Mitochondrial GWA Analysis of Lipid Profile Identifies Genetic Variants to Be Associated with HDL Cholesterol and Triglyceride Levels

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Abstract

It has been suggested that mitochondrial dysfunction has an influence on lipid metabolism. The fact that mitochondrial defects can be accumulated over time as a normal part of aging may explain why cholesterol levels often are altered with age. To test the hypothesis whether mitochondrial variants are associated with lipid profile (total cholesterol, LDL, HDL, and triglycerides) we analyzed a total number of 978 mitochondrial single nucleotide polymorphisms (mtSNPs) in a sample of 2,815 individuals participating in the population-based KORA F4 study. To assess mtSNP association while taking the presence of heteroplasmy into account we used the raw signal intensity values measured on the microarray and applied linear regression. Ten mtSNPs (mt3285, mt3336, mt5285, mt6591, mt6671, mt9163, mt13855, mt13958, mt14000, and mt14580) were significantly associated with HDL cholesterol and one mtSNP (mt15074) with triglycerides levels. These results highlight the importance of the mitochondrial genome among the factors that contribute to the regulation of lipid levels. Focusing on mitochondrial variants may lead to further insights regarding the underlying physiological mechanisms, or even to the development of innovative treatments. Since this is the first mitochondrial genome-wide association analysis (mtGWAS) for lipid profile, further analyses are needed to follow up on the present findings.

Introduction

Cholesterol is a lipid which is vital for the normal functioning of the body [1]. Having an excessively high level of total cholesterol (TC) itself does not cause any symptoms, but it increases the risk of serious health conditions [2]. Cholesterol is carried around the body in the blood by
lipoproteins, in particular Low-density lipoprotein (LDL), High-density lipoprotein (HDL), and very low density lipoprotein (VLDL). LDL carries cholesterol from the liver to the cells. If there is too much cholesterol for the cells to use, it can build up in the artery walls, leading to atherosclerosis [3, 4]. HDL carries cholesterol away from the cells and back to the liver, where it is either broken down or prepared to be excreted from the body as a waste product. High concentrations of HDL particles have protective value against cardiovascular diseases [5]. VLDL contains the highest amount of triglycerides (TG), which have been linked to atherosclerosis and the subsequent risk of heart diseases and stroke [6]. A high TG level combined with a low HDL or high LDL concentration can speed up the process of plaque formation in the arteries resulting in atherosclerosis. The balance of cholesterol levels is important not just for cardiovascular health [7–10] but also for mental health [11, 12]. Control of cholesterol might reduce the brain plaques linked to Alzheimer’s disease [13]. It has been also suggested that an excess of cholesterol in mitochondria can result in mitochondrial dysfunction and impairment of specific carriers (e.g. mitochondrial transport of cellular glutathione) through alterations in the mitochondrial membrane order [14–16]. Moreover, previous findings have associated an excessive accumulation of cholesterol in mitochondria with neurodegeneration and myocardial ischemia injury [17, 18] as well as with an increased mitochondrial damage in cardiovascular tissues [19]. An excess production of reactive oxygen species (ROS) in mitochondria, accumulation of mitochondrial DNA (mtDNA) damage, and progressive respiratory chain dysfunction have been related to atherosclerosis [20–23].

The primary function of mitochondria is to generate large quantities of energy in the form of adenosine triphosphate (ATP). mtDNA is of approximately 16.6 kb and codes for 13 genes of the mitochondrial respiratory chain complexes, 2 ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes that are required for mitochondrial protein synthesis. Mitochondria consume oxygen and substrates to generate the vast majority of ATP while producing ROS, also called free radicals, in the process. An excess of ROS may damage DNA, proteins, and lipids if not rapidly quenched. This damage, termed oxidative stress, has been suggested to influence cholesterol flux [24, 25].

The 13 structural genes are essential for energy production through the process of oxidative phosphorylation (OXPHOS) that consists of five enzyme complexes (I–V). In addition to supplying cellular energy and involvement in oxidative stress, mitochondria also participate in a wide range of other cellular processes, including signal transduction, cell cycle regulation, thermogenesis, and apoptosis. Mitochondrial mutations can be both somatic and inherited through the maternal line [26]. One peculiarity of mtDNA is the heteroplasmy effect which was originally believed to be a rare phenomenon. Since many mtDNA copies are present in a cell and due to their high mutation rate, new mutations may arise among many of other mtDNA, consequently mutant and wild-type mtDNA can co-exist [27]. For this reason there is heterogeneity of mtDNA within an individual, and even within the same cell. The clinical expression of some phenotypes is determined by the relative proportion of wild-type and mutant mitochondrial genetic variants in different tissues [27]. Variants of mtDNA are under a growing scientific spotlight and there is increasing evidence that these mutations play a central role in many human diseases.

Despite the physiological role of cholesterol in mitochondria, the mechanisms involved in the trafficking to this compartment are poorly understood [28]. Genome-wide association studies and human genetic studies have identified a number of genes and genetic regions affecting cholesterol profile (including TC, HDL, LDL) and TG [7, 8, 10, 29–36]. Nevertheless, none of them have investigated the mitochondrial genome. The purpose of the current study was to conduct a mitochondrial GWAS to identify genetic variants influencing cholesterol phenotypes, including TC, HDL, LDL, and TG. In particular, we tested 978 mtSNPs in a population-based sample of 2,815 adults, aged 31–85 years.
Methods

Study design and population
The Cooperative Health Research in the Region of Augsburg (KORA) study is a series of independent population-based epidemiological surveys and follow-up studies of participants living in the region of Augsburg, in southern Germany, an area with demographic and socioeconomic characteristics roughly reflecting those of an average central European population. The study was approved by the local ethics committee (Bayerische Landesärztekammer). All participants are residents of German nationality identified through the registration office and written informed consent was obtained from each participant [37]. The study was approved by the local ethics committee. All participants filled in a self-administrated questionnaire and underwent a standardized personal interview and an extensive medical examination. All procedures were subjected to quality assessment. The study design, sampling method, and data collection have been described in detail elsewhere [38]. The present study includes data of the study KORA F4 (2006–2008) including a total number of 2,815 unrelated individuals. No evidence of population stratification has been found in multiple published analyses using the KORA cohort. Ascertainments of anthropometric measurements and personal interviews, as well as laboratory measurements of persons, from the KORA F4 have been described elsewhere [39]. In order to avoid confounding with insulin-dependent diabetes mellitus, 213 individuals diagnosed with type 2 diabetes were not included in the study, i.e., the 2,815 individuals considered in our analysis do not include persons affected by type 2 diabetes.

Genotyping and genotype calling
DNA was extracted from full blood after the blood draw and then stored at -80°C. Only single-nucleotide polymorphisms located in the mitochondrial genome (mtSNPs) were considered in this study. Genotyping was performed using the following platforms: Affymetrix 6.0 GeneChip array (465 mtSNPs), Affymetrix Axiom chip array (252 mtSNPs), Illumina Human Exome Beadchip array (226 mtSNPs), and Illumina MetaboChip 200K (135 mtSNPs). The Affymetrix 6.0 chip was genotyped only for a subgroup of 1,814 randomly selected participants of KORA F4. All other chips were genotyped for the whole KORA F4 dataset. The number of individuals used in this analysis corresponds to those that passed genotyping QC, have available phenotype information, and are not affected with type 2 diabetes.

Most of the covered mtSNPs have distinct positions identified by different chips. Although the Affymetrix 6.0 is the one containing the largest number of mtSNPs some regions are not well covered. The Illumina Metabochip contains the smallest number of mtSNPs and many regions are uncovered, especially the hypervariable regions of the mtDNA control region (HVR I and HVR II) as well as the CO1 and CO2 genes. However, when all chips are considered together, good overall coverage of the mitochondrial genome is obtained [40]. Standard genotype calling may be controversial when applied to mtSNPs due to the possible occurrence of heteroplasm. mtDNA tends to be heterogeneous in the sense that different mitochondria of an individual can have different genotypes, such that at an mtSNP may not be restricted to 0, 1, or 2 minor alleles. This issue affects the possibility of estimating genotypes and makes the calling algorithms useless. Therefore, whenever one intends to identify susceptibility genes located in the mtDNA it is recommended to account for heteroplasm by using individual-level allele frequencies obtained from intensity values [40] or sequencing data rather than genotype calls obtained by algorithms that were designed for nuclear SNPs.
Cholesterol phenotypes (TC, HDL, LDL) and TG

All KORA F4 participants were subjected to several medical examinations including measures of cholesterol phenotypes and fasting TG. TC levels are based on the HDL, LDL and TG levels (TC = HDL + LDL + 0.2 × TG). There are several advantages to analyzing cholesterol phenotypes as a quantitative phenotype in a representative population-based sample of subjects. The quantitative nature of the phenotype increases the power of the study considerably. The use of a general population sample as KORA F4 reduces the number of subjects taking antilipidemic medication compared with patient groups. The distribution of characteristics of the study population is given in Table 1.

Quality control

Quality control for the signal intensity values was performed for each genotyping chip as described in detail elsewhere [40]. An attempt to remove the chip-specific global background intensity was made by computing, separately for each individual, the 5% quantile intensity and subtracting it from all intensities. In a second step, the intensities were quantile normalized applying the method proposed by Bolstad et al. [41] and implemented in the limma R package [42]. After quantile normalization log₂ intensity ratios, log₂(C0/A), were computed for each individual and an iterative outlier detection procedure was applied [40]. A summary of the quality control results is given in Table 2. From the original number of mtSNPs, 63 (5.8%) mtSNPs Associated with HDL Cholesterol and Triglycerides Levels

Table 1. Distribution of characteristics of the study population.

| Chip              | Affy. 6.0 | Affy. Axiom | Illum. Exome | Illum. Metabochip |
|-------------------|-----------|-------------|--------------|-------------------|
| Sample size       | 1640      | 2721        | 2710         | 2804              |
| Males Females     | 786       | 1426        | 1334         | 1470              |
| Mean age          | 60.4±8.8  | 60.1±8.7    | 55.6±13.2    | 55±13             |
| Mean TC           | 218.5±38.1| 228.5±38.7  | 215±38.6     | 219.2±39.9        |
| Mean HDL          | 51±12.8   | 62.4±14.4   | 50.7±12.4    | 61.8±14.3         |
| Mean LDL          | 139±33.7  | 142.7±35.9  | 139±33.2     | 135±35.9          |
| Mean TG           | 147.7±108.8| 111.8±59.8 | 140.7±102.9 | 104.6±59.5        |

Sample size is based on the particular chip. Total sample size is 2,815 independent individuals. One person may be present on more than one chip. Distributions are presented as means ± standard deviation.

doi:10.1371/journal.pone.0126294.t001

Cholesterol phenotypes (TC, HDL, LDL) and TG

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Table 2. Summary of the quality control.

| Chip              | mtSNPs | mtSNPs excludedUB | Sample size | ISNP | Intensity Ratio | Outliers |
|-------------------|--------|--------------------|-------------|------|----------------|----------|
| Affy. 6.0         | 411    | 0                  | 1,647       | 3    | 230 (<0.05%)   |          |
| Affy. Axiom       | 215    | 37                 | 2,731       | 4    | 42 (<0.05%)    |          |
| Illum. Exome      | 226    | 0                  | 2,721       | 1    | 128 (<0.05%)   |          |
| Illum. Metabo     | 126    | 0                  | 2,815       | 1    | 98 (<0.05%)    |          |

The number of mtSNPs refers to the SNPs that passed QC and were included in the analysis. Several mtSNPs were excluded due to the upper bound cut-off (UB) [77] or because the basepair position was not available in Build 38 (no_B38). Sample size is based on the particular chip. Total sample size is 2,803 independent individuals. One person may be present on more than one chip. ISNP stands for the number of intensity measures per allele. Intensity Ratio represents the total number of intensity measures in the sample (ISNP*2*sample-size*mtSNPs).

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doi:10.1371/journal.pone.0126294.t002
were removed because their position could not be placed in Build 38. For the Axiom chip, 37 mtSNPs (17\%) were removed due to an upper bound cut-off that has been described in detail in our previous paper [40]. A total number of 498 (<0.05\%) intensity ratios were considered to be outliers and removed from the analysis.

**Statistical method**

To approach the presence of heteroplasmy present in the mitochondria we used the raw signals of luminous intensity, where every measurement is associated with a specific mtSNP and represents one of its alleles. The number of measures \( n \) per mtSNP depends on the vendor-specific technology employed on the genotyping chip. To assess association of cholesterol phenotypes with the mtSNPs intensities we applied linear regression analysis using cholesterol levels as outcome. The mtSNP enters the model as a covariate via the log\(_2\)-transformed intensity ratio, \( \log_2(-A/B) \), where \(-A\) and \(-B\) denote the mean intensity over \( n \) measures, or single measure in case of \( n = 1 \), for the A allele and B allele (minor allele), respectively. We center this variable \( (z = \log_2(-A/B) - \mu) \) as well as the additional quantitative covariate age at examination, to improve the convergence properties of the model estimates. Sex is also introduced in the model as covariate with male as a baseline. Each type of genotyping chip needs to be analyzed separately because different chips make use of different technologies, even between chips of the same manufacturer. In each of the analyses \( p \)-values are obtained from a Wald test and adjusted for multiple comparisons applying the Bonferroni correction method with the correction factor being equal to the number of mtSNPs used in the analysis. All the analyses were performed with the statistical software R v3.1.0 [43]. For more details about the statistical method we refer to [40].

**Results**

After QC, a total number of 978 mtSNPs were included in the analysis. The resulting significant \( p \)-values are plotted in Fig 1 for each phenotype. A more detailed figure for each genotyping chip and phenotype is provided in S1 Fig. No significant mtSNPs were obtained for TC. However, when analysing cholesterol subtypes, ten mtSNPs for HDL cholesterol and one mtSNP for TG reached significance after correcting for multiple testing. The association results that remained significant after adjustment for multiple testing \( (P_{\text{adjusted}} \leq 0.05) \) are presented in Table 3. Some of the significant mtSNPs from the Affymetrix chip (mt3336, mt5285, and

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**Fig 1. Illustration by phenotype of the 11 significant mtSNPs after correcting for multiple testing.** On the y axis, the \( p \)-values transformed into the negative of the base 10 logarithm, \(-\log_{10}(p\text{-value})\), are shown. The x-axis represents the mitochondrial genome for each phenotype.

doi:10.1371/journal.pone.0126294.g001
mt14000) were also present in other chips. Although these variants also resulted to be nominal-
ly significant when analyzing the other chips, they lost their significance after adjusting for
multiple testing (see Table 3).

A negative parameter estimate for the mtSNP ($\beta_{SNP} < 0$) indicates that the minor allele is
associated with an increase of the level of cholesterol subtype while a $\beta_{SNP} > 0$ indicates that
the major allele is associated with high levels of cholesterol subtype.

**TG levels**

For TG levels the only significant mtSNP was found in the MT-CYB gene (mt15074T→C). The presence of mt15074T→C heteroplasmy with more C than T alleles results in a higher level of TG than having only T alleles ($\beta_{mt15074T→C} = -14.9$). In the regression model for this mtSNP the estimates of sex and age were both significant with $\beta_{sex} < 0$ and $\beta_{age} > 0$, indicating significantly higher TG levels in males than in females and with older age.

**HDL cholesterol**

Six of the ten significant mtSNPs for HDL cholesterol (mt3336T→G, mt5285A→G,
mt13855C→T, mt13958G→C, mt14000T→A, and mt14580A→G) are located in the NADH

| Chip | Bp | rs_number | Alleles | Point mutation | $\beta_{SNP}$ | $P_{nominal}$ ($P_{adj}$) | Overlap Chip: $P_{nominal}$ | Protein: Gene |
|------|----|-----------|---------|---------------|-------------|---------------- |------------------|-------------|
| **HDL** |
| Affy.6.0 | 3285 | rs28537613 | T→A (n. a.) | - | 3.49 | 2.2x10^{-07} (8.3x10^{-05}) | - | tRNA{Leu(UUR)}: MT-TL1 |
| Affy.6.0 | 3336 | rs28416101 | T→G (0.0033) | missense | 0.91 | 1.2x10^{-06} (4.8x10^{-04}) | Axiom: 0.022 | ND1: MT-ND1; subunit of NADH dehydrogenase, complex I |
| Affy.6.0 | 5285 | rs28357986 | A→G (0.0030) | synonymous | -6.63 | 8.9x10^{-05} (3.6x10^{-02}) | Axiom: 0.013 | ND2: MT-ND2; subunit of NADH dehydrogenase, complex I |
| Affy.6.0 | 6591 | rs28483589 | C→A (n. a.) | missense | -3.09 | 4.5x10^{-05} (1.8x10^{-02}) | - | COI: gene MT-COI; subunit of cytochrome c oxidase, complex IV |
| Affy.6.0 | 6671 | rs1978028 | T→C (0.0189) | synonymous | -2.09 | 9.1x10^{-05} (3.7x10^{-02}) | - | COI: gene MT-COI; subunit of cytochrome c oxidase, complex IV |
| Affy.6.0 | 9163 | rs2298010 | G→A (0.0004) | missense | 4.47 | 1.5x10^{-05} (6.3x10^{-03}) | - | ATP6: MT-ATP6; subunit of ATP synthase, complex V |
| Affy.6.0 | 13855 | rs3925298 | C→T (0.0011) | synonymous | -5.19 | 4.1x10^{-05} (1.7x10^{-02}) | - | ND5: MT-ND5; subunit of NADH dehydrogenase, complex I |
| Illum. Exome | 13958 | rs202081448 | G→C (0.0037) | missense | -2.32 | 1.9x10^{-04} (4.2x10^{-02}) | - | ND5: MT-ND5; subunit of NADH dehydrogenase, complex I |
| Affy.6.0 | 14000 | rs28359185 | T→A (0.0100) | missense | 4.48 | 9.4x10^{-05} (3.9x10^{-03}) | Axiom: 8.3x10^{-03} Exome: 2.1x10^{-03} Metabo: 1.0x10^{-4} | ND5: MT-ND5; subunit of NADH dehydrogenase, complex I |
| Affy.6.0 | 14580 | rs28496897 | A→G (0.0004) | synonymous | 1.87 | 3.5x10^{-05} (1.4x10^{-02}) | - | ND6: MT-ND6; subunit of NADH dehydrogenase, complex I |
| **Triglycerides** |
| Illum. Exome | 15074 | rs201169089 | T→C (n. a.) | missense | -14.9 | 7.5x10^{-05} (1.6x10^{-02}) | - | CYTB: MT-CYB, cytochrome c reductase, complex III |

Genomic position in base pairs (bp), alleles, rs_number, and point mutation are based on the NCBI dbSNP GRCh38 human genome assembly (rCRS, GeneBank ID J01415.2). Alleles are given in terms of major—minor allele. The population minor allele frequency “maf” is based on 2,704 individuals provided by mitomap (http://www.mitomap.org). Note that these allele frequency estimates do not account for the presence of heteroplasmy. An estimated effect size ($\beta_{SNP}$) < 0 indicates that the risk allele is the minor allele. Nominal p-values and adjusted p-values are provided. mtSNPs mt3336, mt5285 and mt14000 are also included in other chips.

Genes: MT-CYB, cytochrome c reductase, complex III

mt14000) were also present in other chips. Although these variants also resulted to be nominal-
ly significant when analysing the other chips, they lost their significance after adjusting for
multiple testing (see Table 3).

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## TG levels

For TG levels the only significant mtSNP was found in the MT-CYB gene (mt15074T→C). The presence of mt15074T→C heteroplasmy with more C than T alleles results in a higher level of TG than having only T alleles ($\beta_{mt15074T→C} = -14.9$). In the regression model for this mtSNP the estimates of sex and age were both significant with $\beta_{sex} < 0$ and $\beta_{age} > 0$, indicating significantly higher TG levels in males than in females and with older age.

## HDL cholesterol

Six of the ten significant mtSNPs for HDL cholesterol (mt3336T→G, mt5285A→G, mt13855C→T, mt13958G→C, mt14000T→A, and mt14580A→G) are located in the NADH
subunit dehydrogenase genes of complex I (\textit{MT-ND1}, \textit{MT-ND2}, \textit{MT-ND5}, and \textit{MT-ND6}), the others are located in the ATP synthase subunit 6 (\textit{MT-ATP6}), cytochrome c oxidase subunit 1 (\textit{MT-CO1}), and tRNA (\textit{MT-TL1}).

Taking the most strongly associated variant mt3285T→A, in the tRNA, \textit{MT-TL1} gene, based on our estimates ($\beta_{mt3285T→A} = 3.49$) HDL cholesterol increases with a higher proportion of T alleles at this locus, making the T allele favourable to HDL. Similar arguments can be applied to the other mtSNPs. The regression models for all significant mtSNPs regarding HDL showed no significant effect for age; however, the estimate of $\beta_{sex}$ was significant with $\beta_{sex}>0$ indicating significantly higher levels of HDL cholesterol in females than in males.

Discussion

We performed a mitochondrial GWA analysis for the lipid profile including TC, TG, HDL cholesterol, and LDL cholesterol. Ten mtSNPs were significant for HDL and one mtSNP for TG. No significant results were observed for TC and LDL cholesterol. The possible role of mitochondria in the regulation of the lipid profile is mainly concerned with ROS production [24, 25].

Triglycerides

\textit{MT-CYB\textsubscript{mt15074T→C}}: Mitochondrially encoded cytochrome b, complex III, catalyzes the transfer of electrons from ubiquinol to cytochrome c and utilizes the energy to translocate protons from inside the mitochondrial inner membrane to outside. Complex I and complex III are considered as the major ROS sources [44]. It has been shown that inhibition of complex III trigger the accumulation of TG in 3T3-L1 cells [45, 46]. Mutations of the \textit{MT-CYB} gene have also been related to exercise intolerance [47–49]. Recently, association of \textit{MT-CYB\textsubscript{15059G→A}} mutation heteroplasmy with essential hypertension has been suggested [50].

Oxidative stress may also result from the metabolic impact of intracellular TG. Lipids and glucose that are not needed for immediate use are stored in adipose tissues and liver in the form of TG in lipid droplets. It has been demonstrated that hyperglycemia (excess of glucose) induces production of ROS [51, 52], which further causes partial inhibition of the electron transport in complex III [52].

The estimates of the regression model with \textit{MT-CYB\textsubscript{mt15074T→C}} variant are in line with the fact that males have higher TG levels than females [53, 54] and increase with older age since as people age and gain weight, TG levels generally increase.

HDL cholesterol

Among the ten mtSNPs identified in this study for HDL cholesterol six are located in NADH genes, the rest are located in the \textit{MT-TL1}, \textit{MT-ATP6}, and \textit{MT-CO1}.

\textit{MT-ND1\textsubscript{mt3336T→G}}, \textit{MT-ND2\textsubscript{mt5285A→G}}, \textit{MT-ND5\textsubscript{mt13855C→T}}, \textit{mt13958G→C}, \textit{mt14000T→A}, and \textit{MT-ND6\textsubscript{mt14580A→G}}: Mitochondrially encoded NADH dehydrogenase subunits, complex I, extracts energy from NADH, produced by the oxidation of sugars and lipids, and traps the energy in a potential difference or voltage across the mitochondrial inner membrane. The potential difference is used to power the synthesis of ATP. Because complex I is central to energy production in the cell, its malfunction may result in a wide range of disorders. Some of them are due to mutations, while others, which result from a decrease in the activity of complex I, or an increase in the production of ROS, are not yet well understood. Despite the fact that one study demonstrated that the polymorphism \textit{MT-ND2\textsubscript{mt5178A→C}} is associated with HDL-C levels in Japanese subjects [55], very little has been reported about relationships between NADH subunits and HDL.
**MT-TL1 mt3285T→A**: Mitochondrially encoded tRNA leucine 1 gene (MT-TL1) provides instructions for making a specific form of tRNA that is designated as tRNA\(^{Leu}({UUUR})\). Mutations of the MT-TL1 gene may play a pathogenic role in the formation of atherosclerotic lesions of human arteries, causing various defects in the protein chains of some tRNA, synthesized directly in the mitochondria. This leads to a decrease in the concentration of these enzymes and their tRNA or total dysfunction, which contributes to the development of oxidative stress and increases the probability of occurrence and development of atherosclerosis [56]. Particularly the variant \(MT-TL1\)\(_{mt3256C→T}\) has been related to atherosclerosis predisposition [57]. There is unequivocal evidence of an inverse association between plasma HDL cholesterol concentrations and the risk of cardiovascular disease, a finding that has led to the hypothesis that HDL protects from atherosclerosis [58].

**MT-ATP6 mt9163G→A**: Mitochondrially encoded ATP synthase, complex V, is an important enzyme that produces most of cellular ATP. Alteration of ATP synthase biogenesis may cause two types of isolated defects: either the enzyme is structurally modified and does not function properly, or it is present in abnormal amounts. In both cases the cellular energy provision is impaired, which leads to a dysregulation of ROS production [59]. The presence of two principal proteins of the mitochondrial ATP synthase, \(β\)-chain and \(α\)-chain, on the surface of human hepatocytes have been associated with HDL catabolism for the control of cholesterolemia [60, 61]. However, how the cell directs these proteins towards the cell surface and how their cell-surface expression is regulated remain unknown and require further investigation.

**MT-CO1 mt6591C→A, mt6671T→C**: Mitochondrially encoded cytochrome c oxidase subunits, complex IV, is a key oxidative enzyme regarded as one of the major regulation sites for the OXPHOS system, controlled by both nDNA and mtDNA. Its catalytic activity is primarily determined by three of the 13 subunits which are encoded by the mtDNA (\(MT-CO1, MT-CO2,\) and \(MT-CO3\)) [62]. The loss of function of this enzyme has been suggested to trigger ROS production, although the increase in radical accumulation rests with non-mitochondrial sources [63]. However, the function of each subunit and the molecular mechanism behind the regulation of the activity of this important protein complex are largely unknown [64]. Although a direct relationship between HDL and variants in the \(MT-CO1\) genes has not been reported yet, a significant inverse correlation between the hepatic \(MT-CO1\) methylated/unmethylated DNA ratio and HDL has been observed [65].

The regression models for all significant mtSNPs identified for HDL cholesterol in this study also corroborate the generally acknowledged fact that females have significantly higher HDL cholesterol values than males [66–68]. Progesterone, anabolic steroids and male sex hormones (testosterone) also lower HDL cholesterol levels while female sex hormones raise HDL cholesterol levels. Age was not significant in our study, meaning that HDL cholesterol levels do not vary with age, a finding also reported from other studies [69, 70]. However TC and LDL cholesterol levels tend to vary with age [70, 71].

It has been suggested that HDL potentially inhibits apoptosis in endothelial cells [72–74]. This effect is paralleled by decreased intracellular generation of ROS and diminished levels of apoptotic markers, suggesting that it can be related to the intracellular antioxidative actions of HDL or HDL components. HDL is also able to inhibit generation of ROS in vitro under conditions of cell culture [74, 75].

Some of the variants identified in this study are missense mutations which lead to an amino acid change, thus being a non-synonymous variant. So, individuals with an excess of missense mutations may carry an appreciable fraction of an altered protein that is responsible for altering the levels of the phenotype. Other variants are synonymous, i.e., they code for the same amino acid. How an excess of synonymous mutations at this locus could impact the levels of HDL cholesterol needs further investigation, since the single nucleotide change leads to an
unchanged protein. However, different codons might lead to different protein expression levels. Based on these findings we hypothesize that levels of HDL and TG are attributable at least in part to mitochondrial polymorphisms. Animal and human data consistently show that mitochondria are altered in aging, leading to increased mutations in mtDNA, decreased expression of some mitochondrial proteins, reduced enzyme activity, and altered respiration with reduced maximal capacity in sedentary adults. The possible role of mitochondria in the regulation of HDL cholesterol and TG is mainly concerned with ROS production. However, the complexity of mitochondrial ROS metabolism suggests that interventions such as the administration of one or a few antioxidants may be too simplistic. A more complete approach to antioxidant therapy might be to decrease ROS generation (for example, by expressing uncoupling proteins) and to upregulate the multilayered endogenous mitochondrial and intracellular antioxidant defense network [76]. However, this will require a considerably better understanding of ROS biology than we have at present.

Conclusions

In summary, our study reports eleven mitochondrial genetic variants, ten of which are significantly associated with HDL cholesterol and one with TG levels, indicating that the presence of heteroplasmy in these variants may influence the balance of HDL cholesterol and TG levels. Although further analyses are needed to follow up on the present results, these findings highlight the important role of the mtDNA among the factors that contribute to the balance of the lipid profile in adults and suggest that variants in the mitochondrial genome may be more important than has previously been suspected.

Supporting Information

S1 Fig. Mitochondrial genome-wide P values by chip and phenotype. On the y axis, p-values transformed into the negative of the base 10 logarithm, \(-\log_{10}(p\text{-value})\), are shown. The x-axis represents the mitochondrial genome, displaying the position and relative size of each of the 13 major mitochondrial genes, 12S and 16S rRNAs, hypervariable region 1 (HVR I), hypervariable region 2 (HVR II) as well as the position of the 22 tRNAs (gray). The left side illustrates the results for TC and the right side illustrates the results for cholesterol subtypes (LDL, HDL, and triglycerides). The dashed lines show the critical values of the pointwise significance level corresponding to \(\alpha = 0.05\).

(TIFF)

Acknowledgments

We thank all members of the field staff involved in the KORA study and we express our appreciation to all study participants.

Author Contributions

Conceived and designed the experiments: AF KS. Performed the experiments: HG MW ER SZ HP. Analyzed the data: AF SR. Contributed reagents/materials/analysis tools: AF KS HG TM AP. Wrote the paper: AF. Critical revision of the manuscript for intellectual content: KS CM.

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