Recessive genetic effects on type 2 diabetes-related metabolites in a consanguineous population

Ayşe Demirkan*1,2,3, Jun Liu*1,4, Najaf Amin1, Jan B van Klinken5, Ko Willems van Dijk5,6, Cornelia M. van Duijn1,4,7

*These authors contributed equally

1 Genetic Epidemiology Subunit, Department of Epidemiology, Erasmus University Medical Center, Rotterdam, the Netherlands
2 Department of Experimental and Clinical Research, Faculty of Bioscience and Medicine, University of Surrey, Guildford, UK
3 Department of Genetics, University Medical Center Groningen, Groningen, the Netherlands
4 Nuffield Department of Population Health, University of Oxford, Oxford, UK
5 Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands
6 Department of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands
7 Leiden Academic Center for Drug Research, Leiden University, Leiden, the Netherlands

*These authors contributed equally

Keywords
Type 2 diabetes, metabolomics, homozygosity, autozygosity

Corresponding author: Cornelia M. van Duijn

Email address: cornelia.vanduijn@ndph.ox.ac.uk

Materials and Correspondence
Correspondence and requests for materials should be addressed to Ayşe Demirkan (email: a.demirkan@surrey.ac.uk) or to Jun Liu (email: jun.liu@ndph.ox.ac.uk) or to or to Cornelia M. van Duijn (email: cornelia.vanduijn@ndph.ox.ac.uk).
Abstract

Autozygosity, meaning inheritance of an ancestral allele in the homozygous state is known to lead bi-allelic mutations that manifest their effects through the autosomal recessive inheritance pattern. Autosomal recessive mutations are known to be the underlying cause of several Mendelian metabolic diseases, especially among the offspring of related individuals. In line with this, inbreeding coefficient of an individual as a measure of cryptic autozygosity among the general population is known to lead adverse metabolic outcomes including Type 2 diabetes (T2DM); a multifactorial metabolic disease for which the recessive genetic causes remain unknown. In order to unravel such effects for multiple metabolic facades of the disease, we investigated the relationship between the excess of homozygosity and the metabolic signature of T2DM. We included a set of 53 metabolic phenotypes, including 47 metabolites, T2DM and five T2DM risk factors, measured in a Dutch genetic isolate of 2,580 people. For 20 of these markers, we identified 29 regions of homozygous (ROHs) associated with the nominal significance of P-value < 1.0 × 10^{-3}. By performing association according to the recessive genetic model within these selected regions, we identified and replicated two intronic variants: rs6759814 located in KCNH7 associated with valine and rs1573707 located in PTPRT associated with IDL-free cholesterol and IDL-phospholipids. Additionally, we identified a rare intronic SNV in TBR1 for which the homozygous individuals were enriched for obesity. Interestingly, all three genes are mainly neuronally expressed and pointed out the involvement of glutamergic synaptic transmission pathways in the regulation of metabolic pathways. Taken together our study underline the additional benefits of model supervised analysis, but also seconds the involvement of the central nervous system in T2DM and obesity pathogenesis.
Introduction

Consanguineous marriages between close relatives as a result of assertive mating is known to cause severe metabolic diseases in the off-spring (Vernon 2015). In addition to that, moderate inbreeding due to isolation in populations has been shown to cause unfavorable outcomes among with cardio-metabolic and neuropsychiatric parameters (Verweij et al. 2014; Howrigan et al. 2016). The previous report shows evidence that inbreeding associates with an increase in blood pressure, glucose and decrease in high-density lipoprotein cholesterol (HDL-C), intelligence quotient (IQ) and height (McQuillan et al. 2012). In the decade, technological advances in metabolomics allow researchers to capture the biochemical status in an organism. As one of the most common metabolic disorders, studying the metabolomics of type 2 diabetes (T2DM) is particularly promising as the deregulation of biochemical processes is involved in the pathophysiology of T2DM. In line with this, many circulating metabolites have been found associated with T2DM: including phospholipids, branch-chain amino-acids and lipoprotein subclasses (Wang et al. 2005; Wang et al. 2011; Liu et al. 2017).

In our previous report, we tested the evidence of recessive SNP effects. We revealed that six out of the eight quantitative metabolite genetic loci showed a recessive rather than an additive effect on the metabolite (Demirkan et al. 2015). This raises an important question of whether such recessive variants are relevant for metabolic diseases such as T2DM and its markers. In order to answer this question and find genetic loci that act under recessive inheritance, we first defined runs of homozygosity (ROHs) that relate to T2DM and its circulating profile in blood in the genetically isolated ERF population and then looked for the causal recessive variants within the loci using coding variants. We focused on a set of 47 metabolites which we selected based on their correlation to inbreeding in the population (Demirkan et al. 2019). On top of that, we additionally studied the five commonly measured metabolic phenotypes, i.e. body-mass index (BMI), waist-hip ratio (WHR), fasting glucose, insulin and homeostatic model assessment for insulin resistance (HOMA-IR), as well as T2DM case/control status in the ERF population.

Methods

Study population

The Erasmus Rucphen Family genetic isolate study (ERF) is a prospective family-based study located in Southwest of the Netherlands. This young genetic isolate was founded in the mid-eighteenth century and minimal
immigration and marriages occurred between surrounding settlements due to social and religious reasons. The study population includes 3,465 individuals that are living descendants of 22 couples with at least six children baptized. Informed consent has been obtained from patients where appropriate. The study protocol was approved by the medical ethics board of the Erasmus Medical Center Rotterdam, the Netherlands (SANTOS et al. 2006). The baseline demographic data and measurements of the ERF participants were collected around 2002 to 2006. All the participants filled out questionnaires on socio-demographics, diseases and medical history and lifestyle factors, and were invited to the research center for an interview and blood collection for biochemistry and physical examinations including blood pressure and anthropometric measurements have been performed. The participants were asked to bring all their current medications for registration during the interview. Venous blood samples were collected after at least eight hours of fasting. Baseline type 2 diabetes was defined according to the fasting plasma glucose ≥ 7.0mmol/L and/or anti-diabetic treatment, yielding 212 cases and 2,564 controls, totaling up to 2,776.

The follow-up data collection of the ERF study took place in May 2016 (9 to 14 years after baseline visit). During the follow up a total of 1,935 participants' records were scanned for the incidence of type 2 diabetes in general practitioner’s databases. Additionally, a questionnaire on type 2 diabetes medication surveyed on 1,232 participants in June 2010 (4 to 8 years after baseline visit) was referred if a participant were not included in May 2016 follow-up. This effort yielded the inclusion of 18 otherwise missed extra cases, yielding a total of 349 cases and 2,427 controls in the current study.

**Metabolite measurements**

Metabolic markers were measured by five different metabolomics platforms using the methods which have been described in earlier publications (DEMIRKAN et al. 2012; GONZALEZ-COVARRUBIAS et al. 2013; DEMIRKAN et al. 2015; DRAISMA et al. 2015; KETTUNEN et al. 2016). In total 562 metabolic markers including sub-fractions of lipoproteins, triglycerides, phospholipids, ceramides, amino acids, acyl-carnitines and small intermediate compounds, which throughout this article will be referred as “metabolites”, were measured either by nuclear magnetic resonance (NMR) spectrometry or by mass spectrometry (MS). The platforms used in this research are: (1) Liquid Chromatography-MS (LC-MS, 116 positively charged lipids, comprising of 39 triglycerides, 47 phosphatidylcholines, 8 phosphatidylethanolamines, 20 sphingolipids, and 2 ceramides, available in up to 2,638 participants) measured in the Netherlands Metabolomics Center, Leiden using the method described...
before (Gonzalez-Covarrubias et al. 2013); (2) Electrospray-Ionization MS (ESI-MS, in total 148 phospholipids and sphingolipids comprising of 16 plasmalogens, 72 phosphatidylcholines, 27 phosphatidylethanolamines, 24 sphingolipids, 9 ceramides, available in up to 878 participants), measured in the Institute for Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany using the method described previously (Demirkan et al. 2012); (3) Small molecular compounds window based NMR spectroscopy (41 molecules comprising of 29 low-molecular weight molecules and 12 amino acids available in up to 2,639 participants) measured in the Center for Proteomics and Metabolomics, Leiden University Medical Center (Demirkan et al. 2015; Verhoeven et al. 2017); (4) Lipoprotein window-based NMR spectroscopy (104 lipoprotein particles size sub-fractions comprising of 28 VLDL components, 30 HDL components, 35 LDL components, 5 IDL components and 6 plasma totals, available in 2,609 participants) measured in the Center for Proteomics and Metabolomics, Leiden University Medical Center and lipoprotein sub-fraction concentrations were determined by the Bruker algorithm (Bruker BioSpin GmbH, Germany) as detailed in Kettunen et al. (Kettunen et al. 2016); (5) AbsoluteIDQ™ p150 Kit of Biocrates Life Sciences AG (153 molecules comprising of 14 amino acids, 91 phospholipids, 14 sphingolipids, 33 acyl-carnitines and hexose available in up to 989 participants) measured as detailed in publication from Draisma et al. (Draisma et al. 2015) and the experiments were carried out at the Metabolomics Platform of the Genome Analysis Center at the Helmholtz Zentrum München, Germany as per the manufacturer’s instructions. The laboratories had no access to phenotype information.

**Genome-wide SNP measurements**

Genotyping in ERF was performed using Illumina 318/370 K, Affymetrix 250 K, and Illumina 6K micro-arrays. All SNPs were imputed using MACH software (www.sph.umich.edu/csg/abecasis/MaCH/) based on the Hapmap2 (release 22, build 36). Individuals were excluded for excess autosomal heterozygosity, mismatches between called and phenotypic gender, and if there were outliers identified by an identical-by-state (IBS) clustering analysis.

**Defining ROHs and regression**

We used Hapmap2 (release 22, build 36) imputations for the extraction of homozygosity per loci per individual. The best guess information used for SNPs with imputation quality ($R^2$) > 0.95. The algorithm used to
define the region-wise homozygosity was developed by the ROHGEN consortium as explained before (CEBALLOS et al. 2018). In brief, the genome was divided into 3 Mb windows (n=992) and for each window, a plink sliding window routine was used to identify the proportion of >1.5 Mb homozygosity within the window. For each window, a maximum of one heterozygous SNP and five missing measurements were allowed. All SNP ids were mapped to the human genome build 19 (hg19) coordinates. The regression analyses were performed using mixed models, adjusting for genetic relatedness using a genomic kinship matrix; age, sex, and first 10 principal components were used as covariates in the model.

**Exome sequence measurements**

Exomes of 1,336 randomly selected individuals from the ERF study cohort were sequenced at the Center for Biomics of the Cell Biology department, at the Erasmus MC, The Netherlands. Sequencing was done at a median depth of 57x using the Agilent version V4 capture kit, on an Illumina Hiseq2000 sequencer, using the TruSeq Version 3 protocol. The sequence reads were aligned to hg19, using Burrows-Wheeler Aligner (BWA) and the NARWHAL pipeline (LI AND DURBIN 2009), (BROUWER et al. 2012) (Li et al. 2009). Subsequently, the aligned reads were processed further, using the IndelRealigner, MarkDuplicates and Table Recalibration tools from the Genome Analysis Toolkit (GATK) (MCKENNA et al. 2010) and Picard (http://picard.sourceforge.net). This was necessary to remove systematic biases and to recalibrate the PHRED quality scores in the alignments. After processing, genetic variants were called, using the Unified Genotyper tool from the GATK (MCKENNA et al. 2010). For each sample, at least four Gigabases of sequence was aligned to the genome. Functional annotations were also performed using the SeattleSeq annotation 138 database. About 1.4 million SNVs were called. After removing variants with low quality, out of Hardy-Weinberg equilibrium (HWE, P-value < 10⁻⁶) and low call rate (< 99%), and samples with a low call rate (< 90%), we retrieved 543,954 very high-quality SNVs in 1,327 individuals.

**Exome chip measurements**

Study participants from the ERF study whose exomes were not sequenced (N = 1,527) were genotyped on the Illumina Infinium HumanExome BeadChip, version 1.1, which contains over 240,000 exonic variants selected from multiple sources together spanning 12,000 samples from multiple ethnicities. Calling was performed with GenomeStudio. We removed subjects with a call rate < 95%, IBS > 0.99 and heterozygote ratio > 0.60. Ethnic
outliers identified using a principal component analysis with 1000 Genomes data and individuals with sex discrepancies were also removed. The SNVs that were monomorphic in our sample or had a call rate < 95% were removed. After quality control, we retrieved about 70,000 polymorphic SNVs in 1,515 subjects.

**Genetic association analyses**

**Exome chip and sequence-based genetic variants**

All tests were performed using RVtests (version 20150630), adjusted by age, sex and familial relatedness using a genome wide-autosomal kinship matrix (ZHAN et al. 2016). Single variant association analyses were performed for variants that have more than five copies of homozygous presentation in the population, using a recessive genetic model.

**Imputation-based genetic variants**

The SNPs with less than five minor allele counts were excluded. The exclusion criteria for SNPs were HWE with P-value < $1.0 \times 10^{-6}$ or SNP call rate < 98%. The associations of the significant metabolites within the genotype in these regions were performed by linear regression with ProbABEL software in both recessive model and dominant model in order to capture the homozygous model for both effect and non-effect alleles. The residuals of the metabolites with age, sex and family-relationship matrix were calculated by the polygenic function in R package GenABEL and used in the region-wide associations. The associations within the regions were performed using the 1000G phase 1, release v3. All SNP ids were mapped to hg19 coordinates. The genotype data of a population-based cohort, Rotterdam Study (IKRAM et al. 2017) (n = 6,291), was also used to replicate the findings.

Candidate genes within the ROH were selected by in-house developed automated pathway search algorithm as explained before (DEMIRKAN et al. 2015). The selection algorithm combines information from GTEx-eQTL, GWAS catalog, ConsensusPathDB (KAMBUROV et al. 2011), UniProtKB (MAGRANE 2011), OMIM (MCKUSICK 1998), TCDB (SAIER JR et al. 2006), ExPASy (GASTEIGER et al. 2003) and KEGG database (KANEHISA AND GOTO 2000) for each genetic loci.

**Results**

**ROH regression analysis**
In total, 47 metabolites selected in ERF population in an earlier report (DEMIRKAN et al. 2019) in addition to T2DM and five T2DM-related phenotypes were included in the ROH regression analysis with 992 windows constructed genome-wide. These analyses yielded 3,334 window-metabolite pairs with association p-value < 0.05. However, none of the associations passed either the false discovery rate (FDR), or Bonferroni threshold defined as correction by the number of windows tested times the independent dimensions in the highly correlated data (LI AND JI 2005) (0.05/ (992 × 27) = 2.02 × 10^{-6}). Out of 3,334, 1,734 association pairs showed consistent direction with the initially calculated association to inbreeding coefficients from our earlier report (DEMIRKAN et al. 2019). We focused on the suggestive top loci with association P-value < 1.0 × 10^{-3} for further analysis. These associations with ROH and phenotypes are given in Table 1. The list included 29 genomic loci identified as full or partial homozygosity within a 3MB, influencing 20 different outcomes, in total making up 51 suggestive pairs of association for follow-up. In some loci, the neighboring regions were correlated indicating that the ROH detected was indeed larger than 3MB window (Supplementary Figure 1). The top significant ROH which is shared by 33 individuals is located at 36 to 39 MB on chromosome 4 and is associated with four small size LDL components (S-LDL-Free cholesterol, S-LDL-phospholipids, S-LDL-cholesterol, S-LDL-ApoB) and included candidate genes TLR and PGM2. The second top ROH shared by 43 individuals is located at 63 to 66 MB on chromosome 3, and is associated with a lifetime risk of T2DM (β = 0.43, P-value=5.43 × 10^{-6}) and included candidate genes PSMD6 and ADAMTS9 (Table 1). ROH located at chromosome 4, 30-33 Mb was highest frequently shared (420 individuals). The strongest association was found between this ROH and S-LDL-Free cholesterol (β = 1.35, P-value = 7.48 × 10^{-5}), followed by S-LDL-cholesterol, S-LDL-phospholipids and S-VLDL-triglycerides. For the rest 26 top ROH the number of carrier individuals ranged between 20 to 118. Distributions of the 29 ROH in ERF population are provided in Supplementary Figure 2.

**Fine mapping within the ROHs**

**Association with exonic variants**

For each locus, the association for the minor allele under the recessive models has been tested, results are shown in Table 2a and Table 2b with sequence and chip-based genotyping sets respectively. We used three steps of variant filtering. First, we adopted a liberal variant list and included all the SNVs that are captured within the sequence data, also including the intergenic and intronic variants that are captured around the exons. For each region, we set up a region-wide Bonferroni threshold based on the number of SNVs tested and based on that we
identified the significantly associated SNVs. By this way five SNVs from exonic sequences were detected inside

genes TTC7A (rs57182920, intronic, with XL-HDL-Cholesterol), FRMD4B (rs73095903, intronic, with M-VLDL-TGs),
CSMD3 (rs72685825, intronic, with S-HDL-ApoA1), PREX1 (rs3746820, synonymous, with IDL-phospholipids),
LAM5 (rs35653162, intronic, with glucose) in addition to one SNV detected inside a non-transcribed pseudogene
EXOC5P1 (rs6551721, exonic, with Phosphatidylcholine diacyl C 40:6) as shown in Table 2 and Supplementary
Table 1. By using the exome chip derived genetic data, we detected three SNVs; one located inside KCNH7
(rs6759814, intronic, with valine), and two from intergenic regions (rs6469084, associated with S-HDL-ApoA1 and
rs6101991 associated with IDL-phospholipids) detected passing the pre-defined region-specific association
thresholds (Table 3 and Supplementary Table 2). Second, we limited the analysis to synonymous and non-
synonymous SNVs stop codons, UTR and splice variants only. By this way, additional two synonymous SNVs located
in OXR1 (rs1681904, with S-HDL-ApoA1) and FRMD4B (rs62254461, with M-VLDL-triglycerides) were found using
the exonic sequences (Table 2). Using the same approach one missense coding SNV in the exome chip was found
associated within KRT15 gene (rs1050784, with T2DM) (Table 3). Third, we filtered the dataset such that we
focused on only to those missense and premature stop codons. However, no SNVs were found associated using
such strict filtering.

**Association with common variants and intergenic regions**

Table 4 and Supplementary Table 3 shows the region-wide association with 1000 genome imputed
genotypes within the candidate ROHs. The locus zoom plots of loci with significant SNPs are given in
Supplementary Figure 3. In total, common genetic variants in six regions passed the region-wide FDR. These
included rs59997916 (intronic MAGI1, with T2DM), rs10866392 (intronic LINCO2506, with S-LDL-Free cholesterol),
rs73240383 (intronic, NWD2, with S-LDL-ApoB), rs71562230 (intergenic near SLC22A16, with S-VLDL-triglycerides),
rs1573707 (intronic, PTPRT, with IDL-Free cholesterol, IDL-phospholipids) and rs75320186 (upstream of BIRC7,
with glucose).

In order to investigate the effect of these variants in the outbred population, Rotterdam Study we set out
a replication. Out of the 18 genetic associations coming either from exome sequencing, exome chip or imputation
sets, we were able to test 12 in the Rotterdam study as 6 of the phenotypes were not available for replication.
(Table 5 and Supplementary Table 4). After correcting for multiple testing, we replicated two intronic genetic variants are located in genes KCNH7 (rs6759814) and PTPRT (rs1573707).

**Shared rare homozygous SNVs within the ROH regions**

In parallel to the association analysis explained above, we searched for overweight (BMI > 25.0 kg/m²), obese (BMI > 30.0 kg/m²) and T2DM cases among the ROH carriers who also share the same rare (MAF < 0.05) genetic variants identified by exome sequence or exome chip. By this way, we found additional 28 rare variants were shared exclusively by the ROH carriers within the genomic regions of interest. After exploring the phenotypes of the individuals homozygous for these exclusive 28 rare SNVs, for 18 of them, we detected an enrichment of cases of T2DM and obesity, clustering among 32 homozygous individuals. Eight out of 32 had T2DM (25.0%), 19 were overweight (59.4%) and 11 were obese (34.4%). Among the 18 rare SNVs, rs116175783 located in TBR1 can also be detected in the Rotterdam Study in eight homozygous persons. Six of them (75.0%) were overweight and one was obese in the Rotterdam Study. In ERF one out of the two homozygous individuals is obese and has T2DM, whereas the other homozygous individual is overweight (Table 6).

**Discussion**

We report an in-depth association mapping effort for a total of 53 selected metabolic phenotypes by using three sets of independently generated genotype data. First, we point out increased homozygosity in 29 genomic loci influencing 20 different outcomes, suggesting 51 outcome-genomic locus pairs to be investigated by more in-depth analysis. By using the recessive genetic model for association testing, we detected and replicated 2 intronic genetic variants inside genes KCNH7 and PTPRT. Additionally, within the ROHs we report 18 rare variants in a group of individuals enriched by cases of obesity and T2DM. Among these 18 rare variants, 17 were specific to ERF population, whereas one (rs116175783, located in TBR1) was also found in the Rotterdam study, and remarkably six out of eight homozygous individuals are overweight.

Evolutionary selection is less effective in eliminating recessive deleterious alleles since it needs two copies to reduce the fitness of the organism, those tend to become more frequent than expected. The most favorable setting to observe the effect of recessive deleterious genetic variants is consanguineous families or inbred populations. Harmful effects of close consanguinity in humans have been shown for several of outcomes including
intelligence (FAREED AND AFZAL 2014), schizophrenia (MANSOUR et al. 2010), bipolar disorder (MANSOUR et al. 2009), hypertension (RUDAN et al. 2003), heart disease (SHAMI et al. 1991), cancer (LEBEL AND GALLAGHER 1989), but notably also for metabolic health (ISAACS et al. 2007) and T2DM (GOSADI et al. 2014). Since consanguinity is strongly associated with risk for multifactorial disease, it is more than likely that unknown homozygous regions of the genome explain a significant portion of familial aggregation among other possible mechanisms (HOPPENBROUWERS et al. 2007). ROHs have been associated with several human traits such as personality (VERWEIJ et al. 2012), schizophrenia (KELLER et al. 2012), short stature (MCQUILLAN et al. 2012) and birth height (VERWEIJ et al. 2014).

To our knowledge, this is the first study investigating metabolomics phenotypes by homozygosity mapping. We show that the homozygosity of the minor C allele of synonymous SNV rs6759814 located in KCNH7 associates with branch chain amino acid valine in our study. KCNH7 belongs to the ERG subfamily of voltage-gated potassium channels and is widely expressed by the central nervous system. Voltage-gated potassium channels have diverse functions including regulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume.

Earlier we showed an association of another intronic genetic variant (rs1474260) in KCNH7 and a polyunsaturated ether-phosphatidylcholine (PCae36:5) in a meta-analysis, using the additive genetic model (DEMIRKAN et al. 2012). The second replicated SNV is another synonymous intronic variant (rs1573707) in PTPRT associated with IDL-Free cholesterol, IDL-phospholipids. PTPRT is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. It is of note that both KCNH7 and PTPRT are neuronal proteins associated with glutamate receptor signaling pathway (p-value=5.5 x 10^-4 for KCNH7 and p-value= 2.6 x 10^-7 for PTPRT gene expression in blood) as classified by Gene Ontology (GO)- biological process terms, and summarized by genenetwork.nl (accession date: 16.4.2019). Additionally, both proteins map to regulation of insulin secretion (p-value=6.6 x 10^-4 for PTPRT and p-value=3.5 x 10^-4 for KCNH7) among the biological processes classified by GO (www.genenetwork.nl, accession date: 16.04.2019). Finally, by looking at shared homozygous SNVs exclusively by the individuals sharing homozygous regions, we show that a rare genetic variant (rs116175783) shared by a total of 10 individuals in both ERF and Rotterdam Study is interesting as all of the individuals are either overweight or obese. TBR1 is also a neuronal protein mainly involved in the regulation of glutamergic synaptic transmission (P-value = 3.1 x 10^-7) associated with behavioral fear response (P-value = 2.7 x
Remarkably, feeding behavior (P-value = 8.1 x 10^{-3}) is one of the top GO terms for this gene. Based on GWAS using additive genetic model TBR1 was found associated with gene-based also associates with sodium in blood (P-value = 3.8 x 10^{-10}), educational attainment (P-value = 1.8 x 10^{-8}), diagnosed High blood pressure (2.7 x 10^{-8}) but also with BMI (6.1 x 10^{-6}) to some degree (http://atlas.ctglab.nl/PheWAS, accession date: 16.04.2019). In our study, the region harboring TBR1 was initially selected because the homozygosity was associated with a decrease in XL-HDL-cholesterol level.

Overall by combining the power of recessive inheritance in a genetically isolated population with a wide range of metabolic pathways, we pointed out several genetic loci that could be of interest for further research. By performing fine mapping within these genetic loci we found and replicated two genetic variants in i.e. KCNH7, PTPRT involved in glutameric synaptic pathways by using recessive genetic association models. In addition, we found a rare SNV in another synaptic gene, TBR1, for which the homozygous carriers are enriched in obese phenotype. Taken together our study underline the additional benefits of model supervised analysis but also seconds the involvement of the central nervous system in T2DM and obesity pathogenesis.
Acknowledgments

ERF was supported by the Consortium for Systems Biology (NCSB) both within the framework of the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO). ERF study as a part of EUROSPAN (European Special Populations Research Network) was supported by European Commission FP6 STRP grant number 018947 (LSHG-CT-2006-01947) and also received funding from the European Community's Seventh Framework Program (FP7/2007-2013)/grant agreement HEALTH-F4-2007-201413 by the European Commission under the program “Quality of Life and Management of the Living Resources” of 5th Framework Program (no. QLG2-CT-2002-01254) as well as FP7 project EUROHEADPAIN (nr 602633). High-throughput analysis of the ERF data was supported by a joint grant from Netherlands Organization for Scientific Research and the Russian Foundation for Basic Research (NWO-RFBR 047.017.043). High throughput metabolomics measurements of the ERF study has been supported by BBMRI-NL (Biobanking and Biomolecular Resources Research Infrastructure Netherlands). Ayse Demirkan is supported by a Veni grant (2015) from ZonMw. Ayse Demirkan, Jun Liu and Cornelia van Duijn have used exchange grants from PRECEDI. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscripts. ERF study is grateful to all study participants and their relatives, general practitioners and neurologists for their contributions and to P. Veraart for her help in genealogy, J. Vergeer for the supervision of the laboratory work and P. Snijders for his help in data collection. The scripts and analysis pipeline used for homozygosity mapping belong to the ROHGEN consortium (https://www.wiki.ed.ac.uk/display/ROHgen/ROHgen2) which ERF is a part of. We thank David Clarke and Jim Wilson for sharing the pipeline.

Author Contributions

A.D, J.L. and C.M.v.D contributed to study design. K.W.v.D. and C.M.v.D contributed to data collection. A.D., J.L., N.A., and J.B.v.K. contributed to data analysis. A.D., J.L. contributed to writing of manuscript. All the authors contributed to critical review of manuscript.

Competing interests

The authors declare no competing interests.
References

Brouwer, R. W., M. C. van den Hout, F. G. Grosveld and W. F. van Ijcken, 2012 NARWHAL, a primary analysis pipeline for NGS data. Bioinformatics 28: 284-285.

Ceballos, F. C., P. K. Joshi, D. W. Clark, M. Ramsay and J. F. Wilson, 2018 Runs of homozygosity: windows into population history and trait architecture. Nat Rev Genet 19: 220-234.

Demirkan, A., P. Henneman, A. Verhoeven, H. Dharuri, N. Amin et al., 2015 Insight in genome-wide association of metabolite quantitative traits by exome sequence analyses. PLoS Genet 11: e1004835.

Demirkan, A., J. Liu, N. Amin, K. W. v. Dijk and C. v. Duijn, 2019 Effect of inbreeding on type 2 diabetes-related metabolites in a Dutch genetic isolate. Unpublished.

Demirkan, A., C. M. van Duijn, P. Ugocsai, A. Isaacs, P. P. Pramstaller et al., 2012 Genome-wide association study identifies novel loci associated with circulating phospho- and sphingolipid concentrations. PLoS Genet 8: e1002490.

Draisma, H. H., R. Pool, M. Kobli, R. Jansen, A. K. Petersens et al., 2015 Genome-wide association study identifies novel genetic variants contributing to variation in blood metabolite levels. Nat Commun 6: 7208.

Fareed, M., and M. Afzal, 2014 Estimating the inbreeding depression on cognitive behavior: a population based study of child cohort. PLoS One 9: e109585.

Gasteiger, E., A. Gattiker, C. Hoogland, I. Ivanyi, R. D. Appel et al., 2003 ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic acids research 31: 3784-3788.

Gonzalez-Covarrubias, V., M. Beekman, H. W. Uh, A. Dane, J. Troost et al., 2013 Lipidomics of familial longevity. Aging Cell 12: 426-434.

Gosadi, I. M., E. C. Goyder and M. D. Teare, 2014 Investigating the potential effect of consanguinity on type 2 diabetes susceptibility in a saudi population. Hum Hered 77: 197-206.

Hoppenbrouwers, I. A., L. M. Cortes, Y. S. Aulchenko, K. Sintnicolaas, O. Njajou et al., 2007 Familial clustering of multiple sclerosis in a dutch genetic isolate. Mult Scler 13: 17-24.

Howrigan, D. P., M. A. Simonson, G. Davies, S. E. Harris, A. Tenesa et al., 2016 Genome-wide autozygosity is associated with lower general cognitive ability. Mol Psychiatry 21: 837-843.

Ikram, M. A., G. G. O. Brusselle, S. D. Murad, C. M. van Duijn, O. H. Franco et al., 2017 The Rotterdam Study: 2018 update on objectives, design and main results. Eur J Epidemiol 32: 807-850.

Isaacs, A., F. A. Sayed-Tabatabaei, Y. S. Aulchenko, M. C. Zillikens, E. J. Sijbrands et al., 2007 Heritabilities, apolipoprotein E, and effects of inbreeding on plasma lipids in a genetically isolated population: the Erasmus Rucphen Family Study. Eur J Epidemiol 22: 99-105.

Kamburov, A., K. Pentchev, H. Galicka, C. Wierling, H. Lehrach et al., 2011 ConsensusPathDB: toward a more complete picture of cell biology. Nucleic Acids Res 39: D712-717.

Kanehisa, M., and S. Goto, 2000 KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28: 27-30.

Keller, M. C., M. A. Simonson, S. Ripke, B. M. Neale, P. V. Gejman et al., 2012 Runs of homozygosity implicate autozygosity as a schizophrenia risk factor. PLoS Genet 8: e1002656.

Kettunen, J., A. Demirkan, P. Wurtz, H. H. Draisma, T. Haller et al., 2016 Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. Nat Commun 7: 11122.

Lebel, R. R., and W. B. Gallagher, 1989 Wisconsin consanguinity studies. II: Familial adenocarcinomatosis. Am J Med Genet 33: 1-6.

Li, H., and R. Durbin, 2009 Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1-6.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan et al., 2009 The Sequence Alignment/Map format and SAMtools. Bioinformatics 25: 2078-2079.

Li, J., and L. Ji, 2005 Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity (Edinb) 95: 221-227.

Liu, J., S. Semiz, S. J. van der Lee, A. van der Spek, A. Verhoeven et al., 2017 Metabolomics based markers predict type 2 diabetes in a 14-year follow-up study. Metabolomics 13: 104.

Magrane, M., 2011 UniProt Knowledgebase: a hub of integrated protein data. Database 2011.

Mansour, H., W. Fathi, L. Klei, J. Wood, K. Chowdari et al., 2010 Consanguinity and increased risk for schizophrenia in Egypt. Schizophr Res 120: 108-112.

Mansour, H., L. Klei, J. Wood, M. Talkowski, K. Chowdari et al., 2009 Consanguinity associated with increased risk for bipolar I disorder in Egypt. Am J Med Genet B Neuropsychiatr Genet 150B: 879-885.
McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis et al., 2010 The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20: 1297-1303.

McKusick, V. A., 1998 Mendelian inheritance in man: a catalog of human genes and genetic disorders. JHU Press.

McQuillan, R., N. Eklund, N. Pirastu, M. Kuningas, B. P. McEvoy et al., 2012 Evidence of inbreeding depression on human height. PLoS Genet 8: e1002655.

Rudan, I., N. Smolej-Narancic, H. Campbell, A. Carothers, A. Wright et al., 2003 Inbreeding and the genetic complexity of human hypertension. Genetics 163: 1011-1021.

Saier Jr, M. H., C. V. Tran and R. D. Barabote, 2006 TCDB: the Transporter Classification Database for membrane transport protein analyses and information. Nucleic acids research 34: D181-D186.

Santos, R. L., M. C. Zillikens, F. R. Rivadeneira, H. A. Pols, B. A. Oostra et al., 2006 Heritability of fasting glucose levels in a young genetically isolated population. Diabetologia 49: 667-672.

Shami, S. A., R. Qaisar and A. H. Bittles, 1991 Consanguinity and adult morbidity in Pakistan. Lancet 338: 954.

Verhoeven, A., E. Slagboom, M. Wuhrer, M. Giera and O. A. Mayboroda, 2017 Automated quantification of metabolites in blood-derived samples by NMR. Analytica Chimica Acta.

Vernon, H. J., 2015 Inborn Errors of Metabolism: Advances in Diagnosis and Therapy. JAMA Pediatr 169: 778-782.

Verweij, K. J., A. Abdellaoui, J. Veijola, S. Sebert, M. Koiranen et al., 2014 The association of genotype-based inbreeding coefficient with a range of physical and psychological human traits. PLoS One 9: e103102.

Verweij, K. J., J. Yang, J. Lahti, J. Veijola, M. Hintsanen et al., 2012 Maintenance of genetic variation in human personality: testing evolutionary models by estimating heritability due to common causal variants and investigating the effect of distant inbreeding. Evolution 66: 3238-3251.

Wang, C., H. Kong, Y. Guan, J. Yang, J. Gu et al., 2005 Plasma phospholipid metabolic profiling and biomarkers of type 2 diabetes mellitus based on high-performance liquid chromatography/electrospray mass spectrometry and multivariate statistical analysis. Anal Chem 77: 4108-4116.

Wang, T. J., M. G. Larson, R. S. Vasan, S. Cheng, E. P. Rhee et al., 2011 Metabolite profiles and the risk of developing diabetes. Nat Med 17: 448-453.

Zhan, X., Y. Hu, B. Li, G. R. Abecasis and D. J. Liu, 2016 RVTESTS: an efficient and comprehensive tool for rare variant association analysis using sequence data. Bioinformatics 32: 1423-1426.
| Loci | ROH regression homozygosity mapping top results with P-value < 1.0 × 10⁻³ |
|------|--------------------------------------------------|
| Chr  | Start   | Stop   | N ROH | Trait                        | β     | seβ   | P-value     | Inbreeding association | Glucose association | Candidate genes | Remarks                                      |
| 2    | 45M     | 48M    | 43    | XL-HDL-cholesterol            | -0.94 | 0.28  | 7.72 × 10⁻⁴ | (-)                    | (-)                | MSH2, PRKCE, SIX2, SIX3, intergenic, FLJ40172, MCFD2, TTC7A | HDL-C GWAS, response to statin therapy |
| 2    | 162M    | 165M   | 34    | Valine*, Isoleucine, Leucine  | 3.69  | 1.00  | 2.29 × 10⁻⁴ | (+)                    | (+)                | DPP4, FAP, KCNH7, GCG, IFI1 | T1DM |
| 3    | 63M     | 66M    | 33    | T2DM                          | 0.43  | 0.09  | 5.43 × 10⁻⁶ | (+)                    | (+)                | PSMD6, ADAMTS9 | T2DM |
| 3    | 66M     | 69M    | 31    | T2DM                          | 0.26  | 0.08  | 6.09 × 10⁻⁶ | (+)                    | (+)                | SUCLG2 | blood metabolite levels |
| 3    | 69M     | 72M    | 28    | M-VLDL-triglycerides*, M-VLDL-phospholipids | 5.00  | 1.42  | 4.34 × 10⁻⁴ | (+)                    | (+)                | PROK2, GPR27, MITF | obesity related |
| 4    | 27M     | 30M    | 41    | M-LDL-ApoB, M-LDL-phospholipids*, S-LDL-Free cholesterol | 4.68  | 1.26  | 2.12 × 10⁻⁴ | (+)                    | (+)                | none known | intergenic region |
| 4    | 30M     | 33M    | 420   | S-LDL-cholesterol, S-LDL-Free cholesterol*, S-LDL-phospholipids, S-VLDL-triglycerides | 1.35  | 0.34  | 7.48 × 10⁻⁵ | (+)                    | (+)                | PCDH7 | |
| 4    | 36M     | 39M    | 33    | LDL-phospholipids, S-LDL-cholesterol, S-LDL-ApoB | 2.08  | 0.45  | 5.02 × 10⁻⁶ | (+)                    | (+)                | TLR1, PGM2 | familial obesity |
| 4    | 39M     | 42M    | 25    | Citrate                       | 1.22  | 0.30  | 4.46 × 10⁻⁵ | (+)                    | (+)                | UCL1 | |
| 4    | 63M     | 66M    | 44    | Phosphatidylcholine diacyl C 40:6 | 11.16 | 3.29  | 7.29 × 10⁻⁴ | (+)                    | (+)                | TECRL | fatty acid biosynthesis |
| 4    | 66M     | 69M    | 118   | VLDL-ApoB                      | 2.95  | 0.85  | 5.63 × 10⁻⁴ | (+)                    | (+)                | intergenic | |
|   | 6 | 105M | 108M | 26 | Phosphatidylcholine diacyl C 40:6 | 17.97 | 5.18 | 5.57 × 10^-4 | (+) | (+) | STAP1, ATG5 | glucose homeostasis |
|---|---|------|------|---|---------------------------------|-------|------|--------------|-----|-----|-------------|------------------|
|   | 6 | 108M | 111M | 42 | S-VLDL-triglycerides             | 0.53  | 0.16 | 6.31 × 10^-4 | (+) | (+) | FOXO3       | glucose homeostasis, obesity |
|   | 6 | 111M | 114M | 34 | S-LDL-ApoB, S-VLDL-triglycerides* | 0.55  | 0.14 | 1.52 × 10^-4 | (+) | (+) | KIAA1919   | glucose transporter, blood metabolite levels |
|   | 8 | 102M | 105M | 31 | S-HDL-ApoA1, S-HDL-phospholipids  | 11.21 | 2.66 | 2.58 × 10^-5 | (+) | (+) | KLF10       | T2DM |
|   | 8 | 105M | 108M | 31 | S-HDL-ApoA1                      | 9.79  | 2.64 | 2.13 × 10^-4 | (+) | (+) | RIMS2       | LDL receptor related |
|   | 8 | 114M | 117  | 64 | S-HDL-ApoA1                      | 9.79  | 2.64 | 2.13 × 10^-4 | (+) | (+) | TRPS1       | HDL-C GWAS |
|   | 8 | 117  | 120  | 49 | S-HDL-ApoA1                      | 8.22  | 2.36 | 5.02 × 10^-4 | (+) | (+) | TNFRSF11B, SLC30A8 | T2DM, obesity, proinsulin, T1DM |
|   | 9 | 15M  | 18M  | 20 | Citrate                          | 1.09  | 0.31 | 3.86 × 10^-4 | (+) | (+) | TTC39B, RPL7P3, SH3GL2 | glycemic control in T1DM, metabolite levels, obesity |
|   | 9 | 78M  | 81M  | 29 | IDL-Free cholesterol             | 1.77  | 0.53 | 8.21 × 10^-4 | (+) | (+) | GCNT1, GNAQ, PCSK9 | |
|   | 10 | 0M   | 3M   | 32 | M-VLDL-triglycerides, M-VLDL-phospholipids* | 2.83  | 0.79 | 3.83 × 10^-4 | (+) | (+) | IDI1, IDI2 | obesity |
|   | 10 | 126M | 129M | 20 | WHR                              | 0.08  | 0.02 | 4.34 × 10^-4 | (+) | (+) | LHPP, METTL10, FAM53B, FANK1, ADAM12, DOCK1 | obesity |
|   | 12 | 15M  | 18M  | 33 | XL-LDL-phospholipids             | -4.35 | 1.29 | 7.31 × 10^-4 | (-) | (-) | MGP, MGST1 | T2DM |
|   | 17 | 39M  | 42M  | 62 | T2DM                             | 0.28  | 0.07 | 2.59 × 10^-5 | (+) | (+) | ACLY, STAT3, AOC3, SOST, G6PC, HCRT, MPP3 | T2DM |
|   | 20 | 27M  | 30M  | 74 | S-LDL-ApoB*, S-LDL-cholesterol, S-LDL-Free | 11.01 | 2.88 | 1.38 × 10^-4 | (+) | (+) | none | immune response |
| Loci, Chr, Start, Stop | Trait | N ROH | β  | seβ | P-value | Inbreeding association | Glucose association | Candidate genes | Remarks |
|------------------------|-------|-------|----|-----|---------|-----------------------|-------------------|-----------------|---------|
| 20 39M 42M 32          | cholesterol, S-LDL-phospholipids |        | 1.28 | 0.35 | 2.67 × 10⁻⁴ (+) (+) |          |                   | MAFB, PLCG1, LPIN3, TOP1 | phospholipid regulation |
| 20 45M 48M 43          | IDL-phospholipids, IDL-Free cholesterol |        | 1.41 | 0.42 | 8.49 × 10⁻⁴ (+) (+) |          |                   | PREX1, SLC2A10, SULF2, EYA2 | phospholipid regulation |
| 20 60M 63M 25          | glucose |        | 1.14 | 0.33 | 6.58 × 10⁻⁴ (+) (+) |          |                   | CDH4, TCFL5 | fasting glucose |
| 22 45M 48M 31          | S-HDL-phospholipids |        | 4.53 | 1.32 | 5.83 × 10⁻⁴ (+) (+) |          |                   | PPARA | phospholipid regulation |

**Loci, Chr, Start, Stop**: indicate the borders of the locus included as predictor in ROH regression; **N ROH**: Number of individuals that are fully or partly homozygous for the indicated locus; **Trait**: metabolomics traits or T2DM and related traits studies as outcomes. **β** and **seβ**: Effect estimate of the homozygous region and its standard error; **P-value**: nominal significance value of the **β**; **Inbreeding association**: The direction of association between inbreeding and the trait; **Glucose association**: The direction of association between glucose and the trait; **Candidate genes**: Genes that are found biologically plausible via automated pathway databases and text mining; **Remarks**: based on pathway databases and text mining. * Leading trait associated with the indicated loci, in case that there is associations with more than one trait.
Table 2. Association with exonic SNVs in the loci (NGS based measurements) using the recessive genetic model

| Chr | Start | Stop | Trait | N total/exonic | Bonferroni P-value total/exonic | Minimum P-value total/exonic | RsID SNV/exonic SNV | Gene |
|-----|-------|------|-------|---------------|-------------------------------|-------------------------------|------------------------|------|
| 2   | 45M   | 48M  | XL-HDL-cholesterol | 98/12 | 5.10 × 10^{-4} / 4.16 × 10^{-3} | 8.94 × 10^{-3} / 3.24 × 10^{-2} | rs57182920 / rs17480869 | TTC7A |
| 3   | 69M   | 72M  | M-VLDL-triglycerides*, M-VLDL-phospholipids | 36/7 | 1.39 × 10^{-3} / 7.14 × 10^{-3} | 1.10 × 10^{-3} / 2.31 × 10^{-3} | rs73095903 / rs62254461 | FRMD4B / FRMD4B |
| 4   | 63M   | 66M  | Phosphatidylincholine diacyl C 40:6 | 8/0 | 6.25 × 10^{-3} / na | 7.86 × 10^{-4} / na | rs6551721 / na | EXOC5P1 |
| 8   | 105M  | 108M | S-HDL-ApoA1 | 41/10 | 1.22 × 10^{-3} / 5.00 × 10^{-3} | 3.78 × 10^{-3} / 3.78 × 10^{-3} | rs1681904 / rs1681904 | OXR1 |
| 8   | 114M  | 117M | S-HDL-ApoA1 | 12/2 | 4.17 × 10^{-3} / 2.5 × 10^{-2} | 3.79 × 10^{-3} / 3.90 × 10^{-1} | rs72685825 / rs2219898 | CSMD3 |
| 20  | 45M   | 48M  | IDL-Free cholesterol, IDL-phospholipids | 67/22 | 7.46 × 10^{-4} / 2.27 × 10^{-3} | 4.52 × 10^{-4} / 4.52 × 10^{-4} | rs3746820 / rs3746820 | PREX1 |
| 20  | 60M   | 63M  | glucose | 186/56 | 2.69 × 10^{-4} / 8.93 × 10^{-4} | 2.79 × 10^{-9} / 3.12 × 10^{-3} | rs35653162 / rs1048802 | LAMA5 |

**Chr, Start, Stop**: indicate the borders of the locus included in association analyses; **Trait**: metabolomics traits or T2DM and related traits studies as outcomes; **N total/exonic**: Number of SNVs that are found to be homozygous at least among 5 individuals / Number of SNVs that are found to be homozygous at least among 5 individuals and have functional annotation indicating synonymous, non-synonymous, stop codon, UTR and splice variant; **Bonferroni P-value total/exonic**: Regional P-value threshold calculated according to the number of total/exonic SNVs; **Minimum P-value total/exonic**: P-value for the association of the top most significant SNV in the
region for all SNVs/ for the exonic SNVs; **RsID total/exonic** of the top most significant SNV in the region for all SNVs/ for the exonic SNVs; **Gene total/exonic** annotated to the top SNV/exonic SNV.
Table 3. Association with exonic SNVs in the loci (chip based measurements) using the recessive genetic model

| Chr | Start | Stop | Trait                          | N total/exonic | Bonferroni P-value total/exonic | Minimum P-value total/exonic SNVs | SNV/exonic SNV | Gene total/exonic SNV |
|-----|-------|------|--------------------------------|----------------|--------------------------------|-----------------------------------|----------------|----------------------|
| 2   | 162M  | 165M | Valine*, Isoleucine, Leucine   | 6/1            | 8.33 × 10⁻³ / 5.00 × 10⁻² | 2.37 × 10⁻³ / 2.32 × 10⁻¹         | rs6759814 / rs17783344             | KCNH7               |
| 8   | 105M  | 108M | S-HDL-ApoA1                    | 5/1            | 1.00 × 10⁻² / 5.00 × 10⁻² | 4.35 × 10⁻³ / 1.55 × 10⁻¹         | rs6469084 / rs2920048             | intergenic           |
| 9   | 78M   | 81M  | IDL-Free cholesterol           | 29/11          | 1.72 × 10⁻³ / 4.54 × 10⁻³ | 5.03 × 10⁻² / 5.03 × 10⁻²         | rs1110222 / rs1110222             | PCSK5               |
| 17  | 39M   | 42M  | T2DM                           | 30/27          | 1.67 × 10⁻³ / 1.85 × 10⁻³ | 1.72 × 10⁻³ / 1.70 × 10⁻³         | rs1050784 / rs1050784             | KRT15               |
| 20  | 39M   | 42M  | IDL-phospholipids              | 15/6           | 3.33 × 10⁻³ / 8.33 × 10⁻³ | 3.21 × 10⁻³ / 2.08 × 10⁻¹         | rs6101991 / rs6142884             | intergenic           |

**Chr, Start, Stop**: indicate the borders of the locus included in association analyses; **Trait**: metabolomics traits or T2DM and related traits studies as outcomes; **N total/exonic**: Number of SNVs that are found to be homozygous at least among 5 individuals / Number of SNVs that are found to be homozygous at least among 5 individuals and have functional annotation indicating synonymous, non-synonymous, stop codon, UTR and splice variant; **Bonferroni P-value total/exonic**: Regional P-value threshold calculated according to the number of total/exonic SNVs; **Minimum P-value total/exonic SNVs**: P-value for the association of the top most significant SNV in the region for all SNVs/ for the exonic SNVs; **RsID total/exonic** of the of the top most significant SNV in the region for all SNVs/ for the exonic SNVs; **Gene total/exonic annotated to the top SNV/exonic SNV.**
Table 4: Association analyses of imputed SNPs within the candidate ROHs using the recessive genetic model

| Chr | Start | Stop  | Trait                  | SNP        | MAF   | P-value     | FDR   | Gene region         |
|-----|-------|-------|------------------------|------------|-------|-------------|-------|---------------------|
| 3   | 63M   | 66M   | T2DM                   | rs59997916 | 0.05  | 4.03 × 10^{-7} | 0.003 | Intronic MAGI1       |
|     |       |       | S-LDL-cholesterol, S-LDL-Free cholesterol*, S-LDL-phospholipids, S-VLDL-triglycerides | rs10866392 | 0.14  | 1.60 × 10^{-5} | 0.039 | Intronic LINCO2506   |
| 4   | 36M   | 39M   | S-LDL-cholesterol, S-LDL-phospholipids, S-LDL-cholesterol, S-LDL-ApoB* | rs73240383 | 0.08  | 1.16 × 10^{-5} | 0.031 | Intronic NWD2        |
| 6   | 108M  | 111M  | S-VLDL-triglycerides   | rs71562230 | 0.06  | 1.34 × 10^{-5} | 0.018 | Intergenic near SLC22A16 |
| 20  | 45M   | 48M   | IDL-Free cholesterol, IDL-phospholipids* | rs1573707  | 0.48  | 5.62 × 10^{-5} | 0.022 | Intronic PTPRT       |
| 20  | 60M   | 63M   | glucose                | rs75320186 | 0.12  | 5.62 × 10^{-7} | 0.001 | Upstream gene variant BIRC7 |

Chr, Start, Stop: indicate the borders of the locus included in association analyses; Trait: metabolomics traits or T2DM and related traits studies as outcomes; SNP: Top most significant SNP in the locus; MAF: Minor allele frequency of the SNP; P-value: P-value for the association of the top most significant SNP in the region; FDR: Region-wide False Discovery Rate; Gene region: Closest gene defined based on Ensembl database look-up; n.d: Not determined.
Table 5 Replication of selected SNVs in Rotterdam Study using the recessive genetic model

| Chr | Start | Stop   | Trait                                             | RsID          | A1 | A2  | β        | P-value        | MAF |
|-----|-------|--------|--------------------------------------------------|---------------|----|-----|----------|----------------|-----|
| 2   | 162M  | 165M   | Valine*, Isoleucine, Leucine                      | rs6759814chip | C  | T   | 0.01    | 1.41 × 10^{-3} | 0.08|
| 20  | 45M   | 48M    | IDL-Free cholesterol, IDL-phospholipids*          | rs15737071000g| C  | T   | 0.01    | 1.99 × 10^{-3} | 0.45|

**Chr, Start, Stop**: indicate the borders of the locus included in association analyses; **Trait**: metabolomics traits or T2DM and related traits studies as outcomes; **RsID**: Top most significant genetic variant selected for replication; **A1**: effect allele, **A2**: non-effect allele; **β**: Effect estimate per 2 copies of A1 versus the rest of the genotypes; **P-value**: P-value for the β; **MAF**: Minor allele frequency of the genetic variant studied. The associations that pass the replication threshold as defined by 0.05/12 (number of genetic variants tested) are marked in italic/bold; **Chip/Seq/1000G**: indicating the genotyping platform of the variant in discovery and replication phase.
| Chr | Start | Stop  | Trait                          | SNVs with CAD >15 | Gene   | #individuals in ERF | Phenotype in ERF                                      | #individuals in RS | Phenotype in RS |
|-----|-------|-------|--------------------------------|--------------------|--------|--------------------|-------------------------------------------------------|--------------------|----------------|
| 2   | 45M   | 48M   | XL-HDL-cholesterol             | rs116175783        | TBR1   | 2                  | 1/2 has T2DM, 1/2 is overweight, 1/2 is obese         | SNV not found      | n.a            |
| 3   | 69M   | 72M   | M-VLDL-triglycerides*, M-VLDL-phospholipids | rs201353723        | MITF   | 3                  | 1/3 has T2DM, 2/3 are obese                           | SNV not found      | n.a            |
| 8   | 102M  | 105M  | S-HDL-ApoA1*, S-HDL-phospholipids | rs202243342        | KLF10  | 3                  | 2/3 are overweight, 1/3 is obese                      | SNV not found      | n.a            |
| 8   | 117M  | 120M  | S-HDL-ApoA1                    | rs2921788, rs2921783, rs6996153 | RAD21, RAD21, RAD21 | 2, 2, 2 | 1/2 has T2DM (same individual)                        | SNV not found      | n.a            |
| 9   | 15M   | 18M   | Citrate                        | rs187988729, rs144166918 | BNC2, CNTLN | 2, 6 | 1/6 T2DM, 1/6 overweight, 1/6 obese                  | SNV not found      | n.a            |
| 12  | 15M   | 18M   | XL-LDL-phospholipids           | rs145989508, rs61744792 | ERP27, intergenic | 2 | None T2DM, none overweight                            | SNVs does not exist | n.a            |
| 17  | 39M   | 42M   | T2DM                           | rs146311306        | ATP6V0A1 | 2                  | None T2DM, none overweight                            | SNV not found      | n.a            |
| 20  | 27M   | 30M   | S-LDL-ApoB*, S-LDL-cholesterol, S-LDL-Free cholesterol, S-LDL-phospholipids | rs183942485        | intergenic | 2                  | 1/2 is overweight, 1/2 is obese                       | SNV not found      | n.a            |
| 20  | 39M   | 42M   | IDL-phospholipids              | rs139346346, rs41277020, 20:39745167, 20:40899151, rs140143834 | CHD6, LPIN3, PTPRT, TOP1, CHD6 | 3, 3, 6, 6, 4 | 2/6 has T2DM, 3/6 are overweight, 3/6 are obese (20:39745167 and 20:40899151) | SNV not found      | n.a            |
| 20  | 45M   | 48M   | IDL-Free cholesterol, IDL-phospholipids* | rs112735799, rs201609680, rs73913405, rs139920008 | KCNB1, PREX1, SULF2, ZMYND8 | 3, 3, 2, 2 | 2/3 overweight (rs112735799 and rs201609680), 2/2 overweight (rs73913405) 2/2 T2DM, 2/2 are obese (rs139920008), | SNV not found      | n.a            |
| 20  | 60M   | 63M   | glucose                        | 20:61914992, rs73316017, rs150148851, 20:60902259, rs41309939, 20:61439121, 20:60963224 | ARFGAP1, BIRC7, COL9A3, intergenic, NKAIN4, OGFR, RPS21 | 3, 3, 2, 2, 3, 2, 4, 4 | 2/3 overweight, 1/3 is obese (20:61914992 and 20:60902259), 1/2 overweight (rs41309939, rs73316017 and rs150148851), 2/4 overweight, 2/4 obese (20:61439121 and 20:60963224) | SNV not found      | n.a            |
| 22  | 45M   | 48M   | S-HDL-phospholipids            | rs148939053        | ARHGAP8, PR, RS, ARHGAP8 | 2                  | 2/2 overweight                                         | Homozygous individuals not found | n.a            |
**Chr, Start, Stop**: indicate the borders of the locus included in association analyses; **Trait**: metabolomics traits or T2DM and related traits studies as outcomes; **SNVs, SNVs with CAD >15**: SNV within the ROH locus that is found exclusively among the individuals with full or partial ROH, SNVs with CADD score >15 are marked in italic/bold; **Gene, candidate gene**: Gene in which the SNV is annotated, biologically candidate genes are marked with italic/bold; **#individuals in ERF**: Number of individuals that are homozygous for the SNV in respective order to the SNV ids; **Phenotype in ERF**: Obesity and T2DM occurrence among the individuals that are homozygous for the indicated SNVs; **#individuals in RS**: Number of homozygous individuals in Rotterdam Study cohort for the indicated SNV; **Phenotype in RS**: Obesity and T2DM occurrence among the individuals that are homozygous for the indicated SNVs, in the Rotterdam Study cohort; * Discovered by exome array, n.a.: Not available