	132	714	429	145	744	313	133	665	328	124	683	248	85	624	0·0184
	Girls	331	115	630	367	120	656	363	128	640	283	99	584	313	114	623	0·0478
Soft drinks	Boys	34	13	120	31	12	94	36	10	132	42	15	162	30	10	83	0·0008
	Girls	22	8	65	18	7	48	27	8	78	28	10	94	24	7	98	<0·0001
Tea	Boys	15	0	79	25	5	104	76	22	276	4	0	25	13	2	53	<0·0001
	Girls	24	4	99	34	7	123	90	24	249	11	0	51	21	0	104	<0·0001

P25, 25th percentile; P75, 75th percentile.
*Kruskal–Wallis test.

were very similar for the food groups bread (105 g and 115 g), cheese (17 g and 15 g), eggs (9 g and 8 g), potatoes (53 g and 51 g), pasta (37 g and 38 g) and fruit (112 g and 110 g). In contrast, the reported intakes of meat (52 g and 25 g), breakfast cereals (25 g and 9 g), dairy products (341 g and 244 g) and fruit/vegetable juice (368 g and 193 g) derived from our FFQ were much higher than the corresponding EsKiMo intake data and may have even overestimated the true intakes. However, absolute intake data are in general hardly comparable because of different food group definitions and aggregation levels. In any case, the main sources of total daily energy intake in our study population were comparable to those reported by the EsKiMo study. Dairy products (15·5 % and 11 %), bread (13·8 % and 14 %), fruit/vegetable juices (5·7 % and 7 %) and cake/pastry (5·0 % and 7 %) were ranked among the first eight food groups in both studies, where the first value given in brackets corresponds to all boys of the

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Table 3 Median nutrient intakes per unit energy in the study population by gender and region

		Total (*n* 3435)			Munich (*n* 1730)			Leipzig (*n* 267)			Wesel (*n* 1284)			Bad Honnef (*n* 154)			
Nutrient	Gender	Mean	P25	P75	Mean	P25	P75	Mean	P25	P75	Mean	P25	P75	Mean	P25	P75	*P* value*
Energy (kJ)	Boys	8971	7349	11 016	8658	7077	10 499	8855	7388	10 991	9472	7707	11 681	8947	7226	10 908	0·0000
	Girls	7708	6295	9352	7604	6250	8991	8261	6519	9948	7854	6334	9698	7432	6261	9557	0·0033
Energy (kcal)	Boys	2143	1756	2631	2069	1691	2508	2116	1765	2626	2262	1841	2791	2139	1727	2606	<0·0001
	Girls	1840	1503	2234	1816	1493	2147	1973	1557	2373	1874	1512	2316	1775	1496	2281	0·0034
Protein (%E)	Boys	14	11	18	14	12	18	14	12	17	15	11	19	14	12	18	<0·0001
	Girls	14	11	18	14	11	17	14	11	17	15	12	19	15	12	20	<0·0001
Carbohydrates (%E)	Boys	54	44	67	55	44	67	56	45	68	54	43	67	57	42	65	<0·0001
	Girls	55	44	68	54	44	66	54	42	68	54	44	68	58	46	72	0·0093
Total fat (%E)	Boys	29	22	37	29	23	37	28	22	36	29	23	38	29	21	37	<0·0001
	Girls	29	22	36	29	22	36	29	23	36	28	23	36	29	24	40	0·0406
SFA (mg/MJ)	Boys	3237	2433	4268	3212	2445	4170	3265	2494	4114	3264	2457	4359	3123	2228	4499	0·0000
	Girls	3163	2425	4080	3138	2388	4098	3324	2536	4015	3146	2401	3955	3237	2493	4316	0·0202
MUFA (mg/MJ)	Boys	2707	2068	3519	2754	2094	3587	2617	2190	3239	2707	2049	3582	2767	2024	3323	0·0013
	Girls	2645	2025	3391	2634	2016	3409	2663	2087	3232	2600	2021	3374	2679	2149	3374	0·1191
PUFA (mg/MJ)	Boys	1130	866	1458	1126	863	1455	1073	861	1268	1150	889	1440	1065	865	1387	<0·0001
	Girls	1140	882	1439	1106	848	1391	1048	813	1327	1183	904	1492	1172	985	1516	<0·0001
Linoleic acid (*n*-6; mg/MJ)	Boys	955	729	1233	959	726	1235	908	707	1085	974	744	1249	905	705	1165	<0·0001
	Girls	960	741	1230	939	714	1185	872	679	1097	1005	766	1287	981	834	1267	<0·0001
Arachidonic acid (*n*-6; mg/MJ)	Boys	21	15	28	20	14	27	19	14	24	22	16	31	21	16	31	<0·0001
	Girls	20	15	28	19	13	26	19	13	24	22	17	32	22	17	30	<0·0001
EPA (*n*-3; μg/MJ)	Boys	3767	2038	6845	3713	2014	6895	4454	2674	8068	3603	2054	6431	3817	1909	7358	0·0284
	Girls	3498	1898	6904	3695	1946	7487	4124	2210	8338	3301	1782	5802	3052	1913	6730	0·0151
DPA (*n*-3; μg/MJ)	Boys	1262	720	2010	1195	676	1939	1491	827	2127	1259	741	2076	1303	766	1853	0·0022
	Girls	1212	691	2068	1199	660	2058	1373	797	2352	1218	703	1993	1068	727	2236	0·1037
Vitamin A (μg/MJ)	Boys	114	75	172	117	77	176	117	77	178	108	72	166	122	88	171	0·6453
	Girls	121	86	183	128	89	188	117	82	195	112	82	169	126	89	203	0·0098
Vitamin E (μg/MJ)	Boys	999	781	1311	1042	802	1374	942	724	1205	959	763	1259	961	835	1207	0·0166
	Girls	1077	835	1382	1092	850	1408	1007	751	1211	1053	820	1352	1183	904	1488	0·3731
Vitamin C (mg/MJ)	Boys	10	7	14	10	7	14	10	6	14	10	7	13	10	7	14	0·2121
	Girls	11	8	15	11	8	15	10	8	15	11	8	16	12	8	16	0·9318

P25, 25th percentile; P75, 75th percentile.
*Kruskal–Wallis test.

Table 4 Mean contribution of food groups to total energy intake in boys and girls

Boys (*n* 1752)		Girls (*n* 1683)	
Food group	Mean contribution to total energy intake (%)	Food group	Mean contribution to total energy intake (%)
Dairy products	15·5	Bread	14·3
Bread	13·8	Dairy products	13·6
Meat products	8·7	Meat products	7·7
Breakfast cereals	6·6	Pasta	7·2
Pasta	6·4	Fruit/vegetable juices	6·1
Fruit/vegetable juices	5·7	Breakfast cereals	5·8
Meat	5·5	Cake and pastry	5·5
Cake and pastry	5·0	Meat	5·3
Chocolate	3·9	Fruit	4·5
Fruit	3·5	Chocolate	3·9
Cheese	3·0	Cheese	3·3
Soft drinks	3·0	Butter	2·9
Butter	2·9	Rice	2·8
Potatoes	2·5	Potatoes	2·6
Rice	2·4	Soft drinks	2·3
Vegetable oil	1·7	Vegetable oil	1·8
Pizza	1·6	Vegetables, salad	1·7
Fish	1·4	Pizza	1·5
Vegetables and salad	1·3	Fish	1·4
Margarine	1·2	Margarine	1·3
Desserts	1·2	Desserts	1·1
Snacks	1·2	Snacks	1·1
Ice cream and fruit gums	0·8	Ice cream and fruit gums	0·8
Eggs	0·7	Eggs	0·7
Nuts and seeds	0·7	Nuts and seeds	0·7

present study population and the second value to 6–11-year-old boys of the EsKiMo study. This general good agreement was also reflected on the nutrient level, as intakes of the macronutrients fat, protein and carbohydrates as well as vitamins E and C corresponded to the values reported by the EsKiMo study. Only total energy intake in boys (8971 kJ (2143 kcal) and 7586 kJ (1813 kcal)) seemed to be overestimated in the present study.

For children, the KiGGS study suggests no persisting dietary differences in intakes between children from eastern and western Germany[24], which is in contrast to our findings. We compared food group intakes between metropolitan areas such as Leipzig in the east and Munich in the west of Germany and observed some significant differences for the food groups margarine, dairy products, eggs, potatoes, fruit, cake/pastry, soft drinks, tea, breakfast cereals, pasta, rice, pizza and vegetable oil. The study centres Wesel and Bad Honnef have been excluded from the analysis although they are located in the west of Germany. Owing to the heterogeneity of the three western study centres, rural/urban and north/west differences would be implied in the east/west comparison. Certainly, the different socio-economic structures of the two study centres might have contributed to the observed regional effects as, for example, parents in Munich (79·6 %) more likely had a high educational level than in Leipzig (59·2 %; see Table 1). Thus, the correlation between parental education and region ($r = 0{\cdot}17$; $P < 0{\cdot}0001$) might have led to residual confounding even after controlling for parental education. As this could also affect the association between parental education and food intake, we calculated gender- and centre-specific cut-off points for defining high food intake for analysing the association with parental education to minimise the described effect.

Comparing present findings with other studies is difficult because various measures for socio-economic position have been used, including education, income or occupation, which have been shown not to serve as adequate proxies for one another[25]. According to the findings of the present study, the intakes of butter, eggs, vegetables/salad, fruit, vegetable oil, meat products, desserts, snacks and soft drinks in children varied by level of parental education. Our findings are thus in line with probably the most consistent evidence of dietary inequalities in adults, showing a lower consumption of fruit and vegetables among socio-economically disadvantaged groups[4]. This has also been reported by a study carried out in German schoolchildren, a part of WHO's cross-sectional survey HBSC (Health Behaviour in School-Aged Children)[26], showing that the impact of the social situation was particularly strong for healthy foods, such as raw vegetables, fruit and wholegrain wheat bread. A Finnish study in children aged 9–15 years also reported that children of families with higher socio-economic status used more fruit than did children of families with lower socio-economic status, where family's socio-economic status was defined according to the father's educational level, his occupation and family income[27]. Contrary to our findings, they reported that high socio-economic status was associated with a higher margarine intake, whereas children of low

Table 5 Results of logistic regression models of parental education, equivalent income and region on children's food intake (defined as the upper 25 % of each gender- and center-specific subgroups)

	Parental education				Equivalent income				Region	
	Medium *v.* low (*n* 888)		High *v.* low (*n* 2236)		Medium *v.* low (*n* 699)		High *v.* low (*n* 2401)		Leipzig *v.* Munich (*n* 267)	
Food group (g/d)	aOR*	95 % CI	aOR*	95 % CI	aOR*	95 % CI	aOR*	95 % CI	aOR†	95 % CI
Bread	0·89	0·61, 1·29	1·16	0·82, 1·65	0·68	0·48, 0·95	0·90	0·66, 1·22	1·05	0·75, 1·48
Butter	1·11	0·73, 1·67	1·88	1·28, 2·78	1·06	0·74, 1·54	1·37	0·98, 1·92	0·99	0·70, 1·40
Margarine	1·09	0·77, 1·54	0·79	0·57, 1·09	0·95	0·68, 1·32	0·79	0·58, 1·07	3·89	2·85, 5·32
Cheese	0·95	0·66, 1·37	1·12	0·80, 1·58	0·76	0·54, 1·05	0·79	0·58, 1·06	1·19	0·85, 1·65
Meat	1·10	0·78, 1·57	0·81	0·58, 1·14	1·04	0·74, 1·46	0·95	0·70, 1·30	1·15	0·82, 1·60
Meat products	0·78	0·55, 1·10	0·64	0·46, 0·88	0·82	0·59, 1·14	0·68	0·50, 0·92	1·03	0·74, 1·44
Breakfast cereals	1·34	0·91, 1·96	1·43	0·99, 2·07	0·84	0·60, 1·17	0·87	0·64, 1·18	0·64	0·45, 0·92
Dairy products	1·06	0·74, 1·50	0·85	0·61, 1·18	0·82	0·59, 1·14	0·78	0·58, 1·05	1·81	1·31, 2·48
Eggs	1·39	0·94, 2·04	1·45	1·01, 2·09	1·17	0·83, 1·65	1·02	0·74, 1·39	1·39	1·00, 1·92
Potatoes	0·92	0·65, 1·31	0·89	0·64, 1·25	0·87	0·63, 1·21	0·74	0·55, 1·00	2·23	1·63, 3·05
Pasta	1·09	0·79, 1·50	1·25	0·92, 1·69	1·07	0·78, 1·46	1·36	1·02, 1·81	0·42	0·29, 0·59
Rice	1·11	0·80, 1·56	1·09	0·80, 1·50	1·23	0·88, 1·72	1·25	0·92, 1·70	0·60	0·43, 0·83
Pizza	0·85	0·61, 1·17	0·77	0·57, 1·05	1·05	0·76, 1·45	0·99	0·74, 1·33	0·27	0·18, 0·41
Fish	0·83	0·58, 1·18	0·85	0·61, 1·19	1·00	0·71, 1·40	0·93	0·68, 1·27	1·08	0·77, 1·50
Vegetables and salad	1·43	0·95, 2·14	1·70	1·16, 2·51	1·07	0·75, 1·52	1·08	0·78, 1·49	0·73	0·51, 1·05
Fruit	1·99	1·27, 3·10	2·44	1·60, 3·74	1·16	0·80, 1·68	1·44	1·03, 2·02	2·32	1·69, 3·18
Cake and pastry	1·16	0·80, 1·68	1·10	0·77, 1·56	0·85	0·61, 1·19	0·78	0·58, 1·06	1·94	1·42, 2·66
Desserts	0·66	0·48, 0·91	0·53	0·39, 0·73	0·88	0·64, 1·22	0·89	0·66, 1·19	1·15	0·84, 1·58
Chocolate	0·88	0·63, 1·24	0·73	0·53, 1·01	1·00	0·71, 1·41	0·95	0·70, 1·29	0·86	0·61, 1·21
Ice cream and fruit gums	1·01	0·71, 1·43	0·83	0·60, 1·17	1·17	0·84, 1·64	0·86	0·63, 1·17	1·18	0·85, 1·63
Snacks	1·07	0·78, 1·48	0·73	0·54, 0·99	0·95	0·70, 1·29	0·77	0·58, 1·03	1·19	0·88, 1·62
Nuts and seeds	1·14	0·79, 1·64	1·14	0·81, 1·61	1·04	0·74, 1·45	0·88	0·65, 1·19	0·78	0·55, 1·11
Vegetable oil	1·28	0·86, 1·92	1·68	1·15, 2·45	1·24	0·86, 1·80	1·37	0·98, 1·92	0·20	0·12, 0·34
Fruit/vegetable juice	1·06	0·74, 1·52	1·00	0·71, 1·40	1·25	0·87, 1·78	1·16	0·84, 1·61	0·84	0·60, 1·19
Soft drinks	0·67	0·49, 0·93	0·44	0·32, 0·59	0·81	0·59, 1·12	0·63	0·47, 0·84	1·44	1·04, 2·00
Tea	1·03	0·71, 1·49	1·14	0·80, 1·61	0·78	0·55, 1·09	0·82	0·60, 1·10	2·55	1·86, 3·49

aOR, adjusted OR.
Low parental education, low family income and the study centre in Munich formed the reference category.
*OR adjusted for age and parental atopy; centre- and gender-specific cut-off points were used to define high intake (upper 25 %).
†OR adjusted for parental education, age and parental atopy; gender-specific cut-off points were used to define high intake (upper 25 %)

socio-economic status had a higher intake of butter. However, although the parental education level was lower in Leipzig compared with Munich, fruit intake was higher in this eastern German city. This result strongly points to the additional impact of further influencing factors on food intake.

Another study showed that among several potential predictors of fruit and vegetable consumption analysed in pre-school children, maternal education emerged to be positively associated with children's vegetable consumption whereas no effect of fruit intake was reported[28]. Although the dependence of healthy food intake, such as fruit and vegetables, on social class has been studied well, the situation is less clear for some of the foods for which significant associations have been described in the present study. Within the ALSPAC (Avon Longitudinal Study of Parents and Children) study[29], a positive association between maternal education and intake of meat products and soft drinks was shown in 18-month-old children. Compared with school age, food choice at this age is completely under the control of parents, which is why parental socio-economic position might have a different effect than later in childhood.

Several possible limitations of the study should be considered. First, it should be noticed that the present findings are based on a cross-sectional analysis within the two cohort studies – LISAplus and GINIplus. Even though study sampling was primarily population-based, the study population is, as almost every cohort study, subject to selection bias, and thus the findings are not representative for Germany. Owing to non-random loss to follow-up, both cohorts on which the present analysis is based underrepresent children from lower social classes. The true social inequalities might therefore even be stronger than reported here. Furthermore, the intakes of a large number of nutrients and foods were examined, many of which are correlated with one another. As a result there is the possibility of type I errors occurring. However, the significant associations that we have found are either similar to previous findings or are in line with our previous hypothesis, which leads us to believe that they are unlikely to be the result of type I error. Certainly, it has to be considered that the FFQ was actually not designed to measure, for example, carbohydrate and protein intakes. However, since total energy intake as well as the percentage contribution to total daily energy intake of carbohydrates and protein shows plausible values, we think that this justifies the analysis of the respective macronutrients. However, it has to be considered that the food item list included only those foods that significantly contribute to the intake of total energy, fatty acids and selected antioxidants.

Unfortunately, we do not have any information on whether fortified foods were consumed. However, according to a recent publication[30], the contribution of fortified foods to total nutrient intake is rather low in Germany, in particular for vitamins E and A. The authors further conclude that the base diet is the major contributor to intakes of all nutrients in Europe. When we included nutrients from supplement intake in our analyses, the numbers did not change substantially, as the rather low frequency of regular supplement use in our study population (<7 %) let assume. However, the rather

Table 6 Prevalence of supplement use in boys and girls

	Boys		Girls	
	n	%	*n*	%
Number of supplements	(*n* 1752)		(*n* 1683)	
0	1631	93·1	1572	93·4
1	101	5·8	80	4·8
≥2	20	1·1	31	1·8
Frequency				
Regular supplement use	121	6·9	111	6·6
Irregular supplement use	161	9·2	155	9·2
Specification (only regular supplement user)*	(*n* 121)		(*n* 111)	
Multivitamins	69	57·0	56	50·5
Vitamin C	26	21·5	28	25·2
Vitamin E	16	13·2	13	11·7
Vitamin B	3	2·5	7	6·3
β-Carotene	1	0·8	4	3·6
Ca	12	9·9	19	17·1
Mg	18	14·9	16	14·4
Fluorine	11	9·0	19	17·1
Zn	12	9·9	13	11·7
Iodine	5	4·1	9	8·1
Fe	4	3·3	8	7·2
Others	35	28·9	30	27·0

*Multiple answers possible.

Table 7 Results of logistic regression models of parental education and equivalent income on children's supplement use

	Parental education						Equivalent income					
	Low (*n* 193)		Medium (*n* 888)		High (*n* 2236)		Low (*n* 335)		Medium (*n* 699)		High (*n* 2401)	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
User *v.* non-user	9	4·7	66	7·4	153	6·8	25	7·5	47	6·7	160	6·7
			aOR*	95 % CI	aOR*	95 % CI			aOR*	95 % CI	aOR*	95 % CI
	1·00		1·62	0·83, 3·55	1·48	0·78, 3·18	1·00		0·89	0·54, 1·50	0·88	0·58, 1·40

aOR, adjusted OR.
*Adjusted for gender, study centre and age.

vague estimation of nutrient intake from supplements and the missing information on fortified foods could have led to misclassification of nutrient intake. However, the statistical models for the association between socio-economic factors and region on food intake are not affected by this matter.

One of the major strengths of the present study was the food selection method underlying the dietary assessment method as described previously[14]. In brief, this newly developed FFQ was designed for the specific purpose of measuring diet, especially fatty acid and antioxidant intake, in school-age children. The food selection method was based on a regression approach using data from 9–11-year old children from the German DONALD Study. The FFQ has been validated against one 24 h dietary recall and has shown good practicability in the past. In summary, even if the FFQ is generally believed to overestimate dietary intakes[31], we believe that this FFQ is valid to rank individuals according to their dietary intakes and to detect differences between levels of socio-economic status. Furthermore, this is one of the few studies in children collecting specific information on dietary supplement use.

In conclusion, we have found that food intake in school-age children differs by the level of parental education and area of living, but the differences cannot be attributed to healthy or unhealthy dietary habits. This suggests that particular efforts should be made to adjust nutrition education programmes to the target population characterised not only in terms of education but also by considering regional particularities.

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References

1. Winkler G, Brasche S & Heinrich J (1997) Trends in food intake in adults from the city of Erfurt before and after the German reunification. *Ann Nutr Metab* **41**, 283–290.
2. Mensink GB & Beitz R (2004) Food and nutrient intake in East and West Germany, 8 years after the reunification – The German Nutrition Survey 1998. *Eur J Clin Nutr* **58**, 1000–1010.
3. Giskes K, Turrell G, van Lenthe FJ *et al.* (2006) A multilevel study of socio-economic inequalities in food choice behaviour and dietary intake among the Dutch population: the GLOBE study. *Public Health Nutr* **9**, 75–83.
4. Giskes K, Avendano M, Brug J *et al.* (2010) A systematic review of studies on socioeconomic inequalities in dietary intakes associated with weight gain and overweight/obesity conducted among European adults. *Obes Rev* **11**, 413–419.
5. Irala-Estevez JD, Groth M, Johansson L *et al.* (2000) A systematic review of socio-economic differences in food habits in Europe: consumption of fruit and vegetables. *Eur J Clin Nutr* **54**, 706–714.
6. Lopez-Azpiazu I, Sanchez-Villegas A, Johansson L *et al.* (2003) Disparities in food habits in Europe: systematic review of educational and occupational differences in the intake of fat. *J Hum Nutr Diet* **16**, 349–364.
7. Darmon N & Drewnowski A (2008) Does social class predict diet quality? *Am J Clin Nutr* **87**, 1107–1117.
8. Sichert-Hellert W & Kersting M (2004) Vitamin and mineral supplements use in German children and adolescents between 1986 and 2003: results of the DONALD Study. *Ann Nutr Metab* **48**, 414–419.
9. Six J, Richter A, Rabenberg M *et al.* (2008) Dietary supplement use among adolescents in Germany. Results of EsKiMo. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* **51**, 1202–1209.
10. von Berg A, Koletzko S, Grubl A *et al.* (2003) The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol* **111**, 533–540.
11. von Berg A, Filipiak-Pittroff B, Kramer U *et al.* (2008) Preventive effect of hydrolyzed infant formulas persists until age 6 years: long-term results from the German Infant Nutritional Intervention Study (GINI). *J Allergy Clin Immunol* **121**, 1442–1447.
12. Heinrich J, Bolte G, Holscher B *et al.* (2002) Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur Respir J* **20**, 617–623.
13. Zutavern A, Brockow I, Schaaf B *et al.* (2006) Timing of solid food introduction in relation to atopic dermatitis and atopic sensitization: results from a prospective birth cohort study. *Pediatrics* **117**, 401–411.
14. Stiegler P, Sausenthaler S, Buyken AE *et al.* (2010) A new FFQ designed to measure the intake of fatty acids and antioxidants in children. *Public Health Nutr* **13**, 38–46.
15. Bohlscheid-Thomas S, Hoting I, Boeing H *et al.* (1997) Reproducibility and relative validity of food group intake in a food frequency questionnaire developed for the German part of the EPIC project. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol* **26**, Suppl. 1, S59–S70.
16. Hartmann BM, Bell S, Vásquez-Caiquedo AL *et al.* (2005) *Der Bundeslebensmittelschlüssel. German Nutrient Data Base.* Karlsruhe: Federal Research Centre for Nutrition and Food (BfEL).
17. Kroke A, Manz F, Kersting M *et al.* (2004) The DONALD Study. History, current status and future perspectives. *Eur J Nutr* **43**, 45–54.

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18. Block G, Woods M, Potosky A *et al.* (1990) Validation of a self-administered diet history questionnaire using multiple diet records. *J Clin Epidemiol* **43**, 1327–1335.
19. Haraldsdottir J (1993) Minimizing error in the field: quality control in dietary surveys. *Eur J Clin Nutr* **47**, Suppl. 2, S19–S24.
20. Hauser R (1998) Adequacy and poverty among the retired. In *Maintaining Prosperity in an Ageing Society. Ageing Working Paper* no. 3.2. Paris: OECD.
21. Sausenthaler S, Kompauer I, Mielck A *et al.* (2007) Impact of parental education and income inequality on children's food intake. *Public Health Nutr* **10**, 24–33.
22. R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
23. Mensink GB, Heseker H, Richter A *et al.* (2007) *Ernährungsstudie als KiGGS-Modul (EsKiMo) – Forschungsbericht*. Berlin, Paderborn: Robert-Koch Institut, Universität Paderborn.
24. Stahl A, Vohmann C, Richter A *et al.* (2009) Changes in food and nutrient intake of 6- to 17-year-old Germans between the 1980s and 2006. *Public Health Nutr* **12**, 1912–1923.
25. Turrell G, Hewitt B, Patterson C *et al.* (2003) Measuring socio-economic position in dietary research: is choice of socio-economic indicator important? *Public Health Nutr* **6**, 191–200.
26. Klocke A (1997) The impact of poverty on nutrition behaviour in young Europeans. In *Poverty and Food in Welfare Societies*, pp. 224–237 [B Kohler, E Feichtinger, E Barlosius, *et al.*, editors]. Berlin: Sigma.
27. Laitinen S, Rasanen L, Viikari J *et al.* (1995) Diet of Finnish children in relation to the family's socio-economic status. *Scand J Soc Med* **23**, 88–94.
28. Cooke LJ, Wardle J, Gibson EL *et al.* (2004) Demographic, familial and trait predictors of fruit and vegetable consumption by pre-school children. *Public Health Nutr* **7**, 295–302.
29. Rogers I & Emmett P (2003) The effect of maternal smoking status, educational level and age on food and nutrient intakes in preschool children: results from the Avon Longitudinal Study of Parents and Children. *Eur J Clin Nutr* **57**, 854–864.
30. Flynn A, Hirvonen T, Mensink GB *et al.* (2009) Intake of selected nutrients from foods, from fortification and from supplements in various European countries. *Food Nutr Res* Suppl. 1, 53.
31. Robinson S, Godfrey K, Osmond C *et al.* (1996) Evaluation of a food frequency questionnaire used to assess nutrient intakes in pregnant women. *Eur J Clin Nutr* **50**, 302–308.

Appendix

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8 Paper 5: *FADS* Variants, Dietary Fatty Acid Intake and Lipids in Children

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FADS1 FADS2 Gene Cluster, PUFA Intake and Blood Lipids in Children: Results from the GINIplus and LISAplus Studies

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Abstract

Background: Elevated cholesterol levels in children can be a risk factor for cardiovascular diseases in later life. In adults, it has been shown that blood lipid levels are strongly influenced by polymorphisms in the fatty acid desaturase (*FADS*) gene cluster in addition to nutritional and other exogenous and endogenous determinants. Our aim was to investigate whether lipid levels are determined by the *FADS* genotype already in children and whether this association interacts with dietary intake of n-3 fatty acids.

Methods: The analysis was based on data of 2006 children from two German prospective birth cohort studies. Total cholesterol, HDL, LDL and triglycerides were measured at 10 years of age. Six single nucleotide polymorphisms (SNPs) of the *FADS* gene cluster were genotyped. Dietary n-3 fatty acid intake was assessed by food frequency questionnaire. Linear regression modeling was used to assess the association between lipid levels, n-3 fatty acid intake and *FADS* genotype.

Results: Individuals carrying the homozygous minor allele had lower levels of total cholesterol [means ratio (MR) ranging from 0.96 ($p = 0.0093$) to 0.98 ($p = 0.2949$), depending on SNPs] and LDL [MR between 0.94 ($p = 0.0179$) and 0.97 ($p = 0.2963$)] compared to homozygous major allele carriers. Carriers of the heterozygous allele showed lower HDL levels [β between −0.04 ($p = 0.0074$) to −0.01 ($p = 0.3318$)] and higher triglyceride levels [MR ranging from 1.06 ($p = 0.0065$) to 1.07 ($p = 0.0028$)] compared to homozygous major allele carriers. A higher n-3 PUFA intake was associated with higher concentrations of total cholesterol, LDL, HDL and lower triglyceride levels, but these associations did not interact with the *FADS1 FADS2* genotype.

Conclusion: Total cholesterol, HDL, LDL and triglyceride concentrations may be influenced by the *FADS1 FADS2* genotype already in 10 year old children. Genetically determined blood lipid levels during childhood might differentially predispose individuals to the development of cardiovascular diseases later in life.

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Introduction

Lipid concentrations in blood are associated with cardiovascular diseases [1–4]. Elevated cholesterol levels during childhood and adolescence increases the risk for cardiovascular diseases in adulthood. Moreover treatments which effectively lower cholesterol levels early in life have been shown to prevent disease manifestation in later life [5–7].

Among other factors, dietary polyunsaturated fatty acid (PUFA) intake can attenuate high blood lipid concentrations [8–10].

In addition to dietary influences, recent genome wide association studies have identified several genetic loci that are associated with blood lipid levels in adults [11–15]. Among the top hits for the determination of lipid levels are polymorphisms in the fatty acid desaturase (*FADS*) gene cluster. Lower total cholesterol [11], low-density lipoprotein (LDL) [11–13], high-density lipoprotein (HDL) [13,14] and higher triglyceride levels [14,15] are all associated with the minor alleles of the tested *FADS* polymorphisms. The genes *FADS1* and *FADS2*, which are both located in this gene cluster, encode the enzymes delta-5-desaturase and delta-6-desaturase, which are involved in the conversion of dietary n-3 and n-6 fatty acids to their longer chain metabolites [16]. Polymorphisms in these genes are associated with n-3 and n-6 fatty acid levels in blood and several tissues [17–23]. Carriers of the minor alleles exhibit increased levels of desaturase substrates and decreased levels of desaturase products. This trend may be the result of lower transcription levels or diminished enzyme conversion rates in individuals carrying the minor alleles [17].

The previously reported strong association of *FADS* polymorphisms with lipid levels in adults makes these genes good candidates for genetic association studies focused on lipid levels in children. Given the role of the gene products in the conversion of dietary fatty acids to biologically important longer chain polyunsaturated fatty acids, which in turn have been shown to influence lipid levels, interaction analysis between genetic polymorphisms and dietary intake of fatty acids for individual lipid levels is of interest.

In this study we investigated whether genetic variations of the *FADS* gene cluster already pre-determines total cholesterol, HDL, LDL and triglyceride levels in 10 year old children and whether genetic variation interacts with dietary intake of n-3 PUFAs to determine individual blood lipid levels.

Results

Information on lipid concentrations in blood and *FADS1 FADS2* genotype was available for 2006 children (1288 (64%) children from the GINIplus study and 718 (36%) children from the LISAplus study). Information on n-3 PUFA intake was only available for 1697 of these children (1100 (65%) children from the GINIplus study and 597 (35%) children from the LISAplus study).

Basic characteristics of the study population are presented in Table 1. Total cholesterol, LDL and triglyceride concentrations were significantly higher in the GINIplus study, whereas HDL concentrations were higher in the LISAplus study.

The genotype and allele frequencies of the six SNPs which were included in the analysis are shown in Table 2. There was no difference in the allele frequency distribution between the GINIplus and LISAplus studies.

Five of the six SNPs (rs174545, rs174546, rs174556, rs174561 and rs3834458) are in high LD with each other. For these five SNPs, the pairwise squared correlations r^2 ranged from 0.83 to 0.99, and Lewontin's D' ranged from 0.98 to 1.

For rs174575, the linkage disequilibrium is lower. The pairwise correlation r^2 for this SNP ranged from 0.49 to 0.66 and Lewontin's D' ranged from 0.77 to 0.96.

Median levels of total cholesterol, HDL, LDL and triglyceride concentrations, stratified by *FADS* genotype, are presented in Table 3. Homozygous minor allele carriers had lower levels of total cholesterol and LDL compared to homozygous or heterozygous major allele carriers. In contrast, triglyceride concentrations were higher in minor allele carriers compared to homozygous major allele carriers. After adjustment for multiple testing ($\alpha_{corr} = 0.025$), these associations remained significant for triglyceride levels and LDL (rs174556 and rs174561).

The results of the linear regression models for total cholesterol, HDL, LDL and triglyceride concentrations, the *FADS* genotype and n-3 PUFA intake are presented in Table 4. Adjusted means ratios for total cholesterol, LDL and triglyceride concentrations and effect estimates for HDL are shown.

The p-values describing the association between n-3 PUFA intake and elevated concentrations of total cholesterol [MR = 1.01 for all six SNPs (p-value ranging from 0.0288 to 0.0884)], LDL [MR from 1.01 ($p = 0.1075$) to 1.02 ($p = 0.0411$)], HDL [$\beta = 0.02$ for all six SNPs (p-value between 0.0126 and 0.0306)] and reduced triglyceride levels [MR between 0.97 ($p = 0.0219$) and 0.98 ($p = 0.0841$)] were statistically significant. However, after correcting for multiple testing, only five (out of six) SNPs for HDL and triglyceride levels and rs3834458 remained significant.

Additional analyses showed similar results for the n-3 PUFAs ALA, EPA, DPA and DHA, which were combined into the total dietary n-3 PUFA intake (data not shown).

There was no association between dietary n-6 PUFA intake and lipid levels (data not shown).

Homozygous minor allele carriers had decreased levels of total cholesterol [MR ranging from 0.96 ($p = 0.0093$) to 0.98 ($p = 0.2949$)] and LDL [MR between 0.94 ($p = 0.0179$) and 0.97 ($p = 0.2963$)] compared to homozygous major allele carriers. After correcting for multiple testing ($\alpha_{corr} = 0.025$), the association remained significant for rs174556 and rs174561 and total cholesterol and LDL.

HDL concentrations were reduced in carriers of the heterozygous genotype compared to homozygous major allele carriers [β between -0.04 ($p = 0.0074$) to -0.01 ($p = 0.3318$)]. These associations remained significant for four SNPs after correction for multiple testing.

Individuals carrying the heterozygous genotype showed significantly increased triglyceride levels compared to homozygous major allele carriers [MR ranging from 1.06 ($p = 0.0065$) to 1.07 ($p = 0.0028$)]. These associations remained significant after correcting for multiple testing. Although homozygous minor allele carriers also showed increased triglyceride levels, these effects did not reach statistical significance. Additional analyses restricted to fasting blood samples did not show substantially different results and the magnitude of the association between *FADS* genotype, n-3 PUFA and lipid concentration was similar. However, this result did not reach statistical significance, likely because of the reduced sample size (Table S1).

For each model, including the *FADS* SNP increased the percentage of explained variance compared to the model without any SNPs (Table S2). The maximal increase in the explained variance was 0.71% for the total cholesterol model (1.98% to 2.69%), 1.28% for the LDL model (from 4.21% to 5.49%), 0.96% for the HDL model (from 7.83% to 8.79%) and 0.48% for the triglycerides model (from 12.38% to 12.86%). Additional analyses stratified by study (GINIplus and LISAplus) showed similar results,

Table 1. Basic characteristics of the study population.

	GINIplus (n = 1288)	LISAplus (n = 718)	Total (n = 2006)	p-value
	Median (25%-Qu.,75%-Qu.) or %			
Gender [% male]	49.0	55.6	51.3	0.0052[1]
Age [weeks]	531 (526,538)	529 (525,536)	530 (525,537)	0.0072[2]
BMI	17 (16,19)	17 (15,18)	17 (16,19)	<0.0001[2]
Fasting blood samples [%]	16.5	21.9	18.4	0.0032[1]
n-3 PUFA intake [mg/MJ]	0.14 (0.13,0.16)	0.14 (0.13,0.16)	0.14 (0.13,0.16)	0.5950[2]
Total energy intake [MJ]	8237 (6900,9907)	8198 (6654,9906)	8231 (6824,9906)	0.4012[2]
Total cholesterol [mmol/L]	4.81 (4.28,5.39)	4.75 (4.30,5.23)	4.79 (4.28,5.32)	0.0422[2]
HDL [mmol/L]	1.20 (1.02,1.41)	1.33 (1.15,1.50)	1.24 (1.06,1.44)	<0.0001[2]
LDL [mmol/L]	2.14 (1.73,2.58)	2.08 (1.72,2.45)	2.12 (1.72,2.53)	0.0225[2]
Triglyceride [mmol/L]	1.24 (0.94,1.67)	1.11 (0.83,1.58)	1.19 (0.90,1.64)	<0.0001[2]

[1]p-value derived from Fisher's exact test.
[2]p-value derived from Wilcoxon rank sum test.
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although again, the lack of statistical significance is likely attributable to the reduced sample size (Figure S1).

There was no significant interaction between n-3 PUFA intake and *FADS1 FADS2* genotype for any of the tested lipid concentrations (data not shown).

Discussion

The present study investigated the association between n-3 PUFA intake and *FADS* genotype with total cholesterol, HDL, LDL and triglyceride concentrations in 10-year-old children from the GINIplus and LISAplus birth cohort studies.

Although not all associations were statistically significant after adjustment for multiple testing, in these children, a higher n-3 PUFA intake was associated with higher total cholesterol, HDL and LDL and lower triglyceride levels. These associations remained significant after adjustment for multiple testing for HDL (five out of six tested SNPs) and triglyceride concentrations (rs3834458).

Minor alleles of *FADS1* and *FADS2* SNPs were significantly associated with higher levels of triglycerides and lower levels of total cholesterol, HDL, and LDL levels. However, not all of these associations reached statistical significance after correcting for multiple testing.

Generally, our results on trends in children are in line with previously published GWA studies that report an association between the minor allele of the tested *FADS* variant with lower total cholesterol [11], LDL [11–13], HDL [13,14] and higher triglyceride [14,15] concentrations in adults. Although the sample size of our cohort is relatively large, failure to reach statistical significance for all SNPs after correcting for multiple testing may be due to a lack of statistical power and small effect sizes. Nevertheless, our effect sizes for total cholesterol and triglycerides are comparable to those reported for adults [11,14]. Bokor et al. [24] investigated the association between *FADS* haplotypes and lipid levels in adolescents, and reported no associations between any of the haplotypes and total cholesterol, HDL or LDL. However, the haplotype carrying the minor allele of rs174546 was significantly associated with higher triglyceride levels and the effect size reported is similar to that observed in our study.

The lack of statistical significance with respect to triglyceride levels in homozygous minor allele carriers may be due to the small size of this group (7% to 12% of the complete sample).

The inclusion of the *FADS* SNPs to the model lead to an increase of explained variance ranging from 0.48% for the triglyceride model to 1.28% for the LDL model, which showed a similar level as reported by several studies [25].

Table 2. Characteristics of the SNPs in the *FADS* gene cluster.

SNP	Alleles	N	Number of subjects with				
	(major/minor)		genotype (%)			allele (%)	
	A/a		aa	Aa	AA	a	A
rs174545	G/C	1829	211 (12%)	793 (43%)	825 (45%)	1215 (33%)	2443 (67%)
rs174546	G/A	1854	211 (11%)	804 (43%)	839 (45%)	1226 (33%)	2482 (67%)
rs174556	G/A	1849	165 (9%)	758 (41%)	926 (50%)	1088 (29%)	2610 (71%)
rs174561	A/G	1867	165 (9%)	766 (41%)	936 (50%)	1096 (29%)	2638 (71%)
rs174575	C/G	1974	139 (7%)	738 (37%)	1097 (56%)	1016 (26%)	2932 (74%)
rs3834458	T/DEL	1971	216 (11%)	855 (43%)	900 (46%)	1287 (33%)	2655 (67%)

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Table 3. Median total cholesterol, HDL, LDL and triglyceride concentrations [mmol/L] in with 25%- and 75%-quantiles stratified by *FADS* genotype (A: major allele/ a: minor allele).

	Total cholesterol	HDL	LDL	Triglyceride
rs174545				
AA	4.81 (4.29,5.34)	1.27 (1.06,1.47)	2.13 (1.74,2.57)	1.14 (0.87,1.57)
Aa	4.77 (4.28,5.30)	1.23 (1.06,1.42)	2.13 (1.74,2.51)	1.23 (0.93,1.67)
aa	4.68 (4.18,5.16)	1.26 (1.09,1.43)	2.01 (1.67,2.47)	1.23 (0.92,1.69)
p-value[1]	0.1189	0.2246	0.0718	**0.0058**
rs174546				
AA	4.82 (4.29,5.34)	1.27 (1.07,1.47)	2.13 (1.74,2.57)	1.14 (0.88,1.57)
Aa	4.77 (4.28,5.31)	1.23 (1.06,1.42)	2.13 (1.74,2.51)	1.24 (0.94,1.68)
aa	4.66 (4.18,5.15)	1.26 (1.09,1.43)	2.01 (1.67,2.47)	1.22 (0.92,1.68)
p-value[1]	0.0793	0.1494	0.0701	**0.0078**
rs174556				
AA	4.83 (4.30,5.38)	1.27 (1.07,1.47)	2.14 (1.74,2.58)	1.15 (0.88,1.58)
Aa	4.76 (4.28,5.30)	1.23 (1.06,1.41)	2.12 (1.73,2.49)	1.23 (0.94,1.69)
aa	4.65 (4.18,5.08)	1.25 (1.09,1.43)	1.98 (1.65,2.38)	1.22 (0.90,1.68)
p-value[1]	0.0263	0.0842	**0.0182**	**0.0071**
rs174561				
AA	4.83 (4.30,5.38)	1.27 (1.07,1.47)	2.14 (1.74,2.59)	1.15 (0.88,1.58)
Aa	4.76 (4.28,5.29)	1.23 (1.06,1.42)	2.12 (1.73,2.49)	1.23 (0.94,1.69)
aa	4.65 (4.18,5.11)	1.25 (1.09,1.43)	2.00 (1.65,2.38)	1.25 (0.92,1.68)
p-value[1]	0.0288	0.1042	**0.0145**	**0.0076**
rs174575				
AA	4.80 (4.28,5.32)	1.26 (1.06,1.46)	2.12 (1.73,2.53)	1.15 (0.88,1.57)
Aa	4.80 (4.31,5.35)	1.23 (1.06,1.43)	2.16 (1.75,2.54)	1.25 (0.93,1.72)
aa	4.63 (4.18,5.08)	1.24 (1.04,1.41)	1.96 (1.68,2.36)	1.24 (0.98,1.70)
p-value[1]	0.0390	0.3346	0.0325	**0.0032**
rs3834458				
AA	4.81 (4.30,5.35)	1.26 (1.06,1.47)	2.13 (1.73,2.56)	1.15 (0.88,1.58)
Aa	4.79 (4.28,5.33)	1.23 (1.06,1.42)	2.16 (1.75,2.53)	1.24 (0.94,1.68)
aa	4.65 (4.23,5.14)	1.25 (1.06,1.43)	2.01 (1.68,2.40)	1.22 (0.94,1.69)
p-value[1]	0.0434	0.2505	0.0414	**0.0081**

[1]p-value derived from Kruskal-Wallis rank sum test. Significance level after correction for multiple testing: $\alpha_{corr} = 0.025$. Values reaching significance after adjustment for multiple testing are highlighted in bold.

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Lipid levels in children are determinants for cardiovascular diseases in adulthood [5–7]. Analysis of the underlying causes for disturbances in lipid metabolism during childhood can contribute to the prevention of cardiovascular diseases later in life. Our study suggests that the *FADS1 FADS2* gene cluster may influence lipid levels in early life.

The underlying causal biological mechanism between an individual's *FADS* genotype and their lipid concentrations is not

Table 4. Results of linear regression models for total cholesterol, HDL, LDL and triglyceride concentrations, *FADS* genotype (A: major allele/ a: minor allele, reference: homozygous major allele) and n-3 PUFA intake (per IQR increase, IQR (n-3 PUFA) = 0.04 mg/ MJ) adjusted for gender, study centre, age, BMI, fasting status and total dietary energy intake [MJ].

	Total cholesterol			HDL			LDL			Triglycerides		
	MR	95% CI	p-value1	Estimate	Sd	p-value1	MR	95% CI	p-value1	MR	95% CI	p-value1
rs174545	n = 1532			N = 1531			n = 1531			n = 1531		
n-3 PUFA	1.01	(1.00, 1.02)	0.0568	0.02	0.01	**0.0193**	1.01	(1.00, 1.03)	0.1023	0.98	(0.96, 1.00)	0.0841
Aa (ref AA)	1.00	(0.98, 1.02)	0.8311	−0.04	0.02	**0.0214**	1.01	(0.98, 1.04)	0.6525	1.06	(1.02, 1.11)	**0.0063**
aa (ref AA)	0.98	(0.95, 1.00)	0.0909	−0.01	0.02	0.6368	0.97	(0.92, 1.02)	0.1758	1.05	(0.97, 1.12)	0.2148
rs174546	n = 1554			n = 1553			n = 1553			n = 1553		
n-3 PUFA	1.01	(1.00, 1.02)	0.0823	0.02	0.01	**0.0147**	1.01	(1.00, 1.03)	0.1072	0.98	(0.95, 1.00)	0.0413
Aa (ref AA)	1.00	(0.98, 1.02)	0.9721	−0.04	0.02	**0.0113**	1.01	(0.97, 1.04)	0.7541	1.07	(1.02, 1.11)	**0.0048**
aa (ref AA)	0.97	(0.95, 1.00)	0.0592	−0.01	0.02	0.6663	0.97	(0.92, 1.02)	0.1777	1.03	(0.96, 1.11)	0.3621
rs174556	n = 1548			n = 1547			n = 1547			n = 1547		
n-3 PUFA	1.01	(1.00, 1.02)	0.0884	0.02	0.01	**0.0173**	1.01	(1.00, 1.03)	0.1075	0.98	(0.95, 1.00)	0.0363
Aa (ref AA)	1.00	(0.98, 1.01)	0.6460	−0.04	0.02	**0.0074**	0.99	(0.96, 1.03)	0.7434	1.07	(1.02, 1.12)	**0.0035**
aa (ref AA)	0.96	(0.93, 0.99)	**0.0093**	0.00	0.03	0.8902	0.94	(0.88, 0.99)	**0.0179**	1.02	(0.94, 1.10)	0.6769
rs174561	n = 1564			n = 1563			n = 1563			n = 1563		
n-3 PUFA	1.01	(1.00, 1.02)	0.0577	0.02	0.01	0.0306	1.01	(1.00, 1.03)	0.0790	0.98	(0.96, 1.00)	0.0533
Aa (ref AA)	1.00	(0.98, 1.01)	0.7075	−0.04	0.02	**0.0094**	1.00	(0.96, 1.03)	0.8039	1.07	(1.02, 1.11)	**0.0041**
aa (ref AA)	0.96	(0.93, 0.99)	**0.0107**	0.00	0.03	0.9700	0.94	(0.89, 0.99)	**0.0207**	1.02	(0.95, 1.11)	0.5278
rs174575	n = 1662			n = 1661			n = 1661			n = 1661		
n-3 PUFA	1.01	(1.00, 1.02)	0.0290	0.02	0.01	**0.0140**	1.02	(1.00, 1.03)	0.0411	0.97	(0.95, 1.00)	0.0258
Aa (ref AA)	1.01	(1.00, 1.03)	0.1070	−0.01	0.01	0.3318	1.03	(0.99, 1.06)	0.1020	1.07	(1.02, 1.11)	**0.0028**
aa (ref AA)	0.98	(0.95, 1.02)	0.2949	−0.01	0.03	0.6907	0.97	(0.91, 1.03)	0.2963	1.07	(0.99, 1.16)	0.0922
rs3834458	n = 1659			n = 1658			n = 1658			n = 1658		
n-3 PUFA	1.01	(1.00, 1.02)	0.0288	0.02	0.01	**0.0126**	1.02	(1.00, 1.03)	0.0422	0.97	(0.95, 1.00)	**0.0219**
Aa (ref AA)	1.00	(0.98, 1.02)	0.9479	−0.03	0.01	0.0324	1.01	(0.98, 1.04)	0.6232	1.06	(1.02, 1.11)	**0.0065**
aa (ref AA)	0.97	(0.95, 1.00)	0.0704	−0.01	0.02	0.6009	0.97	(0.92, 1.01)	0.1674	1.04	(0.98, 1.12)	0.2130

[1]Significance level after correction for multiple testing: $\alpha_{corr} = 0.025$. Values reaching significance after adjustment for multiple testing are highlighted in bold.
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entirely clear. It is likely that the composition of polyunsaturated fatty acids in human tissues, which has been shown to be highly associated with the *FADS* genotype [17], is the direct link between the observed associations. Tanaka et al. [26] presumed that higher concentrations of the precursor fatty acids in minor allele carriers may result in increased membrane fluidity, and thus, in lower LDL. In addition to altered membrane fluidity, differential concentrations of long-chain PUFAs (LC-PUFAs) may lead to a change in the activation of transcription factors such as peroxisome proliferator activating receptor alpha (PPARA). Endogenous LC-PUFAs are natural ligands of PPARA [27], whose activation has been shown to influence the expression of apo-lipoproteins (e.g. ApoAI, Apo-AII, and ApoCIII) and enzymes (lipoprotein lipase) that are involved in the metabolism of lipoprotein particles [28–31].

The effect of *FADS* genotypes on fatty acid levels, which are also influenced by nutrition, leads to further interesting questions. One of which is whether there is a concerted interaction effect between *FADS* genotypes and PUFA intake on lipid levels. To date, few studies have dealt with this issue. Lu et al. [32] reported an association between *FADS* genotype and lipid levels, but only in groups with a high n-3 or n-6 PUFA intake. This result suggests an interaction between genotype and fatty acid intake, although a complete interaction analysis was not performed in this study. In our study, a higher intake of n-3 PUFA was associated with higher total cholesterol, HDL and LDL levels and lower triglyceride concentrations, although these effects were not significant after adjustment for multiple testing. We did not find any association between lipid levels, *FADS* genotype and dietary n-6 PUFA intake (data not shown). In our interaction analysis, we did not find a modification of the effect of n-3 PUFA intake on lipid concentrations in blood by the *FADS* genotype.

Additionally, the number of multiple comparisons has to be considered. In our manuscript, 24 hypotheses are investigated (6 SNPs and 4 traits). A correction for multiple testing is necessary, if several independent hypotheses are tested simultaneously. In the present manuscript, neither the SNPs, nor traits are independent. A more stringent approach, which would account for the number of outcome variables, would lead to a corrected alpha level of 0.0063. Taking this alpha level as a basis, the association between individuals carrying the heterozygous genotype and triglyceride levels compared to homozygous major allele carriers remains significant for five out of six SNPs. However, the reported associations with each of the selected six SNPs with traits were similar, possibly due to high correlation. A chance finding would be obvious, if just one of the tested SNPs were highly statistical significant, where the others were not. This is not the case in our

analyses. Therefore, we do not consider these results as chance finding.

Nevertheless, our results suggest that there is an effect of n-3 PUFA intake as well as an effect of *FADS* genotype on lipid levels, although these effects do not interact with each other. It is known, that the conversion rate from dietary PUFA intake to longer chain metabolites is depending on the *FADS* genotype [17]. Therefore, it might be possible that dietary n-3 PUFA intake as well as the *FADS* genotype have a linear influence on the endogenous PUFA levels. This would be in line with the results presented by Moltó-Puigmartí et al. [22]. They reported lower DHA proportions in plasma phospholipids in women carrying the homozygous minor allele, but the DHA proportions increased with higher intake of fatty fish to a similar extent for all genotypes.

To our knowledge, this is the first study which has examined the complex associations between genetics, diet, and lipid levels in 10 year old children.

In addition to its strength, our study also faces some limitations which must be considered. Although lipid levels were measured from blood samples, the dietary fatty acid intake was assessed by a FFQ. The FFQ used in the present study measured dietary intake over the past 12 months and was validated for the dietary n-3 PUFA intake. Alternatively, it might be interesting to examine the association of *FADS* genes, lipid concentrations and measured fatty acid blood levels. A further limitation of our study is the low proportion of fasting blood samples (18.4%). The presented results were adjusted for fasting status. Additionally, the magnitude of the association between *FADS* genotype, n-3 PUFA and lipid concentration was similar when the sample was restricted to those with information on fasting blood, although statistical significance was lacking due to a reduced sample size (Results for triglyceride levels are presented in Table S1).

In order to increase the power, the two independent studies GINIplus and LISAplus were analyzed together. Additional analyses stratified for the GINIplus and LISAplus studies showed consistent results (Figure S1), except for LDL, although significance was missed due to the reduced sample size. This investigation addressed a complex hypothesis and is based on a specific data situation: Dietary fatty acid intake was assessed using a FFQ, which was especially developed and validated for dietary fatty acid intake in this specific study population [33], blood lipid levels of total cholesterol, HDL, LDL and triglycerides were measured and genotyping of six variants of the *FADS1 FADS2* gene cluster was performed. Nevertheless, the results in the two independent studies GINIplus and LISAplus, which are based on the same methodology, are similar and our results are comparable to those reported for adults.

Our study suggests that the *FADS1 FADS2* gene cluster may affect lipid levels already in childhood. Although the explained variance is low, and can therefore not be used for prevention or prediction purposes, these results underline the hypothesis that there is a causal association between dietary n-3 PUFA intake and lipid levels in children and may help to identify the causal biological mechanism.

However, further studies are needed to investigate the long-term effects of the impact of dietary intervention on the development of cardiovascular diseases, while considering the influence of the *FADS* gene cluster.

Materials and Methods

Study population

Data from two ongoing German birth cohort studies were included in this investigation: the German LISAplus (Life-style Related Factors on the Immune System and the Development of Allergies in Childhood PLUS the influence of traffic emissions and genetics) and GINIplus (German Infant Nutritional Intervention PLUS environmental and genetic influences on allergy development) studies. LISAplus is a population based birth cohort study in which a total of 3097 neonates were recruited between 1997 and 1999 from the German cities of Munich, Leipzig, Wesel and Bad Honnef. The participants were not pre-selected based on family history of allergic diseases [34]. A total of 5991 mothers and their newborns were recruited from Munich and Wesel into the GINIplus study between September 1995 and June 1998. Infants with at least one allergic parent and/or sibling were allocated to the interventional study arm which investigated the effect of different hydrolysed formulas consumed during the first year of life on the development of allergies [35]. All children without a family history of allergic diseases and children whose parents did not give consent for the randomized clinical trial were allocated to the non-interventional arm. Given that the entire current GINIplus study is composed of both the interventional and non-interventional arm, this cohort is also population-based. Detailed descriptions of the LISAplus and GINIplus studies have been published elsewhere [34–36]).

In both studies only individuals with Caucasian German descent were included.

For this analysis, only data from the 10 year follow-up is used. During this follow-up, blood samples were collected and in a subset of almost 20% of the children fasting blood samples could be collected.

For both studies, approval by the local Ethics Committees (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia) and written consent from participant's families were obtained.

Dietary n-3 PUFA intake

A food frequency questionnaire (FFQ) was developed to measure a child's usual food and nutrient intake during one year, and more specifically, to estimate energy, fatty acid and antioxidant intake at 10 years of age. The FFQ comprised a list of 82 food items accompanied by several questions about the preferred fat and energy content of products, preparation methods, diet and food preferences, buying habits and dietary supplement use. The consumption frequencies and portion size estimates were converted to average consumption in grams per day and linked to the German Nutrient Data Base, version II.3.1 [37]. The design of the FFQ, including the selection of the food item list, validation, and the calculation of food and nutrient intake is described in more detail by Stiegler et al. [33].

The intake of n-3 PUFA was calculated by summing a child's daily intake of α-linolenic acid (ALA, 18: 3n-3), eicosapentaenoic acid (EPA, 20: 5n-3), docosapentaenoic acid (DPA, 22: 5n-3) and docosahexaenoic acid (DHA, 22: 6n-3).

Genotyping

Six single nucleotide polymorphisms (SNPs) in the *FADS1 FADS2* gene cluster (rs174545, rs174546, rs174556, rs174561, rs174575 and rs3834458) were genotyped. Five of these variants (rs174545, rs174546, rs174556, rs174561 and rs3834458) are in strong linkage disequilibrium (LD) with each other ($r^2>0.7$, $D'>0.9$) [17], and were selected based on previous publications in adult populations [17,38]. Additionally, we included the rs174575 SNP in order to obtain a better coverage of the *FADS* gene cluster. This SNP was selected based on a previous publication in children [39]. By applying the tagger server program (http://www.broadinstitute.org/mpg/tagger/) in combination with HapMap

(http://hapmap.ncbi.nlm.nih.gov/), we were able to tag 27 additional SNPs between base pair positions 61234329 and 61372379 in the *FADS1 FADS2* gene cluster using three of our original SNPs (rs174545, rs174546 and rs174556). The efficiency was 10.7 fold, however of these new 27 SNPs, rs174561 and rs3834458 could not be used in the analysis as these are not included in the HapMap database. Genotyping of SNPs was conducted using the iPLEX (Sequenom, San Diego, CA, USA) method by means of matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Mass Array; Sequenom) in one laboratory, according to the manufacturer's instructions. Standard genotyping quality control included 10% duplicate and negative samples. The genotyping discordance rate was below 0.3%.

Measurement of lipids

The measurement of serum lipids and lipoproteins was performed using homogenous enzymatic colorimetric methods according to the manufactures instructions (Roche Diagnostics GmbH Mannheim). All parameters and controls were analysed on a Modular Analytics System from Roche Diagnostics GmbH Mannheim.

External controls were used in accordance with the guidelines of the German Society of Clinical Chemistry and Laboratory Medicine.

Statistical analysis

Because of the skewed distribution, total cholesterol, LDL and triglyceride concentrations were naturally log-transformed. Afterwards, linear regression modelling was used to assess the association between log-transformed total cholesterol, HDL, log-transformed LDL and log-transformed triglyceride concentrations, n-3 PUFA intake and *FADS* genotype. Therefore, for HDL, the regression coefficient β (Estimate) with standard deviation (Sd) is shown.

For total cholesterol, LDL and triglyceride, the results are presented as means ratio (MR) with 95% confidence interval (95% CI). The MR can be easily calculated for lognormal distributed variables and is derived by applying the exponential function on the regression coefficient β (i.e. $MR = \exp(\beta)$). It describes the ratio of the mean of the outcome variable in one group compared to the mean of the outcome variable in the reference group. The MR can be interpreted as percentage change in the mean of the outcome variable in one group compared to the reference group adjusted for confounder variables.

The dietary n-3 PUFA intake was included in the regression analyses as nutrient density (n-3 PUFA intake divided by total energy intake). Additionally, the results were adjusted for total energy intake [40]. In order to compare the effect size of high (75% quantile) and low (25% quantile) dietary n-3 PUFA intake, the influence of n-3 PUFA intake is given per interquartile range increase (IQR (n-3 PUFA) = 0.04 mg/MJ).

All regression models were adjusted for gender, study centre (Munich, Wesel, Leipzig and Bad Honnef), age, BMI at 10 years of age and fasting status.

Moreover, in additional analyses, an interaction between n-3 PUFA and *FADS* genotype (reference: homozygous major allele), was included in the linear regression models in order to test whether the effect of dietary n-3 PUFA intake is modified by *FADS* variants.

Statistical significance was defined by a two-sided alpha level of 5%. We corrected for multiple testing according to Nyholt [41]. In brief, this method takes the correlation pattern between the SNPs into account and reduces the number of variables in a set to the effective number of variables and provides thereby an estimate of the number of independent tests.

The alpha level is divided by the number of effective loci (which was computed as two, based on the number of effective loci of the six SNPs in the *FADS* gene cluster), which yields a corrected two-sided alpha level of 0.025 (5%/2 = 2.5%).

Differences between the GINIplus and LISAplus studies were tested using Fisher's exact test or Wilcoxon rank sum test. The association between lipid concentrations and *FADS* genotype was tested using Kruskal-Wallis rank sum test, a nonparametric method to test whether the median of the lipid concentrations is different between the *FADS* genotypes.

Statistical analysis was performed using the statistical software R, version 2.13.1 (http://www.R-project.org) [42].

Supporting Information

Figure S1 Results of linear regression models on total cholesterol, HDL, LDL and triglycerides stratified for the GINIplus and LISAplus studies. Presented are means ratios (total cholesterol, LDL and triglycerides) and effect estimates (HDL) of *FADS* genotype (A: major allele/ a: minor allele, reference: homozygous major allele) and n-3 PUFA intake (per IQR increase, IQR (n-3 PUFA) = 0.04 mg/MJ). All models are adjusted for gender, study centre, age, BMI and total dietary energy intake [MJ]. a) Total cholesterol b) HDL c) LDL d) Triglycerides
(DOC)

Table S1 Results of linear regression models restricted to fasting blood samples for triglyceride concentrations, *FADS* genotype (A: major allele/ a: minor allele, reference: homozygous major allele) and n-3 PUFA intake (per IQR increase, IQR (n-3 PUFA) = 0.04 mg/MJ) adjusted for gender, study centre, age, BMI and total dietary energy intake [MJ].
(DOC)

Table S2 Percentage of variance explained in the models without and with the *FADS* variants.
(DOC)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: SK CPB AvB DB UK B. Schaaf OH JH. Analyzed the data: MS. Wrote the paper: MS. Development of statistical analysis plan: EL JH. Lipid measurement: B. Stach TD JT. Interpretation of the findings: MS EL B. Stach SK CPB AvB DB UK B. Schaaf SR OH AB TD JT BK JH. Read and approved the manuscript: MS EL B. Stach SK CPB AvB DB UK B. Schaaf SR OH AB TD JT BK JH.

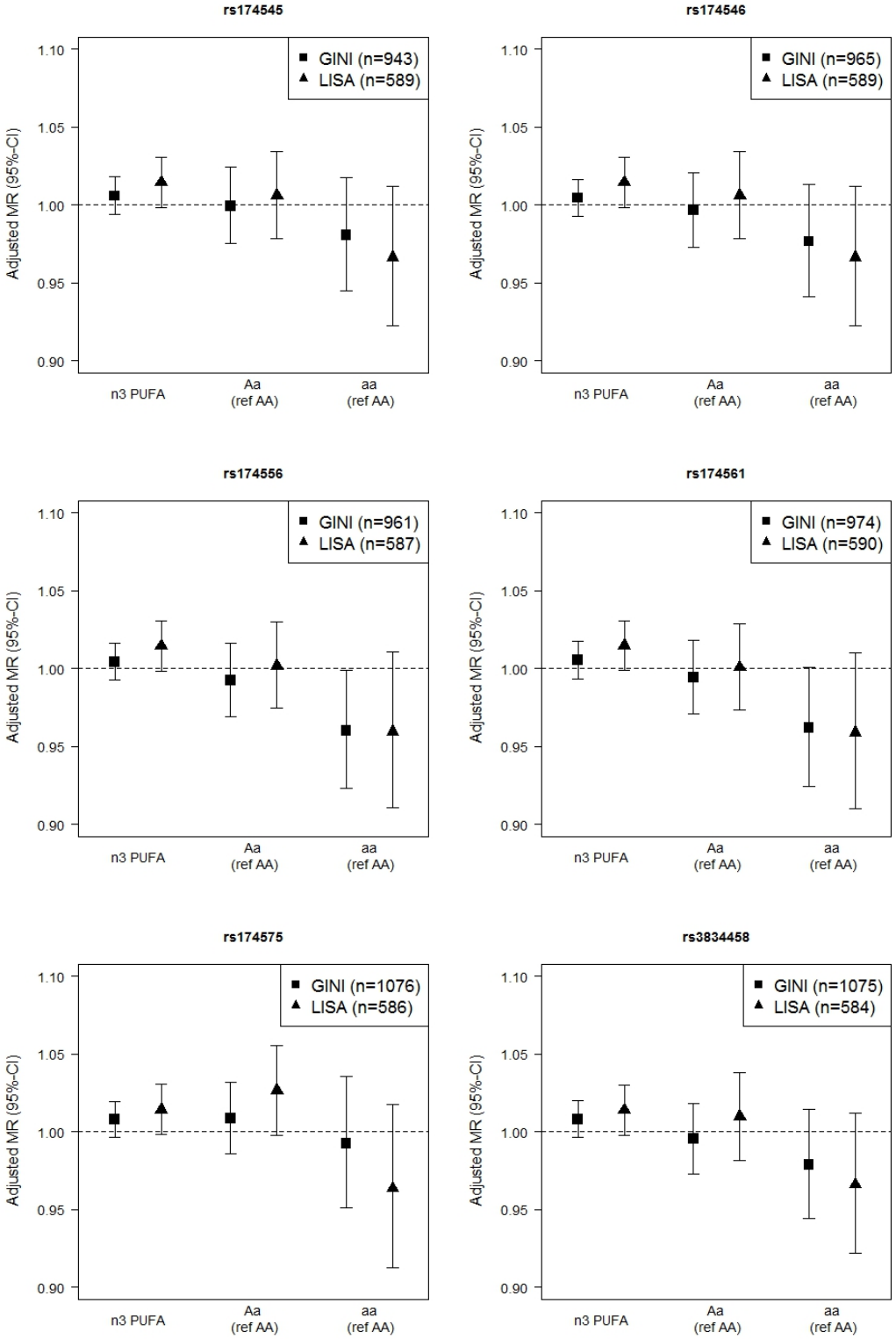
References

1. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J (1961) Factors of risk in the development of coronary heart disease – six year follow-up experience. The Framingham Study. Ann Intern Med 55: 33–50.
2. Miller NE, Miller GJ (1975) Letter: High-density lipoprotein and atherosclerosis. Lancet 1: 1033.
3. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR (1977) High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 62: 707–714.
4. Kareinen A, Viitanen L, Halonen P, Lehto S, Laakso M (2001) Cardiovascular risk factors associated with insulin resistance cluster in families with early-onset coronary heart disease. Arterioscler Thromb Vasc Biol 21: 1346–1352.
5. Newman WP, Freedman DS, Voors AW, Gard PD, Srinivasan SR, et al. (1986) Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis. The Bogalusa Heart Study. N Engl J Med 314: 138–144.
6. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, et al. (1998) Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 338: 1650–1656.
7. McGill HC, McMahan CA, Zieske AW, Sloop GD, Walcott JV, et al. (2000) Associations of coronary heart disease risk factors with the intermediate lesion of atherosclerosis in youth. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Arterioscler Thromb Vasc Biol 20: 1998–2004.
8. Phillipson BE, Rothrock DW, Connor WE, Harris WS, Illingworth DR (1985) Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N Engl J Med 312: 1210–1216.
9. Calabresi L, Villa B, Canavesi M, Sirtori CR, James RW, et al. (2004) An omega-3 polyunsaturated fatty acid concentrate increases plasma high-density lipoprotein 2 cholesterol and paraoxonase levels in patients with familial combined hyperlipidemia. Metabolism 53: 153–158.
10. Mozaffarian D, Micha R, Wallace S (2010) Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. PLoS Med 7: e1000252.
11. Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, et al. (2009) Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat Genet 41: 47–55.
12. Sabatti C, Service SK, Hartikainen AL, Pouta A, Ripatti S, et al. (2009) Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat Genet 41: 35–46.
13. Chasman DI, Paré G, Mora S, Hopewell JC, Peloso G, et al. (2009) Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. PLoS Genet 5: e1000730.
14. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, et al. (2009) Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 41: 56–65.
15. De Silva NMG, Freathy RM, Palmer TM, Donnelly LA, Luan J, et al. (2011) Mendelian randomization studies do not support a role for raised circulating triglyceride levels influencing type 2 diabetes, glucose levels, or insulin resistance. Diabetes 60: 1008–1018.
16. Lattka E, Illig T, Koletzko B, Heinrich J (2010) Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism. Curr Opin Lipidol 21: 64–69.
17. Schaeffer L, Gohlke H, Müller M, Heid IM, Palmer LJ, et al. (2006) Common genetic variants of the *FADS1 FADS2* gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Hum Mol Genet 15: 1745–1756.
18. Xie L, Innis SM (2008) Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. J Nutr 138: 2222–2228.
19. Baylin A, Ruiz-Narvaez E, Kraft P, Campos H (2007) alpha-Linolenic acid, Delta6-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction. Am J Clin Nutr 85: 554–560.
20. Malerba G, Schaeffer L, Xumerle L, Klopp N, Trabetti E, et al. (2008) SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. Lipids 43: 289–299.
21. Lattka E, Rzehak P, Szabó É, Jakobik V, Weck M, et al. (2011) Genetic variants in the *FADS* gene cluster are associated with arachidonic acid concentrations of human breast milk at 1.5 and 6 mo postpartum and influence the course of milk dodecanoic, tetracosenoic, and trans-9-octadecenoic acid concentrations over the duration of lactation. Am J Clin Nutr 93: 382–391.
22. Moltó-Puigmartí C, Plat J, Mensink RP, Müller A, Jansen E, et al. (2010) FADS1 FADS2 gene variants modify the association between fish intake and the docosahexaenoic acid proportions in human milk. Am J Clin Nutr 91: 1368–1376.
23. Koletzko B, Lattka E, Zeilinger S, Illig T, Steer C (2011) Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children. Am J Clin Nutr 93: 211–219.
24. Bokor S, Dumont J, Spinneker A, Gonzalez-Gross M, Nova E, et al. (2010) Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-6 desaturase activities estimated by serum fatty acid ratios. J Lipid Res 51: 2325–2333.
25. Stranger BE, Stahl EA, Raj T (2011) Progress and promise of genome-wide association studies for human complex trait genetics. Genetics 187: 367–383.
26. Tanaka T, Shen J, Abecasis GR, Kisialiou A, Ordovas JM, et al. (2009) Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI Study. PLoS Genet 5: e1000338.
27. Fruchart JC, Duriez P, Staels B (1999) Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. Curr Opin Lipidol 10: 245–257.
28. Hertz R, Bishara-Shieban J, Bar-Tana J (1995) Mode of action of peroxisome proliferators as hypolipidemic drugs. Suppression of apolipoprotein C-III. J Biol Chem 270: 13470–13475.
29. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, et al. (1996) PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J 15: 5336–5348.
30. Vu-Dac N, Schoonjans K, Laine B, Fruchart JC, Auwerx J, et al. (1994) Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element. J Biol Chem 269: 31012–31018.
31. Vu-Dac N, Schoonjans K, Kosykh V, Dallongeville J, Fruchart JC, et al. (1995) Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. J Clin Invest 96: 741–750.
32. Lu Y, Feskens EJ, Dollé ME, Imholz S, Verschuren WM, et al. (2010) Dietary n-3 and n-6 polyunsaturated fatty acid intake interacts with FADS1 genetic variation to affect total and HDL-cholesterol concentrations in the Doetinchem Cohort Study. Am J Clin Nutr 92: 258–265.
33. Stiegler P, Sausenthaler S, Buyken AE, Rzehak P, Czech D, et al. (2010) A new FFQ designed to measure the intake of fatty acids and antioxidants in children. Public Health Nutr 13: 38–46.
34. Heinrich J, Bolte G, Hölscher B, Douwes J, Lehmann I, et al. (2002) Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. Eur Respir J 20(3): 617–623.
35. von Berg A, Koletzko S, Grübl A, Filipiak-Pittroff B, Wichmann HE, et al. (2003) The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. J Allergy Clin Immunol 111(3): 533–540.
36. von Berg A, Krämer U, Link E, Bollrath C, Heinrich J, et al. (2010) Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course – the GINIplus study up to the age of 6 years. Clin Exp Allergy 40(4): 627–636.
37. Hartmann BM, Bell S, Vásquez-Caicedo AL, Götz A, Erhardt J, et al. (2005) Der Bundeslebensmittelschlüssel. German Nutrient Data Base. Karlsruhe.
38. Rzehak P, Heinrich J, Klopp N, Schaeffer L, Hoff S, et al. (2009) Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. Br J Nutr 101: 20–26.
39. Caspi A, Williams B, Kim-Cohen J, Craig IW, Milne BJ, et al. (2007) Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism. Proc Natl Acad Sci U S A 104: 18860–18865.
40. Willett WC, Howe GR, Kushi LH (1997) Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 65: 1220S–1228S; discussion 1229S–1231S.
41. Nyholt DR (2004) A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 74: 765–769.
42. R Development Core Team (2011) R: A Language and Environment for Statistical Computing. Vienna, Austria.

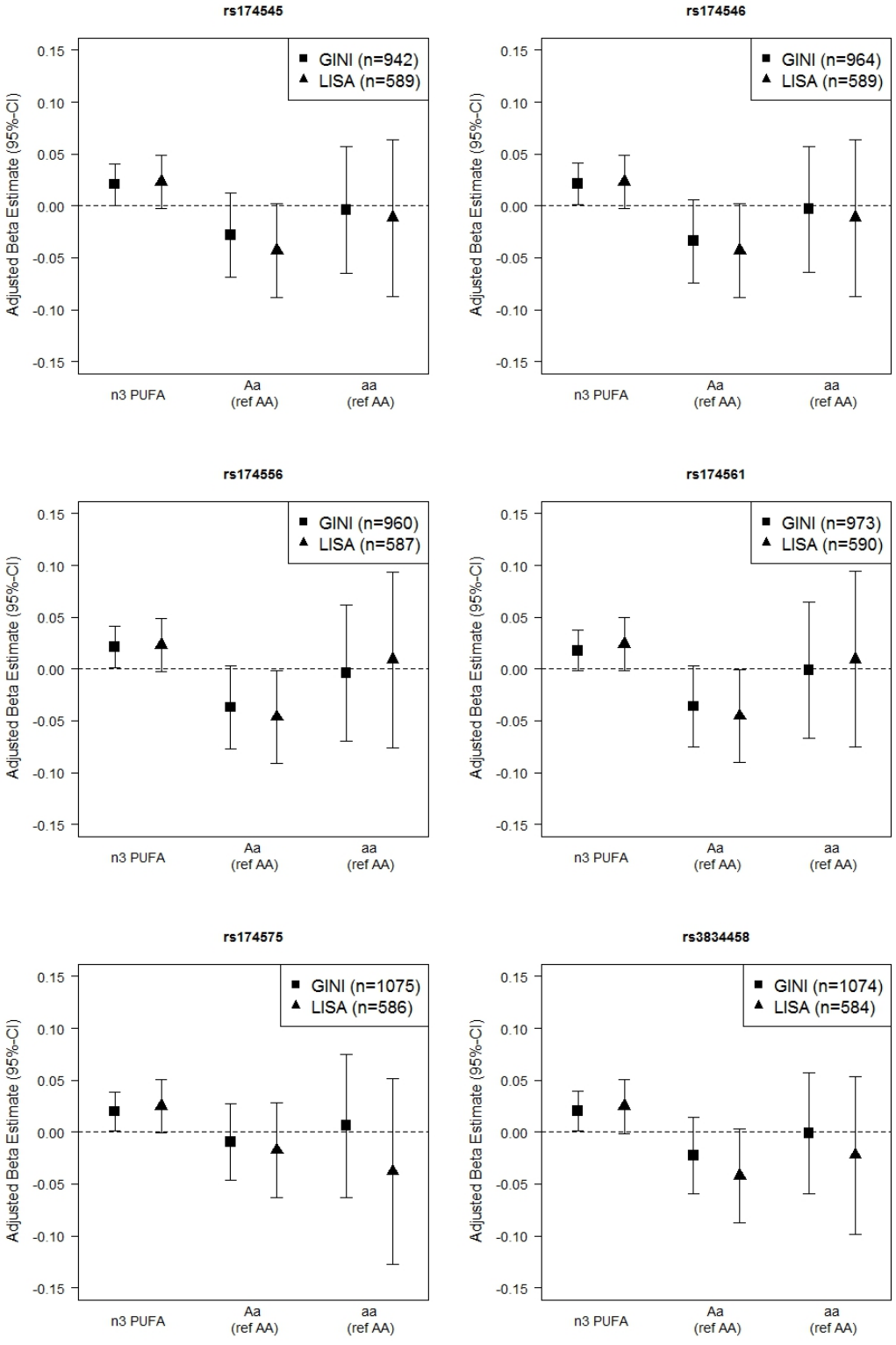
Paper 5: Supplementary Material

Figure S1

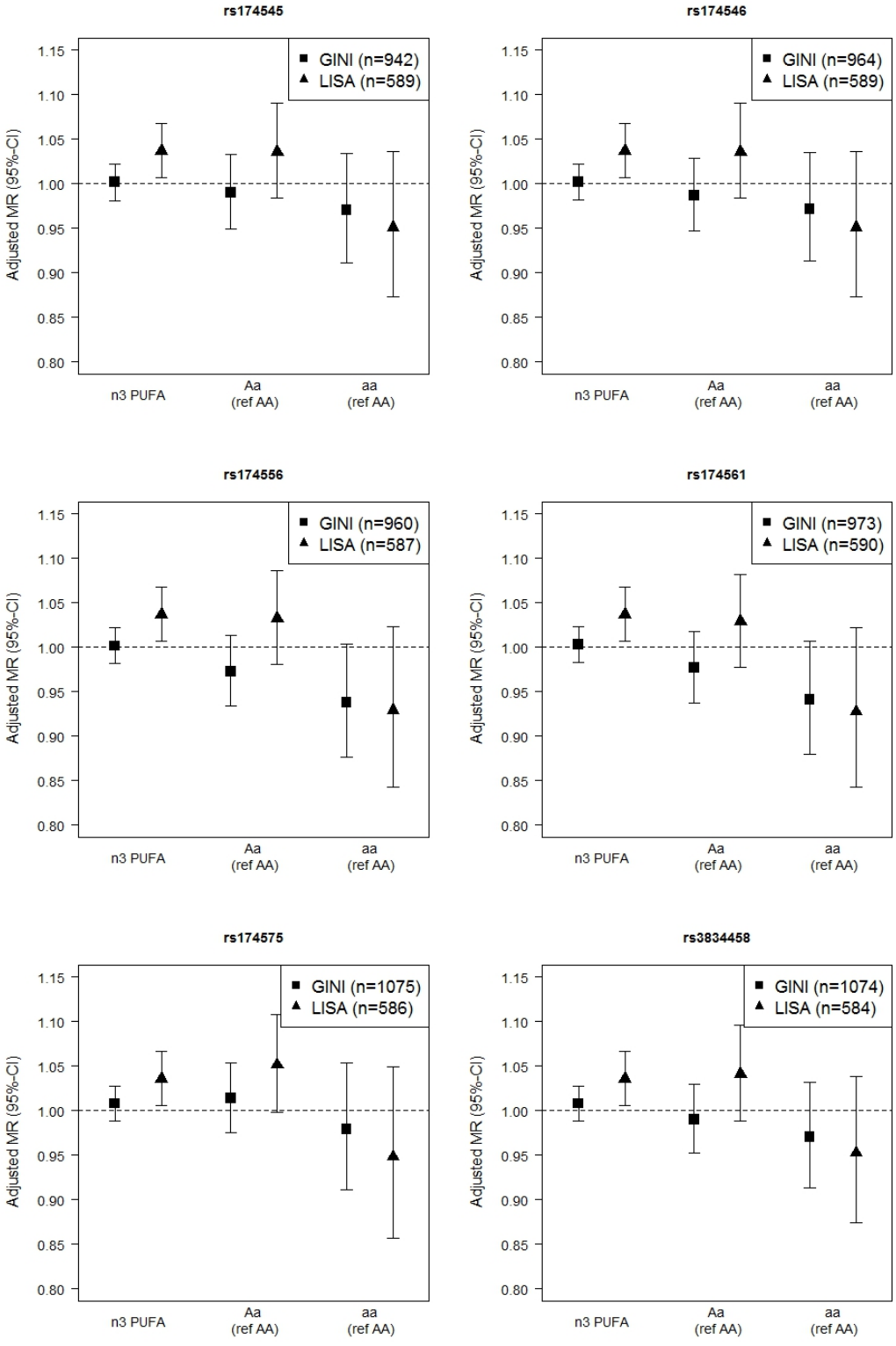
a) Total cholesterol



b) HDL


rs174545
GINI (n=942)
LISA (n=589)
rs174546
GINI (n=964)
LISA (n=589)
rs174556
GINI (n=960)
LISA (n=587)
rs174561
GINI (n=973)
LISA (n=590)
rs174575
GINI (n=1075)
LISA (n=586)
rs3834458
GINI (n=1074)
LISA (n=584)
Adjusted Beta Estimate (95%-CI)
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
n3 PUFA
Aa (ref AA)
aa (ref AA)

c) LDL


rs174545
GINI (n=942)
LISA (n=589)
rs174546
GINI (n=964)
LISA (n=589)
rs174556
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LISA (n=587)
rs174561
GINI (n=973)
LISA (n=590)
rs174575
GINI (n=1075)
LISA (n=586)
rs3834458
GINI (n=1074)
LISA (n=584)
Adjusted MR (95%-CI)
1.15
1.10
1.05
1.00
0.95
0.90
0.85
0.80
n3 PUFA
Aa
(ref AA)
aa
(ref AA)

d) Triglycerides

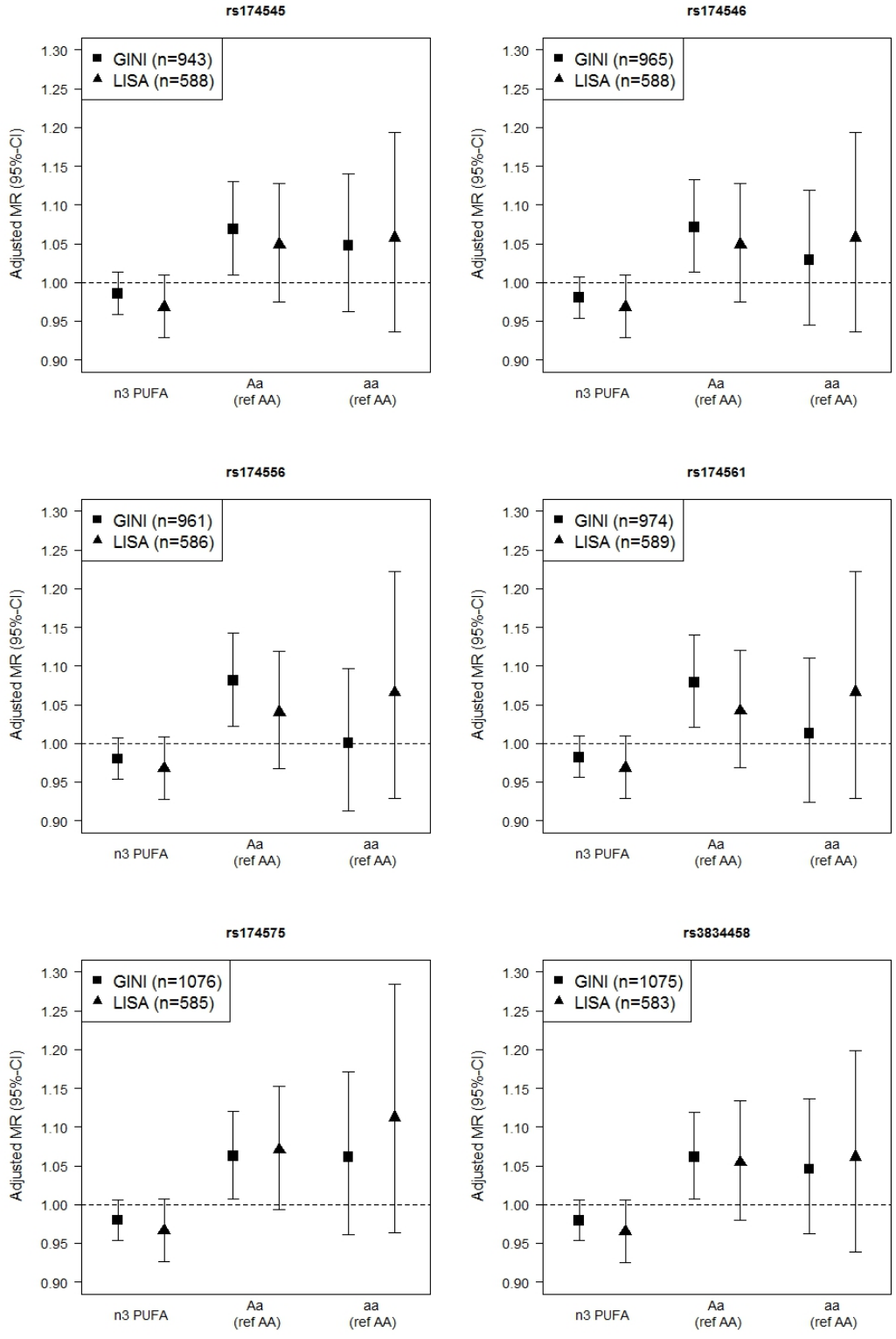

Table S1

	MR	95% CI	p-value
rs174545	n=293		
n-3 PUFA	0.97	(0.94, 1.01)	0.1573
Aa	1.02	(0.93, 1.11)	0.7443
aa	1.09	(0.93, 1.27)	0.2813
rs174546	n=296		
n-3 PUFA	0.97	(0.93, 1.01)	0.1486
Aa	1.02	(0.94, 1.12)	0.6004
aa	1.05	(0.91, 1.23)	0.4927
rs174556	n=296		
n-3 PUFA	0.97	(0.93, 1.01)	0.1429
Aa	1.02	(0.93, 1.12)	0.6167
aa	1.04	(0.88, 1.23)	0.6426
rs174561	n=298		
n-3 PUFA	0.97	(0.93, 1.01)	0.1310
Aa	1.02	(0.93, 1.12)	0.6457
aa	1.04	(0.88, 1.23)	0.6504
rs174575	n=316		
n-3 PUFA	0.97	(0.94, 1.01)	0.1342
Aa	0.99	(0.91, 1.08)	0.8606
aa	1.02	(0.86, 1.21)	0.7950
rs3834458	n=315		
n-3 PUFA	0.97	(0.94, 1.01)	0.1331
Aa	1.03	(0.94, 1.12)	0.5267
aa	1.06	(0.92, 1.23)	0.4059

Table S2

	Total cholesterol	LDL	HDL	Triglycerides
Without SNP	1.98%	4.21%	7.83%	12.38%
rs174545	2.38%	5.26%	8.16%	12.40%
rs174546	2.44%	5.30%	8.45%	12.72%
rs174556	2.53%	5.43%	8.56%	12.86%
rs174561	2.69%	5.49%	8.38%	12.47%
rs174575	2.66%	5.19%	8.59%	12.54%
rs3834458	2.60%	5.09%	8.79%	12.44%
Max. difference	0.71%	1.28%	0.96%	0.48%

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Work experience

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Publications

Publications included in this thesis

M Standl, S Sausenthaler, E Lattka, S Koletzko, C-P Bauer, H-E Wichmann, *et al.* *FADS* gene variants modulate the effect of dietary fatty acid intake on allergic diseases in children. *Clin Exp Allergy*, 2011, 41, 1757–1766.

M Standl, S Sausenthaler, E Lattka, S Koletzko, C-P Bauer, H-E Wichmann, *et al.* *FADS* gene cluster modulates the effect of breastfeeding on asthma. Results from the GINIplus and LISAplus studies. *Allergy*, 2012, 67, 83–90.

L Paternoster*, M Standl*, CM Chen, A Ramasamy, K Bønnelykke, L Duijts, *et al.* Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. *Nat Genet*, 2012, 44, 187–192.
* These authors contributed equally to this work.

S Sausenthaler*, M Standl*, A Buyken, P Rzehak, S Koletzko, C-P Bauer, *et al.* Regional and socio-economic differences in food, nutrient and supplement intake in school-age children in Germany: results from the GINIplus and the LISAplus studies. *Public Health Nutr*, 2011, 14, 1724–1735.
* These authors contributed equally to this work.

M Standl, E Lattka, B Stach, S Koletzko, C-P Bauer, A von Berg, *et al.* *FADS1 FADS2* Gene Cluster, PUFA Intake and Blood Lipids in Children. Results from the GINIplus and LISAplus Studies. *PLoS ONE*, 2012, 7, e37780.

Further publications

P Rzehak, C Thijs, M Standl, M Mommers, C Glaser, E Jansen, *et al.* Variants of the *FADS1 FADS2* gene cluster, blood levels of polyunsaturated fatty acids and eczema in children within the first 2 years of life. *PLoS ONE*, 2010, 5, e13261.

G Kohlboeck, C Glaser, C Tiesler, H Demmelmair, M Standl, M Romanos, *et al.* Effect of fatty acid status in cord blood serum on children's behavioral difficulties at 10 y of age: results from the LISAplus study. *Am J Clin Nutr*, 2011, 94, 1592–1599.

HR Taal, B St Pourcain, E Thiering, S Das, DO Mook-Kanamori, ..., M Standl, ..., *et al.* Common variants at 12q15 and 12q24 are associated with infant head circumference. *Nat Genet*, 2012, 44, 532–538.

Erklärung

Hiermit erkläre ich, Marie Standl, dass ich die vorliegende Dissertation selbstständig angefertigt habe. Ich habe mich außer der angegebenen keiner weiterer Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen. Ich habe bisher noch keinen Promotionsversuch unternommen, und die vorliegende Dissertation wurde nicht in gleicher oder ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht.

München, 10. 07. 2013

Marie Standl