Human Molecular Genetics, 2018, Vol. 27, No. 3 546–558

doi: 10.1093/hmg/ddx413
Advance Access Publication Date: 27 November 2017
Association Studies Article

ASSOCIATION STUDIES ARTICLE

Genome-wide meta-analysis identifies novel determinants of circulating serum progranulin

Anke Tönjes[1,†], Markus Scholz[2,3,†], Jacqueline Krüger[4], Kerstin Krause[1], Dorit Schleinitz[1], Holger Kirsten[2,3], Claudia Gebhardt[1], Carola Marzi[5,6,7], Harald Grallert[5,6,7], Claes Ladenvall[8], Henrike Heyne[9], Esa Laurila[8], Jennifer Kriebel[5,6,7], Christa Meisinger[6,7], Wolfgang Rathmann[10], Christian Gieger[5,6], Leif Groop[8], Inga Prokopenko[11,12,13], Bo Isomaa[14,15], Frank Beutner[16], Jürgen Kratzsch[16], Antje Fischer-Rosinsky[17], Andreas Pfeiffer[17,18], Knut Krohn[19], Joachim Spranger[17], Joachim Thiery[16], Matthias Blüher[1,4], Michael Stumvoll[1,4] and Peter Kovacs[4,*]

[1]Department of Medicine, University of Leipzig, Leipzig 04103, Germany, [2]Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig 04107, Germany, [3]LIFE Research Center, University of Leipzig, Leipzig 04103, Germany, [4]Leipzig University Medical Center, IFB AdiposityDiseases, University of Leipzig, Leipzig 04103, Germany, [5]Research Unit of Molecular Epidemiology, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg 85764, Germany, [6]German Research Center for Environmental Health, Institute of Epidemiology II, Helmholtz Center Munich, Neuherberg 85764, Germany, [7]German Center for Diabetes Research (DZD e.V.), Neuherberg 85764, Germany, [8]Department of Clinical Sciences, Diabetes and Endocrinology, Lund University and Lund University Diabetes Centre, CRC at Skåne University Hospital, Malmö 20502, Sweden, [9]Institute of Human Genetics, University of Leipzig, Leipzig 04103, Germany, [10]German Diabetes Center, Institute of Biometrics and Epidemiology, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf 40225, Germany, [11]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK, [12]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford OX3 7LE, UK, [13]Department of Genomics of Common Diseases, Imperial College London, London SW7 2AZ, UK, [14]Department of Social Services and Healthcare, Jakobstad 68601, Finland, [15]Folkhälsan Research Centre, Helsinki 00290, Finland, [16]Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig 04103, Germany, [17]Department of Endocrinology, Diabetes and Nutrition, Charité-Universitätsmedizin, Berlin 10117, Germany, [18]Department of Clinical Nutrition, German Institute of Human Nutrition, Nuthetal 14558, Germany and [19]Interdisciplinary Centre for Clinical Research, University of Leipzig, Leipzig 04103, Germany

*To whom correspondence should be addressed at: IFB Adiposity Diseases, University of Leipzig, Liebigstrasse 21, D-04103 Leipzig, Germany. Tel: +49 3419715892; Fax +49 3419715979; Email: peter.kovacs@medizin.uni-leipzig.de

[†]These authors contributed equally.

Received: June 12, 2017. **Revised:** November 3, 2017. **Accepted:** November 22, 2017

Abstract

Progranulin is a secreted protein with important functions in processes including immune and inflammatory response, metabolism and embryonic development. The present study aimed at identification of genetic factors determining progranulin concentrations. We conducted a genome-wide association meta-analysis for serum progranulin in three independent cohorts from Europe: Sorbs ($N = 848$) and KORA ($N = 1628$) from Germany and PPP-Botnia ($N = 335$) from Finland (total $N = 2811$). Single nucleotide polymorphisms (SNPs) associated with progranulin levels were replicated in two additional German cohorts: LIFE-Heart Study (Leipzig; $N = 967$) and Metabolic Syndrome Berlin Potsdam (Berlin cohort; $N = 833$). We measured mRNA expression of genes in peripheral blood mononuclear cells (PBMC) by micro-arrays and performed mRNA expression quantitative trait and expression-progranulin association studies to functionally substantiate identified loci. Finally, we conducted siRNA silencing experiments *in vitro* to validate potential candidate genes within the associated loci. Heritability of circulating progranulin levels was estimated at 31.8% and 26.1% in the Sorbs and LIFE-Heart cohort, respectively. SNPs at three loci reached study-wide significance (rs660240 in *CELSR2-PSRC1-MYBPHL-SORT1*, rs4747197 in *CDH23-PSAP* and rs5848 in *GRN*) explaining 19.4%/15.0% of the variance and 61%/57% of total heritability in the Sorbs/LIFE-Heart Study. The strongest evidence for association was at rs660240 ($P = 5.75 \times 10^{-50}$), which was also associated with mRNA expression of *PSRC1* in PBMC ($P = 1.51 \times 10^{-21}$). *Psrc1* knockdown in murine preadipocytes led to a consecutive 30% reduction in progranulin secretion. In conclusion, the present meta-GWAS combined with mRNA expression identified three loci associated with progranulin and supports the role of *PSRC1* in the regulation of progranulin secretion.

Introduction

Progranulin is an 88kDa precursor protein of the family of secreted granulins involved in angiogenesis, tumorigenesis but also in neurodegenerative and metabolic disease (1). Moreover, progranulin is proposed to be a marker of chronic inflammation in obesity and type 2 diabetes characterized by omental adipose tissue macrophage infiltration (2). Progranulin has been shown to affect plasma lipoprotein metabolism, risk for atherosclerosis and chronic inflammatory status (3–5). However, exact mechanisms linking all these diverse functions have not been elucidated so far.

Progranulin is encoded by the granulin gene (*GRN*) on chromosome 17q. Mutations in *GRN* result in reduced serum progranulin (6) and have been shown to be associated with frontotemporal dementia (FTD) and Alzheimer's disease (7–10). However, even in non-carriers of the *GRN* mutations, there is a marked variation in circulating progranulin concentrations, thus challenging studies aiming at identification of further genetic factors involved in progranulin biology. A previous genome-wide scan in 533 subjects revealed an association of common variation in the *CELSR2/PSRC1/MYBPHL/SORT1* locus on chromosome 1p with serum progranulin concentrations. Functional analyses employing overexpression and knockdown of *SORT1 in vitro* suggested this gene to be the major genetic regulator of serum progranulin levels (11).

Although the above-mentioned genome-wide association study (GWAS) implied a strong genetic impact on circulating progranulin, a sufficiently powered GWAS for serum progranulin concentration in metabolically well characterized cohorts is still lacking. Therefore, we conducted a meta-analysis of GWAS for serum progranulin in three independent cohorts: the Sorbs ($N = 848$) and KORA ($N = 1628$) from Germany and the PPP-Botnia ($N = 335$) from Finland. We further replicated our findings in two independent cohorts from Germany [LIFE-Heart from Leipzig with $N = 967$ and MeSyBePo (Metabolic Syndrome Berlin Potsdam) with $N = 833$]. To identify and elucidate the role of candidate genes in the regulation of progranulin secretion, we performed further *in vitro* and *in vivo* analyses using gene-expression data of peripheral blood mononuclear cells (PBMC). These comprised gene-expression quantitative trait locus mapping (eQTL), gene-expression association analysis and corresponding causal inference. Finally, we employed *in vitro* functional assays to validate potential target genes within the associated loci.

Results

Association of serum progranulin concentrations with obesity and parameters of glucose metabolism in the Sorbs cohort

Serum progranulin concentrations were positively correlated with obesity measures (% of body fat, BMI, WHR), fasting insulin, insulin resistance (HOMA-IR, Stumvoll ISI index) and LDL-cholesterol. Interestingly, serum progranulin concentrations were also positively associated with adipocyte fatty acid binding protein (AFABP) and chemerin levels but not with vaspin and adiponectin concentrations (Table 1). There was no correlation between progranulin levels and renal function (Table 1).

Estimation of the heritability of progranulin concentration

The significant degree of relatedness observed in the Sorbs cohort allowed us to estimate the heritability of progranulin concentration, which was 31.8%, using a mixed-model approach proposed by Amin *et al.* and Aulchenko *et al.* (12,13). A similar heritability was estimated for the LIFE-Heart Study (26.1%).

Genome-wide meta-analysis for association with serum progranulin concentrations

No general inflation of meta-analysis statistics was observed ($\lambda = 1.007$ for fixed effect model, $\lambda = 0.82$ for random effects model). Based on the random effects model, 7 SNPs on a unique locus of chromosome 1p13.3 reached genome-wide significance ($P < 5.0 \times 10^{-8}$) in the meta-analysis of Sorbs ($N = 848$), KORA ($N = 1628$) and PPP ($N = 335$, Table 2). All of these SNPs were consistently associated with $P < 10^{-4}$ in all three cohorts with the same direction of the effect (Supplementary Material, Table S2). The strongest evidence for association was observed for rs660240

within the *Cadherin, EGF LAG Seven-Pass G-Type Receptor 2 (CELSR2)—Proline/Serine-Rich Coiled-Coil 1 (PSRC1)—Myosin Binding Protein H-Like (MYBPHL)* locus ($P = 4.22 \times 10^{-15}$, beta=-0.161, Table 2 and Fig. 2). All other SNPs of this locus with $P < 5.0 \times 10^{-8}$ were in strong to moderate linkage disequilibrium (LD) with rs660240 (minimal $r^2 = 0.69$ in HapMap CEU). When adjusting progranulin levels for rs660240, the SNPs at this locus were no longer significant in the Sorbs cohort (minimal P-value 0.37), i.e. no independent associations of other SNPs were detected at this locus. The results of the subgroup analyses in males and females were consistent with data in the total cohort (data not shown). No significant interaction of rs660240 and sex was observed ($P = 0.78$ in the Sorbs cohort).

Of note, a total of 144 SNPs in 24 regions reached P-values $< 5 \times 10^{-5}$ according to the random effects model. Summary statistics including comprehensive annotation with respect to nearby genes, known eQTL data and links to other GWAS hits can be found in Supplementary Material, Table S2.

Table 1. Association of serum progranulin concentrations with anthropometric parameters and markers of glucose metabolism, serum lipids and renal function in the Sorbs cohort

Trait	P-value	ß
%body fat (adj. age, sex)	**6.85×10^{-3}**	0.059
BMI	**0.056**	0.107
WHR (adj. age, sex)	**1.85×10^{-3}**	0.415
WHR (adj. age, sex, BMI)	**0.012**	0.322
fasting insulin	**0.012**	0.041
HOMA-IR	**5.07×10^{-3}**	0.043
Stumvoll Index 1	0.053	−0.077
Stumvoll Index 2	**6.13×10^{-3}**	−0.129
AUC glucose	**5.39×10^{-5}**	0.145
AFABP4	**0.047**	0.034
chemerin	**9.71×10^{-6}**	0.123
vaspin	0.647	−0.004
adiponectin	0.591	0.014
LDL-cholesterol	**1.36×10^{-3}**	0.100
GFR (MDRD)	0.882	0.008

Associations were assessed in a linear regression model adjusting for age, sex and BMI (except for analysis of BMI itself). HOMA-IR was calculated as published by Matthews *et al.* (46). The Stumvoll Index refers to Stumvoll $ISI_{(0 \text{ and } 120\text{min})}$ and Stumvoll $ISI_{(0 \text{ and } 30 \text{ min})}$ (47). Glomerular filtration rate (GFR) was calculated using the MDRD formula. ß - Beta coefficient of linear regression model.

Replication of association signals

Second stage replication analysis in the LIFE-Heart Study

We replicated the respective top-hits of the above-mentioned 24 loci in 967 independent samples of the LIFE-Heart Study (14). All of these 24 SNPs identified at the first-stage GWAS were either directly measured or imputed with high quality, i.e. no proxies were required. Three SNPs were robustly replicated with P-values $< 1 \times 10^{-5}$ with the same direction of effect as in the meta-GWAS. Statistics of the replicated SNPs are displayed in Table 2. Statistics of all SNPs analysed in LIFE-Heart can be found in Supplementary Material, Table S3. Briefly, replication analysis revealed a clear support of our results at chromosome 1 for the locus *CELSR2-PSRC1-MYBPHL-SORT1* with similar effect sizes as observed for the Sorbs and KORA cohort. In addition, we were able to replicate the hits rs4747197 on chromosome 10q22.1 ($P = 1.4 \times 10^{-6}$) and rs5848 on chromosome 17q21.32 ($P = 5.6 \times 10^{-8}$), both with similar effect sizes as in the initial meta-GWAS (Table 2).

Third stage replication analysis in Berlin cohort (MeSyBePo)

The three SNPs successfully replicated in LIFE-Heart were finally tested in the Berlin cohort applying a significance threshold of 5%/3. All SNPs fulfilled this requirement and the effect sizes were similar to those of LIFE-Heart and the meta-analysis (Table 2 and Supplementary Material, Table S4). A meta-analysis including all 5 studied cohorts (3 discovery + 2 replication cohorts) resulted in a $P = 5.7 \times 10^{-50}$ for rs660240, $P = 2.2 \times 10^{-12}$ for rs4747197 in *CDH23* (Cadherin-Related 23 – Chr. 10q22.1) and $P = 5.3 \times 10^{-14}$ for rs5848 (in *GRN*—Granulin—Chr. 17q21.32) (all random effects model, Table 2 and Fig. 3).

The replicated SNPs explain 19.4% (rs660240: 16.7%, rs4747197: 1.6%, rs5848: 1.0%) of progranulin variance in the Sorbs, i.e. more than 60% of the heritability is covered by these SNPs. Again these findings are supported by the LIFE-Heart Study (total explained variance: 15.0%, rs660240: 10.5%, rs4747197: 1.9%, rs5848: 2.6%, total explained heritability: 57%) and MeSyBePo (total explained variance: 11.7%, rs660240: 10.1%, rs4747197: 0.7%, rs5848: 0.9%, heritability estimates are not available for this study).

Comparison with the GWAS catalogue

In total, we robustly identified three independent genetic associations with progranulin. Comparing these hits with published

Table 2. SNPs associated with circulating progranulin levels at study-wise significance

			rs660240 (Chr.1p13.3: 109817838, eff/non-eff allele: C/T)			rs4747197 (Chr.10q22.1: 73566203, eff/non-eff allele: A/T)			rs5848 (Chr.17q21.32: 39785770, eff/non-eff allele: T/C)		
Stage	Cohort	N	eff AF	beta	P	eff AF	beta	P	eff AF	beta	P
1	Sorbs	848	0.801	0.183	5.01×10^{-31}	0.236	−0.0572	1.07×10^{-4}	0.321	−0.0518	1.08×10^{-3}
1	KORA	1628	0.784	0.126	5.89×10^{-9}	0.251	−0.027	1.82×10^{-1}	0.252	−0.071	4.65×10^{-3}
1	PPP-Botnia	335	0.781	0.185	8.91×10^{-7}	0.327	−0.0705	2.29×10^{-2}	0.195	−0.0489	3.73×10^{-1}
1-Meta			–	**0.161**	**4.22×10^{-15}**	–	**−0.0494**	**8.50×10^{-6}**	–	**−0.0566**	**1.31×10^{-5}**
2[a]	LIFE-Heart	967	0.787	0.162	5.01×10^{-26}	0.232	−0.0661	1.38×10^{-6}	0.292	−0.0779	5.60×10^{-8}
3[b]	MeSyBePo	833	0.784	0.193	6.92×10^{-18}	0.255	−0.0561	1.19×10^{-2}	0.338	−0.0670	3.36×10^{-3}
Combined			–	**0.169**	**5.75×10^{-50}**	–	**−0.0563**	**2.16×10^{-12}**	–	**−0.0665**	**5.29×10^{-14}**

SNP positions are given according to hg19. Effect directions and allele frequencies are given for the effect allele. Effect size corresponds to coefficient of regression model. Chr: chromosome. eff AF: Allele frequency of effect allele. We also present the results of the replication analyses and combined analysis of all cohorts.

[a]Adjusted for age, sex, BMI, fasting status and prevalent coronary artery disease. SNPs were considered as replicated at this stage if $P < 1 \times 10^{-5}$.

[b]Adjusted for age, sex and BMI; a P-value cut-off of 5%/3 was applied accounting for three independent association tests.

GWAS via the GWAS catalogue (downloaded at 2016/06/01) revealed that rs660240 was associated with several phenotypes of lipid metabolism and cardiovascular disease. This SNP was also listed in the first previously reported progranulin GWAS study (11). The SNP rs5848 was found to be in some LD with rs708382 (r^2=0.59) showing an association with platelet count (15). However, the latter association was not replicated so far. In the GWAS catalogue, no correlated GWAS hits were found for rs4747197. All results can be found in Supplementary Material, Table S2.

Gene-expression QTL studies

To further elucidate possible functional roles of the three genetic variants detected, we performed analyses of genome-wide PBMC gene-expression data from micro-arrays in Sorbs and in the LIFE-Heart Study.

EQTL results of $N = 894$ Sorb individuals and $N = 4299$ individuals from the LIFE-Heart Study including comparisons with the literature are shown in Supplementary Material, Table S5. Analysis revealed eight eQTLs reaching genome-wide cis/trans-specific false discovery rate < 5% in at least one cohort and at least nominal significance in the other cohort with the same direction of effect. Of note, all genome-wide significant eQTLs of the smaller Sorbs study were also found in the LIFE-Heart Study. Statistics of these eQTLs are summarized in Table 3. No eQTLs were found for rs5848. Four eQTLs were found for both rs4747197 and rs660240, respectively. The eight eQTLs correspond to six unique genes. Two of these, *PSAP* and *PSRC1*, were represented by two correlated transcripts each [*PSAP*: $r = 0.82$ in Sorbs, $r = 0.63$ in LIFE-Heart, *PSRC1*: $r = 0.62$ in Sorbs, $r = 0.61$ in LIFE-Heart]. The strongest eQTLs of rs660240 and rs4747197 were ILMN_1671843 (*PSRC1*) and ILMN_1783149 (*CDH23*), respectively (Table 3).

In the next step, we analysed the correlation between expression levels of regulated genes and progranulin levels in 784 Sorbs and 882 individuals of LIFE-Heart for which both, gene-expression data and progranulin data were available. Four correlations were detected (Table 4). Results for all eQTL transcripts can be found as Supplementary Material, Table S6.

Whereas a significant inverse correlation of the two *PSRC1* transcripts with progranulin was observed for both cohorts, a significant inverse correlation of *PSAP* and *CDH23* transcripts was only observed in LIFE-Heart. For *PSAP* we observed an opposite trend in Sorbs while for *CDH23* the trend was the same but failed to achieve statistical significance.

Mediation analysis of gene expression

The above-mentioned associations complete four association triangles of the form SNP ⇒ progranulin, SNP ⇒ gene expression, and finally, gene expression ⇒ progranulin. The triangles have been used for Mendelian randomization to establish causal chains of the form SNP ⇒ gene expression ⇒ progranulin facilitating a mechanistic interpretation of the observed associations (Table 4 and Supplementary Material, Table S7). By Mendelian randomization analysis, causal effects of one of the *PSRC1* transcripts ($P = 1.56 \times 10^{-3}$) and the *PSAP* transcript ($P = 4.01 \times 10^{-2}$) were detected in the LIFE-Heart cohort suggesting mechanistic effects of the SNPs rs660240 and rs4747197, respectively. Moreover, *PSRC1* explained part of the progranulin association of rs660240 ($P = 1.19 \times 10^{-3}$). However, no causal effects could be inferred in the Sorbs cohort.

Causal inference of progranulin and related phenotypes

In the Sorbs cohort, we assessed whether the observed relations of progranulin with other obesity or metabolic traits studied in Table 1 are un-confounded, i.e. whether there is a causal link. We performed a Mendelian randomization analysis using rs660240 as instrumental variable. Significant causal relationships were observed for LDL-cholesterol (LDLC) ($P = 1.4 \times 10^{-4}$), WHR ($P = 0.0074$), AUC of glucose ($P = 0.013$) and Stumvoll Index 2 ($P = 0.046$) (Supplementary Material, Table S8). Analysing LDLC in more detail, progranulin explained the observed association of rs660240 with LDLC ($P = 0.0033$) but not vice versa ($P = 0.37$).

Functional analyses of the PSRC1 locus

Based on eQTL and mediation analyses, we validated the most plausible target gene *PSRC1* of the associated variant rs660240 by employing knockdown experiments *in vitro*. Treatment of murine 3T3-L1 preadipocytes with respective siRNA resulted in a >60% reduction in *Psrc1* mRNA expression (Fig. 4). *Psrc1* knockdown led to a consecutive reduction in progranulin secretion of approximately 30% (Fig. 4).

Discussion

In the present study, we performed a comprehensive genome-wide meta-analysis of progranulin serum concentrations in three cohorts comprising a total sample size of 2811 individuals. Top-hits were replicated in two independent cohorts totaling 1, 800 samples. By this approach, we identified three genetic loci (*CELSR2-PSRC1-MYBPHL-SORT1*, *CDH23-PSAP*, *GRN*) showing

Table 3. eQTLs of PBMCs in Sorbs and the LIFE-Heart Study

				Sorbs ($N = 894$)		LIFE-Heart ($N = 4, 299$)	
SNP*	ILMN-ID	Gene	cis/trans	beta	P value	beta	P value
rs660240	ILMN_1671843	*PSRC1*	Cis	−0.106	7.46×10^{-22}	−0.0901	1.96×10^{-77}
rs660240	ILMN_1696003	*GNAI3*	Cis	−0.0568	1.30×10^{-4}	−0.0475	1.59×10^{-10}
rs660240	ILMN_2100085	*WDR47*	Cis	−0.0412	1.49×10^{-3}	−0.0208	3.42×10^{-4}
rs660240	ILMN_2315964	*PSRC1*	Cis	−0.0878	3.99×10^{-21}	−0.0714	8.80×10^{-67}
rs4747197	ILMN_1698038	*FAM188B*	Trans	0.0307	1.90×10^{-7}	0.0208	1.11×10^{-15}
rs4747197	ILMN_1749109	*PSAP*	Cis	−0.0391	4.29×10^{-4}	−0.0158	1.08×10^{-5}
rs4747197	ILMN_1783149	*CDH23*	Cis	0.126	2.33×10^{-13}	0.288	6.40×10^{-167}
rs4747197	ILMN_2355559	*PSAP*	Cis	−0.0252	2.13×10^{-2}	−0.0199	3.85×10^{-8}

Same effect alleles as in Table 2 were used.

robust associations with comparable effect sizes among all studies and combined P-values $<2\times10^{-12}$. To unravel functional mechanisms, GWAS analyses were accompanied by genome-wide gene-expression analyses and corresponding mediation analyses. We found evidence for at least one causal chain where the SNP-effect is partly mediated by the expression of the gene. Finally, we performed functional studies in cell cultures which supported the role of *PSRC1* in the regulation of progranulin secretion.

Progranulin is a secreted protein with important functions in several processes; including immune and inflammatory response and embryonic development (16). It is implicated in various disease states such as cancers (17), neurodegenerative diseases (18) and rheumatoid arthritis (19). It has also been shown that serum progranulin is an adipokine induced by TNF-α and dexamethasone which is increased in blood and adipose tissue of obese mouse models and which can be normalized with treatment of insulin-sensitizing agent pioglitazone (20). Consistently, serum progranulin concentrations are increased in patients with type 2 diabetes (2). Our GWAS confirmed the *CELSR2-PSRC1-MYBPHL-SORT1* locus carrying polymorphisms significantly associated with circulating progranulin with rs660240 as the strongest hit. The previous GWAS in 533 subjects by Carrasquillo *et al.* identified rs646776 near the sortilin gene (*SORT1*) as a regulator of progranulin levels in humans (11). The authors supported their finding *in vitro* by showing that knockdown of *SORT1* significantly increased extracellular progranulin levels in HeLa cells. Moreover, rs660240 is an eQTL of *SORT1* in the liver (21). Rs646776 is in strong LD with our top-hit rs660240 ($r^2>0.99$). Therefore, our finding is consistent with the published GWAS with respect to the identified region. However, our data points to *PSRC1* as additional, possibly independent candidate regulator of circulating progranulin: we found the top-hit rs660240 to be associated with *PSRC1* expression in PBMCs and showed that the gene expression of *PSRC1* is inversely correlated with progranulin. This association partly explained the observed SNP-progranulin association. According to GTEx database, rs660240 associates with the expression of *PSRC1* in brain cortex, esophagus, liver, PBMC, pancreas, skin and testis too (22). We observed a positive correlation of *SORT1* and *PSRC1* gene expression in blood. Yet, *SORT1* gene expression in blood could not explain the SNP-progranulin association in our study. In line with this observation, the correlation of *SORT1* and *PSRC1* is markedly decreased if adjusting for confounders in our model (Supplementary Material, Fig. S1) further supporting an independent functional role of *PSRC1*, which was further addressed in *in vitro* knockdown experiments.

Summarizing these results, we suggest *PSRC1* as another plausible candidate of the observed association of rs660240 with serum progranulin. It is possible that both candidate genes (*PSRC1* and *SORT1*) are relevant in a tissue-specific manner as eQTLs of rs660240 and gene expressions of the genes are markedly different in various tissues (Supplementary Material, Fig. S2). In line with this, *PSRC1* and *SORT1* were described as causally associated with cholesterol levels, obesity, diabetes and atherosclerosis suggested to operate in a conserved subnetwork in transcriptional networks in mouse and human (23). Systematic functional studies targeting genes within this locus are warranted to unravel the contributions of these genes in more detail.

Using the RegulomeDB (24), we further prioritized which of the SNPs at the Chr. 1p31.1 locus that significantly associated with progranulin levels is most likely functionally relevant. According to RegulomeDB, rs12740374, which is in almost perfect LD with rs660240 (r^2=0.996) and also strongly associated with progranulin in our data, is supposed to affect binding of multiple transcription factors, one of them TCF7L2, a well-established genetic risk factor for type 2 diabetes (24). Rs12740374 has also the strongest Eigen-PC score at this locus, a measure describing the functional relevance of an SNP (Supplementary Material, Table S2) (25).

Of note, *PSRC1* encodes a proline-rich protein that is a target for regulation by the tumor suppressor protein p53 (26). The encoded protein plays an important role in mitosis by recruiting and regulating microtubule depolymerases that destabilize microtubules (27,28). It remains to be determined whether progranulin's role in cancer development might be attributed to *PSRC1* as would be suggested by these genetic findings.

Also noteworthy, despite missing genome-wide significance in the discovery meta-analysis, rs4747197 (in *CDH23*) and rs5848 (in *GRN*) showed consistent effects across all studies and were replicated in two independent German cohorts with robust combined P-values of 2.16×10^{-12} and 5.29×10^{-14}, respectively. *GRN* encodes for granulin and is therefore a highly plausible candidate for the observed association. The corresponding SNP showed high values for the Eigen-PC corroborating a potential functional role of the mutation (Supplementary Material, Table S2). No eQTL effect of rs5848 on *GRN* expression was found in blood. The lack of eQTL-effects between the progranulin-associated SNPs and progranulin RNA levels might be attributed to the posttranslational progranulin modifications.

Table 4. Gene-expression association analysis in Sorbs and LIFE-Heart and causal analysis

				Sorbs ($N=784$)				LIFE-Heart ($N=882$)			
SNP	ILMN-ID	gene	cis/trans	beta (GE assoc.)[a]	P val (GE assoc.)[b]	P value (MR)[c]	P value (exp. SNP-effect)[d]	beta (GE assoc.)[a]	P value (GE assoc.)[b]	P value (MR)[c]	P value (exp. SNP-effect)[d]
rs660240	ILMN_1671843	*PSRC1*	cis	−0.157	**1.54×10^{-3}**	6.52×10^{-1}	6.73×10^{-1}	−0.300	**3.50×10^{-9}**	**1.56×10^{-3}**	**1.19×10^{-3}**
rs660240	ILMN_2315964	*PSRC1*	cis	−0.196	**7.77×10^{-4}**	8.86×10^{-1}	5.57×10^{-1}	−0.239	**7.56×10^{-5}**	1.23×10^{-1}	6.41×10^{-2}
rs4747197	ILMN_1749109	*PSAP*	cis	0.0663	1.36×10^{-1}	3.07×10^{-1}	1.61×10^{-1}	−0.130	**4.01×10^{-2}**	**1.07×10^{-2}**	9.68×10^{-1}
rs4747197	ILMN_1783149	*CDH23*	cis	−0.0450	1.35×10^{-1}	4.56×10^{-1}	2.31×10^{-1}	−0.0653	**1.01×10^{-3}**	1.13×10^{-1}	5.63×10^{-2}

Significant associations are displayed in bold.

[a]Beta coefficient of gene-expression association with progranulin.

[b]P-value of gene-expression association with progranulin.

[c]P-value of Mendelian randomization.

[d]P-value of SNP-effect explained by gene expression.

Furthermore, it should be noted that the present study only tested the mRNA expression in PBMC whereas progranulin is prominently expressed also in other metabolically active organs such as adipose tissue or liver, so that potential eQTL-effects cannot be ruled out. Indeed, according to GTEx database, several eQTL-effects of rs5848 on *GRN* expression were observed in other tissues.

The situation for the 10q22.1 locus (rs4747197) is less clear. First, this SNP showed the highest deleteriousness estimate of all associated SNPs of that locus underpinning its possible functional role (25). The SNP maps within the coding sequence of *CDH23*. The strongest eQTL in blood was observed for *CDH23* (29). But another cis regulation (*PSAP*) and a variety of trans regulations were described for this SNP in the literature (30,31). In our data of Sorbs and LIFE-Heart, we confirmed the eQTLs of *CDH23 (cis)*, *PSAP* (cis) and *FAM188B* (trans). Gene expression association analysis revealed an inverse correlation of *PSAP* with progranulin and evidence for causality was found by Mendelian randomization analysis in the LIFE-Heart cohort. This is in line with Zhou *et al.* who proposed *PSAP* as a regulator of progranulin in mice (32) and Nicholson *et al.* showing that *PSAP* influences monomer and oligomer composition of progranulin (33).

In conclusion, the present meta-analysis is the largest GWAS of European cohorts targeting genes involved in progranulin regulation. Whereas the previously reported locus of rs660240 was confirmed already in our discovery meta-analysis, the gene-expression analysis and *in vitro* functional studies added a novel candidate gene (*PSRC1*) here. Two additional loci were established with robust genome-wide significance in the final meta-analysis. While for rs5848 *GRN* is the natural candidate, our eQTL and gene-expression association analyses suggest two plausible candidates for rs4747197, namely *CDH23* as strongest eQTL or *PSAP* because of an inverse causal correlation with serum progranulin. Our data may promote new directions in progranulin research and so, in the pathophysiology of metabolic and neurodegenerative diseases, cancer and inflammation.

Materials and Methods

Subjects

Sorbs cohort

The cohort was recruited from the self-contained Sorbs population in Germany (34–37). Phenotyping included standardized questionnaires for past medical history and family history, measurement of anthropometric data [weight, height, waist-to-hip ratio (WHR)], and an oral glucose tolerance test (OGTT). Glucose was quantified by the Hexokinase method (Automated Analyser Modular, Roche Diagnostics, Mannheim, Germany) and serum insulin was assessed using the AutoDELFIA Insulin assay (PerkinElmer Life and Analytical Sciences, Turku, Finland). Progranulin serum concentrations were measured using a commercially available ELISA kit (Progranulin human ELISA Kit AdipoGen, AdipoGen Inc., Korea) according to the manufacturer's instructions. Blood samples were taken in the morning after an overnight fast and stored at −80 °C until analyses. The lowest level of progranulin detectable by the assay was 0.032 ng/ml. The degree of precision of the ELISA system in terms of coefficient of variance of intra-assay was 3–6.9% (6 samples tested 8 times), that of inter-assays was 4.7–7.3% (6 samples tested 5 times).

Detailed phenotypic information is provided in Supplementary Material, Table S1. The study was approved by the ethics committee of the University of Leipzig. Informed consent has been given by all participants before taking part in the study.

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 in the region of Augsburg in Germany. All participants are residents of German nationality identified through registration. All subjects gave written informed consent (38). The present study includes data of the KORA follow-up study F4 (2006–2008) which has been approved by the local ethics committee. 1814 randomly selected participants of KORA F4 were selected for the genome-wide scan. 1628 subjects without type 2 diabetes were included in the present study. Progranulin serum concentrations were determined using a commercially available ELISA (Progranulin human ELISA Kit AdipoGen, AdipoGen Inc., Korea). Further phenotypic information is given in Supplementary Material, Table S1.

Prevalence, prediction and prevention of diabetes – Botnia Study

The Prevalence, Prediction and Prevention of diabetes (PPP)–Botnia Study is a population-based study from the Botnia region of western Finland (39). In the present study, 335 subjects with normal glucose tolerance from PPP that also participated in the Diabetes Genetics Initiative (DGI) were included (40). Progranulin serum concentrations were measured using a commercially available ELISA kit (Mediagnost, Reutlingen, Germany).

The study was approved by the Ethics Committee of Helsinki University Hospital, Finland and all participants gave their written informed consent.

LIFE-Heart Study

This cohort includes individuals with suspected coronary artery disease due to clinical symptoms (non-invasive testing) assigned to coronary angiography. Details of the study can be found elsewhere (14). Progranulin was measured by a commercially available ELISA (Mediagnost, Reutlingen, Germany) on the DSX automatic ELISA test system. The analytical sensitivity of the assay was 0.018 ng/ml. Intra and inter-assay coefficients of variation ($N = 16$) were <5.7% at a concentration of 19.5 ng/ml and <4.4% at a concentration of 55.2 ng/ml progranulin and for a serum dilution of 1: 41.

The study has been approved by the Ethics Committee of the Medical Faculty of the University Leipzig, Germany (Reg. No 276–2005) and is registered at ClinicalTrials.gov (NCT00497887). Written informed consent was obtained from all participants before taking part in the study.

Berlin cohort (Metabolic Syndrome Berlin Potsdam)

This study was used as final replication stage according to our study design (Fig. 1). Subjects were recruited in a cross-sectional approach with focus on traits of the metabolic syndrome. Assessment of anthropometry included weight, height, waist and hip circumference, and skin fold measurements. All laboratory parameters were measured in fasting blood samples. A total of 833 Caucasian individuals from the MeSyBePo (Metabolic Syndrome Berlin Potsdam) study population from the region of Berlin/Potsdam, Germany, were included in the present study. Progranulin serum concentrations were measured using a commercially available ELISA kit (Mediagnost, Reutlingen, Germany).

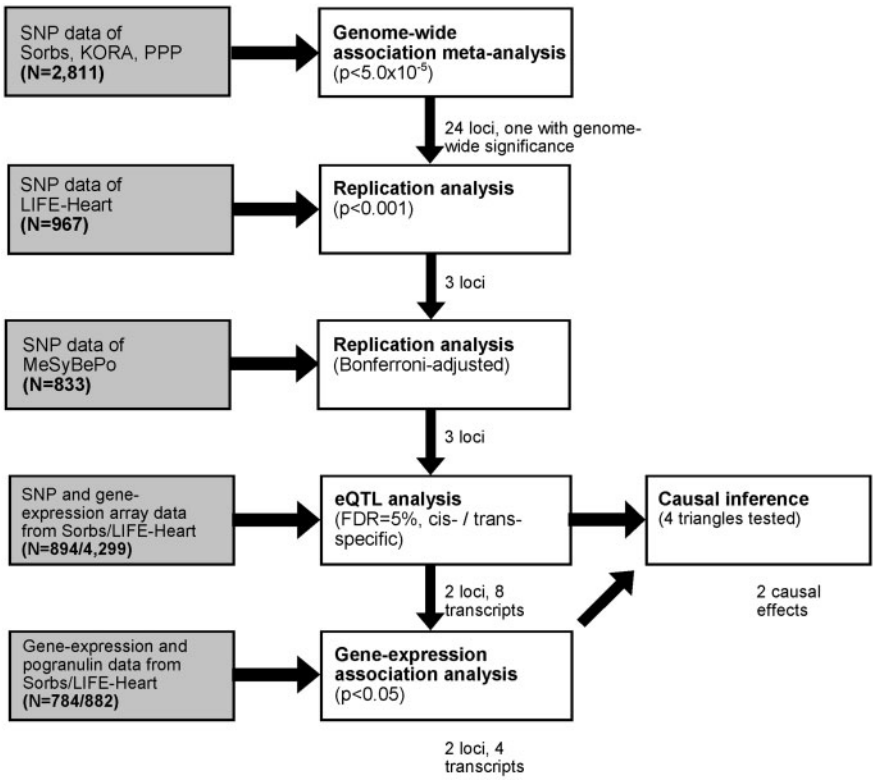

Figure 1. Study design.

The study was approved by the ethics committees of the University of Potsdam and the Charité-University (Medical Department), Berlin, Germany and all subjects gave written informed consent.

Phenotypic information of all cohorts is provided in Supplementary Material, Table S1. We would like to acknowledge that progranulin serum levels vary between the study cohorts, which is in large part explained by different commercially available ELISAs used for measurement (Mediagnost Inc. for PPP, MeSyBePo and LIFE-Heart, Adipogen Inc. for KORA and Sorbs). Accordingly, values are more similar between cohorts using the same measurement technique.

Genotyping, quality control and genotype imputations

Sorbs cohort

Genotyping was performed either by 500K Affymetrix GeneChip or by Affymetrix Genome-Wide Human SNP Array 6.0. The BRLMM algorithm (Affymetrix, Inc) was applied for the 500K array and the Birdseed algorithm was used for the Genome-Wide Human SNP Array 6.0. Quality control was conducted as described previously (35). Genotype imputation was performed separately for individuals genotyped with the two different assays using IMPUTE v2.1.2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html; date last accessed November 29, 2017). The reference panel was HapMap2 CEU, Release 24, dbSNP-build 126, NCBI 36. As proposed by Roshyara et al. (41) no SNP filtering was performed prior to imputation but a number of quality filters were applied thereafter (see Supplementary Material, Table S1 for more details). We applied a 'drop one in' procedure to avoid bias by the relatedness structure within the Sorbs cohort (37). We performed one PCA for each Sorbian individual together with 50 most unrelated HapMap CEU individuals based on genotype data as explained in Gross *et al.* (35). Resulting eigenvectors of CEU individuals were averaged over all iterations. Individuals were considered as ethnical outliers if the distance from the mean of the respective eigenvector of at least one of the first 10 eigenvectors exceeded 6 standard deviations. Based on these filters three individuals were discarded from association analysis (final N = 974). A total of 848 non-diabetic subjects had combined genetic, progranulin and covariate data making them eligible for the first-stage GWAS. Details are provided in Supplementary Material, Table S1.

KORA

Subjects were genotyped at the Helmholtz Genome Analysis Center using Affymetrix 6.0 arrays. The Birdseed 2 calling algorithm was applied. Imputations were conducted with IMPUTE v0.4.2 on HapMap Phase 2 CEU as a reference panel (Supplementary Material, Table S1). We applied the following post-imputation SNP filters: imputation quality $r^2 \geq 0.4$, minor allele frequency > 1% and for called variants Callrate > 98% and $P(\text{HWE}) > 10^{-6}$.

PPP

Subjects were genotyped at the Broad Institute using the Affymetrix GeneChip® Human Mapping 500K Array Set. The BRLMM algorithm was applied. Imputations were conducted with MACH on HapMap Phase 2 CEU as the reference panel (Supplementary Material, Table S1).

LIFE-Heart Study

Genotyping was performed using the Affymetrix Axiom Technology with custom option (Axiom-CADLIFE) or

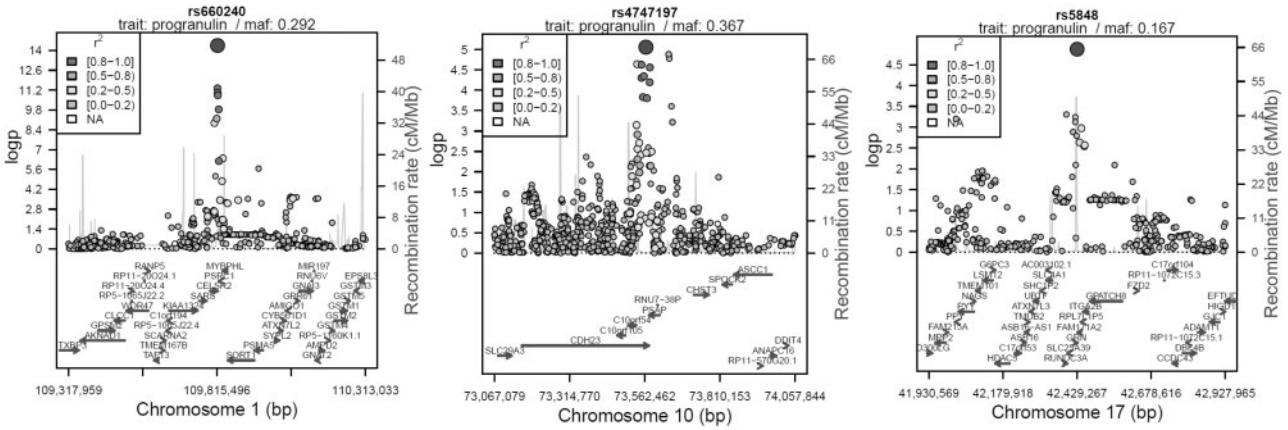

Figure 2. Regional association plot for the top-hits of association with progranulin levels.

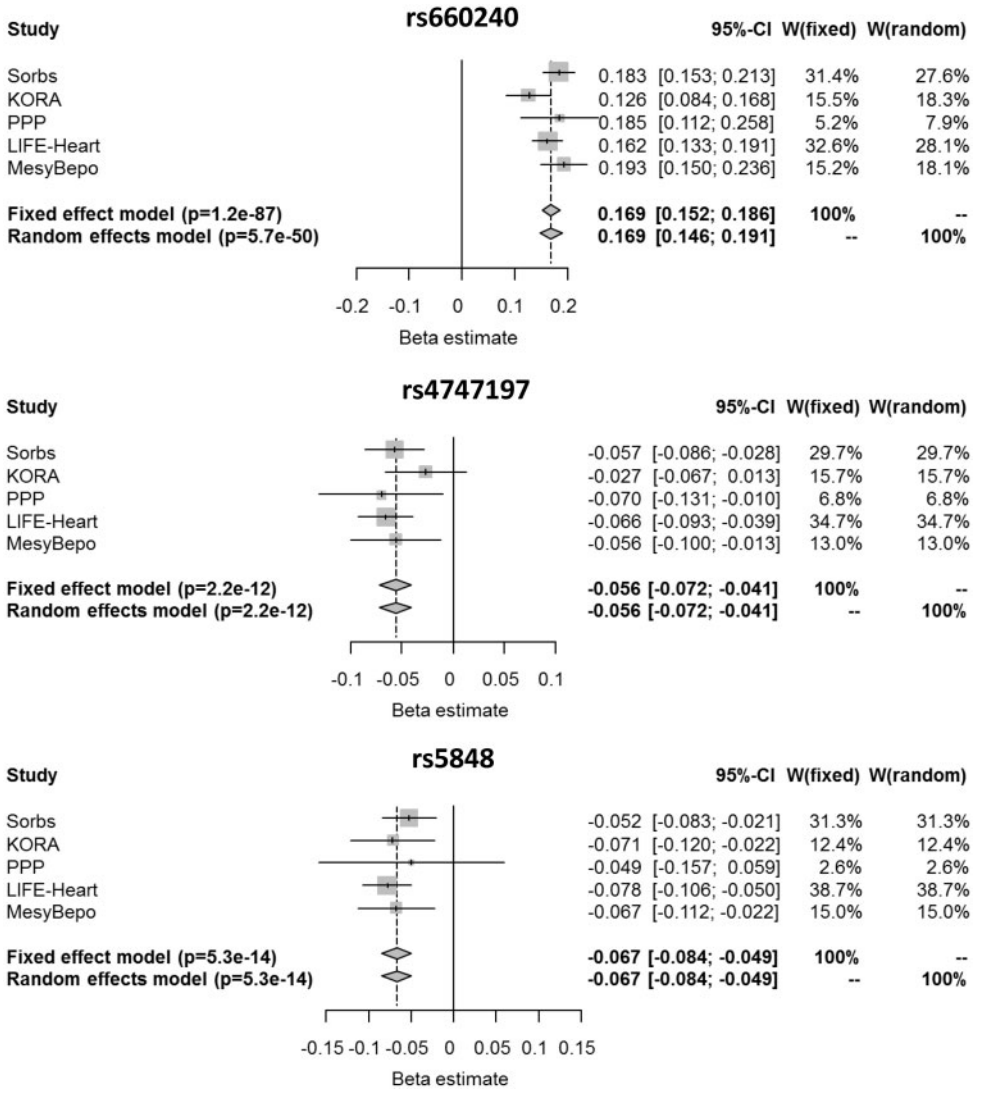

Figure 3. Meta-analysis of the three replicated hits of the present study. Association statistics pooled over the three discovery studies (Sorbs, KORA, PPP) and the two replication studies (LIFE-Heart, MeSyBePo).

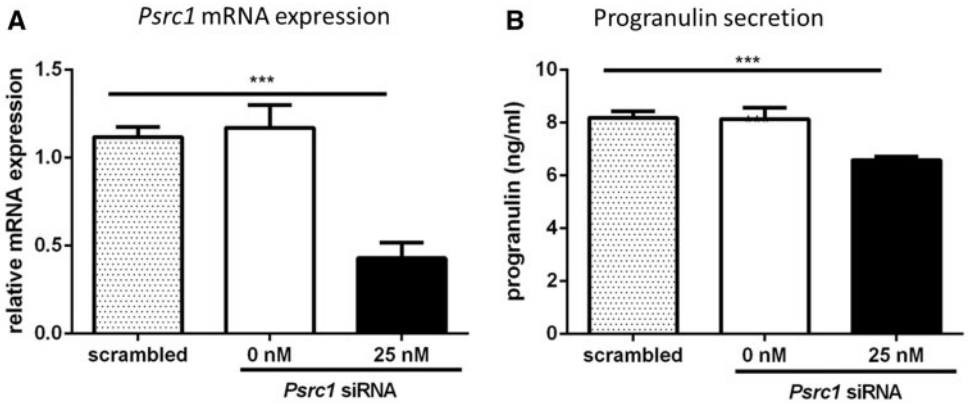

Figure 4. Decrease in *Psrc1* mRNA expression reduces the amount of progranulin in the media of cultured 3T3-L1 cells. (**A**) siRNA mediated knockdown of *Psrc1* in 3T3-L1 cells; (**B**) Progranulin levels in the supernatant of cultured 3T3-L1 cells are significantly decreased after siRNA mediated *Psrc1* silencing; Values represent the average relative expression calculated from three independent experiments. Each sample was performed in duplicate. Bars represent the standard deviation of the mean from the three independent experiments. A Student's t-test was used to determine significance of the averaged *Psrc1* expression values and progranulin levels compared with the scrambled siRNA controls (*** $P < 0.001$).

Axiom-CEU. Genotype calling was conducted with Affymetrix Power Tools version 1.17.0 and 1.16.1 respectively. Quality control criteria for individual samples included call rate (>97%), sex-mismatch, cryptic relatedness, and outliers of principal components analysis (6SD criterion of Eigenstrat software) (42). SNP quality control comprised call rate ≥97%, Affymetrix cluster metrices as recommended by the company (FLD, HetSO, HomRO), P-value of exact Hardy-Weinberg equilibrium test $\geq 10^{-6}$, P-value of plate association $\geq 10^{-7,}$ and minor allele frequency >0%. For imputation, we used the intersection of QC SNPs of the two arrays. A total of 504 071 SNPs could be matched to the 1000Genomes reference phase 1, release 3 (http://mathgen.stats.ox.ac.uk/impute/data_download_1000G_phase1_integrated.html; date last accessed November 29, 2017). Imputation was performed using SHAPEIT v2 and IMPUTE2 v2.3.2.

A total of 5700 patients with genotype data were available. For 967 non-diabetic subjects, combined genotype, progranulin and covariate data were available for subsequent analysis. Since subjects included in this study were in part not fasting and were diagnosed with clinical coronary artery disease, we opted to use this sample as a replication cohort rather than including it in the meta-GWAS, although genome-wide data are available.

Berlin cohort (Metabolic Syndrome Berlin Potsdam-MeSyBePo)

Selected SNPs were genotyped using the TaqMan® SNP Genotyping assays according to the manufacturer's protocol (Applied Biosystems, Inc., Foster City, CA). To validate the reproducibility of genotyping, a random subset (about 5%) of the sample was re-genotyped. Genotypes matched in 100% of the samples. 833 individuals with both, genetic data and phenotypic traits including co-variables and progranulin measurements were available here.

Gene-expression studies

Gene-expression profiling in PBMCs in the Sorbs and LIFE-Heart

PBMCs were extracted from blood samples. Gene expression was assessed using Illumina Human HT-12 v4 as described in detail elsewhere (29,43).

Pre-processing of RNA microarray data

Pre-processing of RNA microarray data relied on the intensities of up to 47 323 transcripts derived from Illumina BeadStudio without background correction and normalization measured in 1029 individuals of the Sorbs cohort and 4509 individuals of the LIFE-Heart cohort. Pre-processing comprehended of (a) exclusion of individuals with an atypically low number of expressed genes [median – 3 interquartile ranges (IQR) of the cohort's values], (b) quantile normalization and log2- transformation, (c) exclusion of individuals with atypical gene-expression profiles (Euclidian distance to average expression larger than median + 3 or 4 IQR for Sorbs and LIFE-Heart, respectively) and excluding also individuals with atypical values of internal quality control-parameters (calculated as Mahalanobis distance of quality control probes included on the HT-12 v4 chip, a distance with a larger value than median + 3 IQR was used as cut-off), (d) adjusting for batch effects due to different ybridization chips with Empirical Bayes estimates (44). A total of 924 individuals from the Sorbs cohort and 4368 individuals of the LIFE-Heart cohort fulfilled all quality criteria.

Statistical analyses

Overall study design

We performed a three stage association analysis. First, we performed a genome-wide meta-analysis of the cohorts Sorbs, KORA and PPP applying a less stringent cut-off of $P < 5 \times 10^{-5}$. Top-hits of identified loci were replicated in the LIFE-Heart Study applying a P-value cut-off of $P < 0.001$. SNPs replicated with the same direction of effect were finally tested in MeSyBePo applying a stringent Bonferroni-corrected significance cut-off. Genome-wide association analysis was accompanied by eQTL and gene-expression association analysis of PBMCs in Sorbs and LIFE-Heart (Fig. 1).

Association between circulating progranulin levels and anthropometric traits

All non-normally distributed parameters were logarithmically transformed. Linear regression analyses adjusted for age, sex, and BMI (only age and sex for BMI analysis) were performed using SPSS software version 20.0 (SPSS, Chicago, IL). Since the discovered associations are potential sources of confounding,

we performed additional adjustments of progranulin levels regarding these factors. This had no relevant impact on the association results of our top-hits (results not shown).

Genome-wide association analysis

Progranulin serum levels were log-transformed prior to analysis. Genetic associations were assessed using linear regression analyses of allele doses adjusted for age, sex and BMI. In the Sorbs cohort, we additionally adjusted for relatedness using mixed effect models (function 'polygenic' of the 'GenABEL' package of R) (12,13). Genome-wide analysis was performed in the studies Sorbs, KORA and PPP. For meta-analysis, SNPs with allele frequencies differing by more than 20% between cohorts and with violations of quality criteria in any of the studies were excluded resulting in a total of 2 069 113 SNPs analysed in the present study. Prior to meta-analysis, effect sizes of single studies were corrected for inflation by applying genomic control (Sorbs cohort: $\lambda = 1.0$, KORA: $\lambda = 1.0$, PPP: $\lambda = 1.108$). The relatively high inflation in PPP could not be explained by any kind of hidden sub-structure in the data and only appeared for this specific trait but not for others (43). Standardized effects of single studies were combined using fixed and random effects models (function 'metagen' of the 'meta' package of R). *P*-values of both models are reported but the more conservative random effects models were used as primary selection criterion for subsequent replications. Q-statistics were applied to assess heterogeneity between studies.

Replication analysis

LIFE-Heart Study. Top SNPs to be replicated in this cohort were retrieved from the LIFE-Heart Study genotype data. We analysed additive models of progranulin-SNP associations adjusting for age, sex and BMI as in the cohorts of the meta-analysis. We furthermore adjusted for fasting status and disease status (coronary artery disease case/control status). A *P*-value cut-off of 0.001 was applied at this stage.

BERLIN cohort (Metabolic Syndrome Berlin Potsdam). SNPs significant in LIFE-Heart (rs660240, rs4747197, rs5848) were de novo genotyped in this cohort. Associations were assessed for the additive mode of inheritance adjusting for age, sex and BMI.

Expression QTL analysis

The three successfully replicated SNPs rs660240, rs4747197 and rs5848 were subjected to genome-wide expression QTL (eQTL) analysis. For $N = 894$ Sorbs and $N = 4299$ LIFE-Heart samples, combined SNP and gene-expression data were available for that purpose. Gene-expression data were adjusted for age, sex, percentage of monocytes and lymphocytes. In the Sorbs we also adjusted for the relatedness structure. eQTLs were reported if achieving genome-wide significance in one of the cohorts (cis/trans-specific false discovery rate of $<5\%$) (29) and at least nominal significance in the other cohort. We also analysed whether our SNPs are in LD with eQTLs reported in the literature.

Mediation analysis

We tested whether the observed SNP-progranulin associations were mediated by gene expressions of the genes identified by our eQTL analyses. First, we analysed, whether the respective gene expressions are correlated with serum progranulin levels. Then, we performed a Mendelian randomization analysis of a causal (un-confounded) relation of the regulated PBMC gene-expression and progranulin serum levels using the corresponding eQTN (expression quantitative trait nucleotide) as instrumental variable. To account for possible direct SNP effects on progranulin, we modified the Mendelian randomization analyses accordingly (45). From progranulin, we subtracted a potential direct SNP-effect, independent of the potential gene-expression effect, and performed Mendelian randomization analyses with the modified phenotype. In simulation analyses, this approach worked well when the direct SNP-effect was strong and the confounding of gene expression and target phenotype was weak. The latter is striven by adjusting progranulin and gene expression for their strongest co-variables as described above. Moreover, we tested whether the observed association between gene expression and progranulin explains at least part of the observed SNP to progranulin association. This is performed by analysing whether the absolute value of the beta coefficient of the SNP is reduced when gene expression is included into the regression model. Standard errors of the differences of corresponding beta coefficients were calculated by Jackknifing and one-sided tests were applied. A total of 784 Sorbs subjects and 882 LIFE-Heart patients were available for this type of analysis.

We also analysed the causal link between progranulin and a variety of obesity and metabolic traits (Table 1) by Mendelian randomization analyses. We used the SNP rs660240 showing the strongest association with progranulin as instrumental variable for that purpose. Here, a direct impact of the SNPs on the phenotypes of interest is less problematic since the SNP is not described as GWAS hit for most of the phenotypes considered in the present study. LDLC is an exception. Thus, we decided to use our above-mentioned strategy for that trait.

These analyses are considered as supplemental to our primary association analysis. Therefore, we refrained from correction for multiple testing here.

Functional consequences of the associated SNPs in RegulomeDB

Potential functional consequences of the associated SNPs were searched by using the RegulomeDB (24). Briefly, RegulomeDB is a database that annotates SNPs with known and predicted regulatory elements in the human genome, such as DNAase hypersensitivity, binding sites of transcription factors, and promoter regions.

In vitro functional analyses

Small interfering RNA mediated knockdown of Psrc1 in vitro

For RNAi mediated knockdown of Psrc1 the murine preadipocyte cell line 3T3-L1 was used. 3T3-L1 cells were maintained in DMEM supplemented with 10% fetal bovine serum. For knockdown, cells were seeded into 6-well plates and cultured for 48 h.

RNAi was performed using the FlexiTube GeneSolution GS84722 (Qiagen, Hilden, Germany). ON TARGET plus Accell GAPDH siRNA human served as positive control and ON TARGET plus Non-targeting pool was used as a negative control (both from Thermo Scientific, Schwerte, Germany). Transfection experiments were carried out using DharmaFECT1 (Thermo Scientific, Hilden, Germany) according to the manufacturer's instructions. Cells were transfected without (0 nM) or with 25 nM siRNA and incubated 48 h (for qPCR) or 72 h (for protein expression and secretion). Following incubation, supernatants

were removed and cells were washed with PBS. Supernatants and cells were frozen in liquid nitrogen and stored at −80 °C.

RNA extraction and quantitative real-time-PCR

RNA was extracted using the InviTrap® Spin Tissue RNA Mini Kit (STRATEC Molecular GmbH, Berlin, Germany). One μg of RNA was incubated with 0.5 μg random primers (Promega, Fitchburg, USA) in a total volume of 13.5 μl for 5 min at 70 °C and cooled to 4 °C. Following primer incubation, a mixture consisting of 200 U Moloney murine leukemia virus reverse transcriptase (Promega, Fitchburg, USA), 4 μl of 5xM-MLV-RT Buffer, 0.5 mM dNTPs (Promega, Fitchburg, USA) and 0.5 μl Ribolock RNase-Inhibitor (Thermo Scientific, Waltham, USA) was added. Reverse transcription was performed at 37 °C for 60 min and 94 °C for 5 min. For the detection of murine Psrc1 gene expression the TaqMan probe Mm00498358_m1 (Life Technologies, Carlsbad, USA) was used according to the manufactureŕs instructions. The expression of GAPDH or Psrc1 was calculated by the delta-delta CT method using ACTB or 36B4 as reference gene. Relative gene expression was compared with scrambled siRNA treated cells set as baseline level.

Progranulin measurements by ELISA

For the detection of progranulin in supernatants of murine 3T3L-1 cells, the progranulin mouse ELISA kit (AdipoGen, Inc., Korea) was used. The degree of precision of the ELISA system in terms of intra-assay and inter-assay coefficient of variance (percentage) was less than 5.2% and 6.8%, respectively. Spike recovery and linearity were in a range of 90% to 103% and 0.3 to 0.6 μg/ml, respectively. Furthermore, the ELISA was specific for mouse progranulin and did not cross-react with rat or human progranulin. In addition, quality controls were included in all ELISA measurements, and results were within the expected range.

Supplementary Material

Supplementary Material is available at *HMG* online.

Acknowledgements

We thank all those who participated in the studies. We would like to acknowledge excellent technical assistance by Beate Gutsmann, Manuela Quandt and Melanie Schuricht for Sorbs.

The KORA authors acknowledge the contribution of Peter Lichtner, Gertrud Eckstein, Guido Fischer, Norman Klopp, Nicole Spada, and all members of the Helmholtz Zentrum München genotyping staff for generating the SNP data, as well as all members of the field staff who were involved in the planning and conducting of the KORA Augsburg studies. Furthermore, the authors thank the Augsburg registry team for the acquisition of the follow-up data. Finally, the authors express their appreciation to all study participants.

The skillful assistance of the Botnia Study Group is gratefully acknowledged for Prevalence, Prediction and Prevention of Diabetes (PPP)-Botnia Study.

We would like to acknowledge excellent technical assistance by Katrin Horn, Janne Pott and Arnd Gross for LIFE-Heart Study.

Conflict of Interest statement. None declared.

Funding

Sorbs

German Research Council [SFB-1052 'Obesity mechanisms' B01 to M.B., B03 to P.K., C01 to A.T., A01 to M.S.; SPP 1629 TO 718/2– 1 to A.T.]; the German Diabetes Association; the DHFD (Diabetes Hilfs- und Forschungsfonds Deutschland); and the Federal Ministry of Education and Research (BMBF), Germany, [IFB Adiposity Diseases FKZ: 01EO1501, AD2–060E to P.K. and AD2–06E99 to P.K.].

KORA

Helmholtz Center Munich, German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria, a grant from the BMBF to the German Center for Diabetes Research (DZD).

Prevalence, Prediction and Prevention of Diabetes (PPP)-Botnia Study

Sigrid Juselius Foundation; the Folkhälsan Research Foundation; the Signe and Ane Gyllenberg Foundation; the Finnish Diabetes Research Foundation; the Foundation for Life and Health in Finland; the Finnish Medical Society; the Ollqvist Foundation; the Närpes Health Care Foundation; the Swedish Research Council [Linné grant No. 31475113580, Linnaeus Centre of Excellence grant Dnr 2006– 37, Dnr 2010–3490 to L.G.]; the Wallenberg Foundation; the Broad Institute; and the Novartis Pharma.

LIFE-Heart Study

LIFE – Leipzig Interdisciplinary Research Cluster of Genetic Factors, Clinical Phenotypes and Environment (LIFE Center, University of Leipzig). LIFE is funded by the European Union, by the European Regional Development Fund (ERFD), the European Social Fund and by the Free State of Saxony within the framework of the excellence initiative.

Berlin cohort (Metabolic Syndrome Berlin Potsdam-MeSyBePo)

A research group of the BMBF (Molecular Nutrition) to J.S., a Heisenberg-Professorship [SP716/2–1] of the Deutsche Forschungsgemeinschaft (DFG), and a graduate school of the DFG [GK1208].

References

1. Nguyen, A.D., Nguyen, T.A., Martens, L.H., Mitic, L.L. and Farese, J. (2013) Progranulin: at the interface of neurodegenerative and metabolic diseases. *Trends Endocrinol.Metab.*, **24**, 597–606.
2. Youn, B.S., Bang, S.I., Klöting, N., Park, J.W., Lee, N., Oh, J.E., Pi, K.B., Lee, T.H., Ruschke, K., Fasshauer, M. *et al.* (2009) Serum progranulin concentrations may be associated with macrophage infiltration into omental adipose tissue. *Diabetes*, **58**, 627–636.
3. Kojima, Y., Ono, K., Inoue, K., Takagi, Y., Kikuta, K.i., Nishimura, M., Yoshida, Y., Nakashima, Y., Matsumae, H., Furukawa, Y. *et al.* (2009) Progranulin expression in

advanced human atherosclerotic plaque. *Atherosclerosis*, **206**, 102–108.
4. Linsel-Nitschke, P., Heeren, J., Aherrahrou, Z., Bruse, P., Gieger, C., Illig, T., Prokisch, H., Heim, K., Doering, A., Peters, A. *et al.* (2010) Genetic variation at chromosome 1p13.3 affects sortilin mRNA expression, cellular LDL-uptake and serum LDL levels which translates to the risk of coronary artery disease. *Atherosclerosis*, **208**, 183–189.
5. Yoo, H.J., Hwang, S.Y., Hong, H.C., Choi, H.Y., Yang, S.J., Choi, D.S., Baik, S.H., Blüher, M., Youn, B.S. and Choi, K.M. (2013) Implication of progranulin and C1q/TNF-related protein-3 (CTRP3) on inflammation and atherosclerosis in subjects with or without metabolic syndrome. *PLoS One*, **8**, e55744.
6. Finch, N., Baker, M., Crook, R., Swanson, K., Kuntz, K., Surtees, R., Bisceglio, G., Rovelet-Lecrux, A., Boeve, B., Petersen, R.C. *et al.* (2009) Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. *Brain*, **132**, 583–591.
7. Baker, M., Mackenzie, I.R., Pickering-Brown, S.M., Gass, J., Rademakers, R., Lindholm, C., Snowden, J., Adamson, J., Sadovnick, A.D., Rollinson, S. *et al.* (2006) Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature*, **442**, 916–919.
8. Cruts, M., Gijselinck, I., van der Zee, J., Engelborghs, S., Wils, H., Pirici, D., Rademakers, R., Vandenberghe, R., Dermaut, B., Martin, J.J. *et al.* (2006) Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature*, **442**, 920–924.
9. Gass, J., Cannon, A., Mackenzie, I.R., Boeve, B., Baker, M., Adamson, J., Crook, R., Melquist, S., Kuntz, K., Petersen, R. *et al.* (2006) Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. *Hum. Mol. Genet.*, **15**, 2988–3001.
10. Minami, S.S., Min, S.W., Krabbe, G., Wang, C., Zhou, Y., Asgarov, R., Li, Y., Martens, L.H., Elia, L.P., Ward, M.E. *et al.* (2014) Progranulin protects against amyloid [beta] deposition and toxicity in Alzheimer's disease mouse models. *Nat. Med.*, **20**, 1157–1164.
11. Carrasquillo, M.M., Nicholson, A.M., Finch, N., Gibbs, J.R., Baker, M., Rutherford, N.J., Hunter, T.A., DeJesus-Hernandez, M., Bisceglio, G.D., Mackenzie, I.R. *et al.* (2010) Genome-wide screen identifies rs646776 near sortilin as a regulator of progranulin levels in human plasma. *Am. J. Hum. Genet.*, **87**, 890–897.
12. Amin, N., van Duijn, C.M., Aulchenko, Y.S. and Heutink, P. (2007) A genomic background based method for association analysis in related individuals. *PLoS One*, **2**, e1274.
13. Aulchenko, Y.S., de Koning, D.J. and Haley, C. (2007) Genomewide rapid association using mixed model and regression: a fast and simple method for genomewide pedigree-based quantitative trait loci association analysis. *Genetics*, **177**, 577–585.
14. Beutner, F., Teupser, D., Gielen, S., Holdt, L.M., Scholz, M., Boudriot, E., Schuler, G., Thiery, J. and Federici, M. (2011) Rationale and design of the Leipzig (LIFE) Heart Study: phenotyping and cardiovascular characteristics of patients with coronary artery disease. *PLoS One*, **6**, e29070.
15. Gieger, C., Radhakrishnan, A., Cvejic, A., Tang, W., Porcu, E., Pistis, G., Serbanovic-Canic, J., Elling, U., Goodall, A.H., Labrune, Y. *et al.* (2011) New gene functions in megakaryopoiesis and platelet formation. *Nature*, **480**, 201–208.
16. Tolkatchev, D., Malik, S., Vinogradova, A., Wang, P., Chen, Z., Xu, P., Bennett, H.P.J., Bateman, A. and Ni, F. (2008) Structure dissection of human progranulin identifies well-folded granulin/epithelin modules with unique functional activities. *Prot. Sci.*, **17**, 711–724.
17. He, Z. and Bateman, A. (2003) Progranulin (granulin-epithelin precursor, PC-cell-derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. *J. Mol. Med.*, **81**, 600–612.
18. Cruts, M. and Van Broeckhoven, C. (2008) Loss of progranulin function in frontotemporal lobar degeneration. *Trends Genet.*, **24**, 186–194.
19. Tang, W., Lu, Y., Tian, Q.-Y., Zhang, Y., Guo, F.-J., Liu, G.-Y., Syed, N.M., Lai, Y., Lin, E.A., Kong, L. *et al.* (2011) The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. *Science*, **332**, 478–484.
20. Matsubara, T., Mita, A., Minami, K., Hosooka, T., Kitazawa, S., Takahashi, K., Tamori, Y., Yokoi, N., Watanabe, M., Matsuo, E.i. *et al.* (2012) PGRN is a key adipokine mediating high fat diet-induced insulin resistance and obesity through IL-6 in adipose tissue. *Cell Metab.*, **15**, 38–50.
21. Musunuru, K., Strong, A., Frank-Kamenetsky, M., Lee, N.E., Ahfeldt, T., Sachs, K.V., Li, X., Li, H., Kuperwasser, N., Ruda, V.M. *et al.* (2010) From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus. *Nature*, **466**, 714–719.
22. Lonsdale, J., Thomas, J., Salvatore, M., Phillips, R., Lo, E., Shad, S., Hasz, R., Walters, G., Garcia, F., Young, N. *et al.* (2013) The Genotype-Tissue Expression (GTEx) project. *Nat. Genet.*, **45**, 580–585.
23. Schadt, E.E., Molony, C., Chudin, E., Hao, K., Yang, X., Lum, P.Y., Kasarskis, A., Zhang, B., Wang, S., Suver, C. *et al.* (2008) Mapping the genetic architecture of gene expression in human liver. *PLoS Biol.*, **6**, e107.
24. Boyle, A.P., Hong, E.L., Hariharan, M., Cheng, Y., Schaub, M.A., Kasowski, M., Karczewski, K.J., Park, J., Hitz, B.C., Weng, S. *et al.* (2012) Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.*, **22**, 1790–1797.
25. Ionita-Laza, I., McCallum, K., Xu, B. and Buxbaum, J.D. (2016) A spectral approach integrating functional genomic annotations for coding and noncoding variants. *Nat. Genet.*, **48**, 214–220.
26. Lo, P.K., Chen, J.Y., Lo, W.C., Chen, B.F., Hsin, J.P., Tang, P.P. and Wang, F.F. (1999) Identification of a novel mouse p53 target gene DDA3. *Oncogene*, **18**, 7765–7774.
27. Jang, C.Y. and Fang, G. (2009) The N-terminal domain of DDA3 regulates the spindle-association of the microtubule depolymerase Kif2a and controls the mitotic function of DDA3. *Cell Cycle*, **8**, 3165–3171.
28. Jang, C.Y., Coppinger, J.A., Yates, J.R., III. and Fang, G. (2011) Mitotic kinases regulate MT-polymerizing/MT-bundling activity of DDA3. *Biochem. Biophys. Res. Commun.*, **408**, 174–179.
29. Kirsten, H., Al-Hasani, H., Holdt, L., Gross, A., Beutner, F., Krohn, K., Horn, K., Ahnert, P., Burkhardt, R., Reiche, K. *et al.* (2015) Dissecting the genetics of the human transcriptome identifies novel trait-related trans-eQTLs and corroborates the regulatory relevance of non-protein coding loci. *Hum. Mol. Genet.*, **24**, 4746–4763.
30. Westra, H.J., Peters, M.J., Esko, T., Yaghootkar, H., Schurmann, C., Kettunen, J., Christiansen, M.W., Fairfax, B.P., Schramm, K., Powell, J.E. *et al.* (2013) Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat. Genet.*, **45**, 1238–1243.
31. Rotival, M., Zeller, T., Wild, P.S., Maouche, S., Szymczak, S., Schillert, A., Castagne, R., Deiseroth, A., Proust, C., Brocheton, J. *et al.* (2011) Integrating genome-wide genetic

variations and monocyte expression data reveals trans-regulated gene modules in humans. *PLoS Genet.*, **7**, e1002367.
32. Zhou, X., Sun, L., Bastos de Oliveira, F., Qi, X., Brown, W.J., Smolka, M.B., Sun, Y. and Hu, F. (2015) Prosaposin facilitates sortilin-independent lysosomal trafficking of progranulin. *J. Cell Biol.*, **210**, 991–1002.
33. Nicholson, A.M., Finch, N.A., Almeida, M., Perkerson, R.B., van Blitterswijk, M., Wojtas, A., Cenik, B., Rotondo, S., Inskeep, V., Almasy, L. *et al.* (2016) Prosaposin is a regulator of progranulin levels and oligomerization. *Nat. Commun.*, **7**, 11992.
34. Breitfeld, J., Tönjes, A., Böttcher, Y., Schleinitz, D., Wiele, N., Marzi, C., Brockhaus, C., Rathmann, W., Huth, C., Grallert, H. *et al.* (2013) Genetic variation in the vaspin gene affects circulating serum vaspin concentrations. *Int. J. Obes. (Lond)*, **37**, 861–866.
35. Gross, A., Tönjes, A., Kovacs, P., Veeramah, K.R., Ahnert, P., Roshyara, N.R., Gieger, C., Rueckert, I.M., Loeffler, M., Stoneking, M. *et al.* (2011) Population-genetic comparison of the Sorbian isolate population in Germany with the German KORA population using genome-wide SNP arrays. *BMC Genet.*, **12**, 67.
36. Tönjes, A., Dünnebeil, J., Schleinitz, D., Koriath, M., Rayner, N.W., Dietrich, K., Enigk, B., Böttcher, Y., Krohn, K., Kovacs, P. *et al.* (2009) Genetic variation in GPR133 is associated with height: genome wide association study in the self-contained population of Sorbs. *Hum. Mol. Genet.*, **4**, 4662–4668.
37. Veeramah, K.R., Tönjes, A., Kovacs, P., Gross, A., Wegmann, D., Geary, P., Gasperikova, D., Klimes, I., Scholz, M., Novembre, J. *et al.* (2011) Genetic variation in the Sorbs of eastern Germany in the context of broader European genetic diversity. *Eur. J. Hum. Genet.*, **19**, 995–1001.
38. Wichmann, H.E., Gieger, C. and Illig, T. (2005) KORA-gen–resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen*, **67**, S26–S30.
39. Isomaa, B., Forsen, B., Lahti, K., Holmstrom, N., Waden, J., Matintupa, O., Almgren, P., Eriksson, J.G., Lyssenko, V., Taskinen, M.R. *et al.* (2010) A family history of diabetes is associated with reduced physical fitness in the Prevalence, Prediction and Prevention of Diabetes (PPP)-Botnia study. *Diabetologia*, **53**, 1709–1713.
40. Saxena, R., Voight, B.F., Lyssenko, V., Burtt, N.P., de Bakker, P.I., Chen, H., Roix, J.J., Kathiresan, S., Hirschhorn, J.N., Daly, M.J. *et al.* (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science*, **316**, 1331–1336.
41. Roshyara, N.R., Kirsten, H., Horn, K., Ahnert, P. and Scholz, M. (2014) Impact of pre-imputation SNP-filtering on genotype imputation results. *BMC Genet.*, **15**, 88.
42. Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A. and Reich, D. (2006) Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.*, **38**, 904–909.
43. Tönjes, A., Scholz, M., Breitfeld, J., Marzi, C., Grallert, H., Gross, A., Ladenvall, C., Schleinitz, D., Krause, K., Kirsten, H. *et al.* (2014) Genome wide meta-analysis highlights the role of genetic variation in RARRES2 in the regulation of circulating serum chemerin. *PLoS Genet.*, **10**, e1004854.
44. Johnson, W.E., Li, C. and Rabinovic, A. (2007) Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics*, **8**, 118–127.
45. Burkhardt, R., Kirsten, H., Beutner, F., Holdt, L.M., Gross, A., Teren, A., Tönjes, A., Becker, S., Krohn, K., Kovacs, P. *et al.* (2015) Integration of genome-wide SNP data and gene-expression profiles reveals six novel loci and regulatory mechanisms for amino acids and acylcarnitines in whole blood. *PLoS Genet.*, **11**, e1005510.
46. Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. and Turner, R.C. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **28**, 412–419.
47. Stumvoll, M., Van Haeften, T., Fritsche, A. and Gerich, J. (2001) Oral glucose tolerance test indexes for insulin sensitivity and secretion based on various availabilities of sampling times. *Diab. Care*, **24**, 796–797.