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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 ($\alpha_{corr}$ = 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)

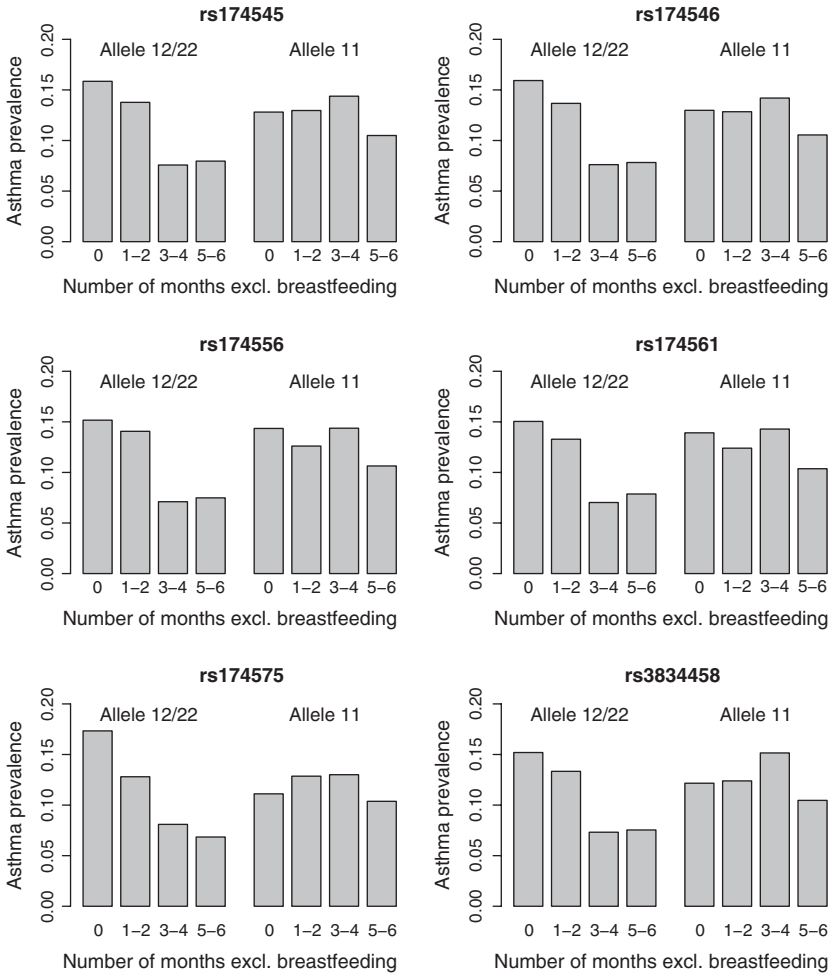


**Figure 1** Asthma prevalence stratified by *FADS* genotype and breastfeeding (1: major allele, 2: minor allele).

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 ($\alpha_{corr}$ = 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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