Abstract

Hypercholesterolemia is characterized by high plasma LDL cholesterol and often caused by genetic mutations in LDL receptor (LDLR), APOB, or proprotein convertase subtilisin/kexin type 9 (PCSK9). However, a substantial proportion of hypercholesterolemic subjects do not have any mutations in these canonical genes, leaving the underlying pathobiology to be determined. In this study, we investigated to determine whether combining plasma metabolomics with genetic information increases insight in the biology of hypercholesterolemia. For this proof of concept study, we combined plasma metabolites from 119 hypercholesterolemic females with genetic information on the LDL canonical genes. Using hierarchical clustering, we identified four subtypes of hypercholesterolemia, which could be distinguished along two axes represented by triglyceride and large LDL particle concentration. Subjects with mutations in LDLR or APOB preferentially clustered together, suggesting that patients with defects in the LDLR pathway show a distinctive metabolomics profile. In conclusion, we show the potential of using metabolomics to segregate hypercholesterolemic subjects into different clusters, which may help in targeting genetic analysis.—Zhang, X., A. Rimbert, W. Balder, A. H. Zwinderman, J. A. Kuivenhoven, G. M. Dallinga-Thie, and A. K. Groen. Use of plasma metabolomics to analyze phenotype-genotype relationships in young hypercholesterolemic females. J. Lipid Res. 2018. 59:2174–2180.

Supplementary key words hypercholesterolemia • triglyceride • low density lipoprotein • genetics • metabolomics

Hypercholesterolemia due to a high concentration of plasma LDL cholesterol has been shown to be a causal factor in accelerating atherosclerosis in a plethora of studies (1, 2). The liver plays a pivotal role in the regulation of cholesterol metabolism. It secretes cholesterol packaged in VLDL particles that are subsequently converted into IDL and LDL particles, largely by the action of different lipases in the periphery (3). A key step in the uptake of cholesterol is the internalization of LDL via the LDL receptor (LDLR) (4). Mutations in the LDLR as well as mutations in genes encoding APOB or proprotein convertase subtilisin/kexin type 9 (PCSK9) are causally related with hypercholesterolemia (5). These genetic mutations, however, do not explain all hypercholesterolemic cases. For instance, in the UK pilot cascade project, 403 of 635 (63.5%) hypercholesterolemic subjects did not have mutations in LDLR, APOB, or PCSK9 (6). In a recent large scale study designed to evaluate the prevalence of a familial hypercholesterolemia (FH) mutation among individuals with severe hypercholesterolemia (7), only 24 of 1,386 subjects with LDL cholesterol above 5 mmol/l were identified to have mutations in these three canonical genes. Although the prevalence of genetically defined hypercholesterolemia varies across studies (8), a substantial proportion of hypercholesterolemic subjects do not have mutations in LDLR, APOB, or PCSK9. A major reason for this finding could be the presence of disease-causing mutations in other genes involved in cholesterol homeostasis either affecting the LDLR pathway or other yet to be defined mechanisms. Interestingly, whole exome sequencing of a cohort with FH subjects

Abbreviations: FH, familial hypercholesterolemia; LDLR, LDL receptor; PC, principal component; PCA, principal component analysis; PCSK9, proprotein convertase subtilisin/kexin type 9; wGRS, weighted genetic risk score.

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without mutations in LDLR, APOB, and PCSK9 did not identify novel causal mutations (9).

Recently, we analyzed a cohort of 119 young females with plasma LDL cholesterol above the 99th percentile for their age. In 20 hypercholesterolemic females, we identified 12 causal heterozygous mutations in LDLR and one causal heterozygous mutation in APOB (10). In the 99 remaining females, we found eight subjects carrying a variant in LDLR or APOB with unknown clinical significance (10). This left us with 91 females that suffered from hypercholesterolemia caused by either a polygenic (11) or epigenetic (12) mechanism, or the presence of a pathogenic variant in yet unknown genes. To get further insight into the underlying pathobiology of hypercholesterolemia of unknown origin, we performed plasma metabolite analysis on all of the 119 hypercholesterolemic females. We hypothesized that mutations in genes belonging to the same metabolic pathway (e.g., the LDLR pathway) should render a similar plasma metabolome. This analysis differentiated four subgroups, which could be distinguished along two axes represented by plasma triglyceride and large LDL particle concentration.

MATERIALS AND METHODS

Participants

The selection of the participants (N = 119) in this study is described in detail elsewhere (10). In brief, these women were apparently healthy, aged 25–40 years, and had a plasma LDL cholesterol level above 4.7 mmol/l. Exclusion criteria were diagnosis described in detail elsewhere (10). In brief, these women were with 91 females that suffered from hypercholesterolemia caused by either a polygenic (11) or epigenetic (12) mechanism, or the presence of a pathogenic variant in yet unknown genes. To get further insight into the underlying pathobiology of hypercholesterolemia of unknown origin, we performed plasma metabolite analysis on all of the 119 hypercholesterolemic females. We hypothesized that mutations in genes belonging to the same metabolic pathway (e.g., the LDLR pathway) should render a similar plasma metabolome. This analysis differentiated four subgroups, which could be distinguished along two axes represented by plasma triglyceride and large LDL particle concentration.

Next generation sequencing

With a custom target sequencing array developed based on the SureSelect capture system, we sequenced the coding regions of 11 genes, including LDLR, APOB, PCSK9, LDLRAP1, APOE, ABCG5, LIPA, STAP1, MTTP, ANGPTL3, and SAR1B to assess a monogenic cause of hypercholesterolemia. If a mutation had a minor allele frequency below 0.1% in the Genome of the Netherlands (13), it was considered a rare mutation. Mutations that are verified to cause hypercholesterolemia were listed in our previous publication (10). Detection of copy number variations was performed using Copy Number Variation Detection in Next-generation Sequencing Gene (CoNvDINGs) panels (14). Detected copy number variations were validated using either multiplex ligation-dependent probe amplification or by long-range PCR or real-time PCR (10).

Genetic risk score calculation

To study a possible polygenic cause of hypercholesterolemia, we calculated the weighted genetic risk score (wGRS). The Global Lipid Genetic Consortium meta-analysis of genome-wide association studies identified 95 loci affecting LDL cholesterol concentration (15). Among these loci, 12 SNPs had the highest power to discriminate between FH mutation-negative individuals and the general population (11, 16). For each individual, we calculated the wGRS using the weighted sum of the risk allele (the LDL cholesterol-raising allele) (10). The weights used were the corresponding per-allele effect in plasma LDL cholesterol changes reported by the Global Lipid Genetic Consortium (15).

Lifestyle score calculation

To investigate the association between lifestyle and plasma metabolome in hypercholesterolemic females, we used a recently described healthy lifestyle score (17). Points were given for the major lifestyle parameters, including smoking status and eating habits. The details were described in our previous publication (10). In short, a maximum of four points reflects a very healthy lifestyle: the smaller the score, the less healthy the lifestyle. The minimum point is zero.

Metabolite measurements

Fasting plasma samples were routinely collected by Lifelines (www.lifelines.nl) and stored at −80 °C until analysis on the Nightingale metabolomics platform (Nightingale Health, Finland). This platform includes 225 metabolic features, including lipids, lipoproteins, fatty acids, amino acids, and glycolysis precursor molecules (listed on https://nightingalehealth.com/biomarkers), using a NMR spectroscopy platform (18, 19).

Statistical analysis

To explore the subtypes of hypercholesterolemia, we performed hierarchical clustering based on the plasma metabolomics data. Because the metabolomics data contains measurements of different units, we first scaled the data so that every variable had mean 0 and standard deviation 1. Next, we ran the hierarchical clustering with the function, hclust, from R (https://cran.r-project.org/). We used Euclidean distance as the dissimilarity measure and complete linkage as the similarity measure between the clusters. The dendrogram was made by using the gg dendro function and ggplot2 (20) R package. Finally, we cut the dendrogram into four clusters by using the cutree function in R.

To identify the cluster corresponding to hypercholesterolemia due to defects in the LDLR pathway, we performed principal component analysis (PCA) on the metabolomics data. Because the data contains measurements of different units, we converted the metabolomics data into ranks, so that every metabolite had a value ranging between 1 and 119. We then calculated the covariance matrix and performed eigenvector decomposition. Entries of every eigenvector are also called loadings. Based on the loadings, we identified metabolites that most correlated to the first and second principal components (PCs) by calculating the Spearman correlation coefficients.

To evaluate associations between genetic risk/lifestyle scores and metabolite concentrations, we applied a nonparametric method, namely, the Kendall’s tau correlation test. We reported the Kendall’s tau correlation coefficient and P value. A P value below 0.05 is considered significant.

RESULTS

A group of 119 young women with hypercholesterolemia, defined as plasma LDL cholesterol levels above the

| TABLE 1. Characteristics of 119 hypercholesterolemic females |
|------------------------------------------------------------|
|                  | Clinical Chemistry | Nightingale Metabolomics | Spearman Correlation Coefficients |
| LDL cholesterol (mmol/l) | 5.25 ± 0.50         | 2.27 ± 0.26               | 0.66                              |
| Total cholesterol (mmol/l) | 7.17 ± 0.64         | 5.57 ± 0.43               | 0.68                              |
| Triglyceride (mmol/l) | 1.50 ± 0.68         | 1.45 ± 0.47               | 0.96                              |
| HDL cholesterol (mmol/l) | 1.39 ± 0.28         | 1.47 ± 0.22               | 0.84                              |
| ApoB (g/l) | 1.25 ± 0.14         | 1.10 ± 0.11               | 0.78                              |

Data are expressed as mean ± SD; N = 119; Age (year), 32.90 ± 4.37; BMI, 27.9 ± 5.10.
99th percentile for their age, was selected from the Life-
lines cohort. The baseline characteristics are presented in 
Table 1. To analyze the underlying pathobiology of the hy-
percholesterolemic phenotype, plasma metabolomics was 
performed using the Nightingale platform. Although the 
absolute values measured in the Nightingale platform are 
lower than the conventional measured plasma lipids, the 
two measurements showed a similar pattern (Table 1). A 
summary of all the results of metabolite analysis is pre-
presented in supplemental Table S1. Hierarchical clustering 
analysis of the metabolomics data set revealed three main 
clusters and one cluster containing only one sample (Fig. 1). 
The size of clusters 1, 2, 3, and 4 was 43, 15, 60, and 1, 
respectively.

To analyze the divergence of the different clusters, we 
rang PCA. The first and second PC explained 38% and 
21% of the total variance of the metabolic variables across 
the 119 individuals, respectively (Fig. 2). To understand 
which metabolites corresponded to the first and second 
PC the most, we calculated the Spearman correlation co-
efficients between original variables and PCs (supple-
mental Table S2). We observed that plasma triglyceride 
and large LDL particle concentration were the most cor-
related variables with the PC1 (Spearman correlation co-
efficient \( \rho = -0.988 \)) and PC2 (Spearman correlation co-
efficient \( \rho = -0.978 \)), respectively. Therefore, we used these 
two variables to represent the axes of PC1 and PC2 (Fig. 3). 
Our next question was whether the four clusters derived
from the hierarchical clustering analysis (Fig. 1) were indeed separated by PC1 and PC2. To answer that, we added the hierarchical clustering results to the scatterplot (Fig. 3). Inspection reveals that the females in cluster 3 are separated from the other groups by showing a high plasma large LDL particle concentration coupled with relatively low plasma triglyceride, suggesting a defect in hepatic LDL uptake.

Because we sequenced LDLR, APOB, and PCSK9 in all subjects, we could verify whether the females with known heterozygous mutations in the LDLR pathway plotted in the region of cluster 3. Indeed, from 20 subjects with
heterozygous mutations in \textit{LDLR} or \textit{APOB}, 15 subjects were located in cluster 3 (Fig. 4). The other five carriers were found in cluster 1 (n = 3) and cluster 2 (n = 2). In addition, we identified eight women who were heterozygous carriers of a novel variant in \textit{LDLR} or \textit{APOB} from which the pathogenicity has not yet been determined. Five of these eight subjects were positioned in cluster 3 and three in cluster 1 (Fig. 5).

To improve our understanding of the underlying pathobiology of the elevated plasma LDL cholesterol in the remaining 91 women, we calculated the wGRS and lifestyle score, and assessed the associations between both scores and plasma concentrations of large LDL particles and triglyceride. As shown in supplemental Figs. S1 and S2, no relation could be demonstrated between both scores and plasma large LDL particle concentration (wGRS: Kendall tau correlation coefficient $-0.017$, $P = 0.80$; lifestyle score: Kendall tau correlation coefficient $-0.04$, $P = 0.57$). Both scores showed moderate association with plasma triglyceride concentration (wGRS: Kendall tau correlation coefficient $-0.156$, $P = 0.02$; lifestyle score: Kendall tau correlation coefficient $-0.198$, $P = 0.0099$).

**DISCUSSION**

In the current study, we showed that combining plasma metabolomics data with genetic information can improve our understanding of the origin of severe hypercholesterolemia in young healthy women. These analyses may help with the diagnosis and personalized treatment of patients with hypercholesterolemia in which no causal mutations in the canonical LDL genes can be identified.

Metabolic profiling has been used in a large number of cohort studies to assess the value of circulating metabolites in prediction of risk for cardiovascular events (21, 22). More specifically, metabolomics has been used to study associations between circulating metabolites and statin usage (23), CETP inhibition (24), and PCSK9 inhibition (25), generating insight into the broad metabolic effects of these interventions. Nightingale metabolomics data contain not only concentrations in different units but also other quantities, such as ratios, percentages, degrees of saturation, and lipoprotein particle size. Therefore, in the current study, we scaled all the metabolic variables to make them have equal importance in the hierarchical clustering.

The hierarchical clustering analysis revealed four clusters in the 119 hypercholesterolemic females with plasma LDL cholesterol above the 99th percentile for their age. We hypothesized that mutations in genes belonging to the same metabolic pathway (e.g., the LDLR pathway) should render a similar plasma metabolome (one cluster). The PCA revealed that plasma triglyceride and large LDL particle concentrations were the major discriminators for the four clusters. Because cluster 3 is characterized by a high concentration of large LDL particles and relatively low triglyceride in plasma, we hypothesized that this cluster represented the hypercholesterolemia due to defective LDL clearance. Incorporation of the genetic information provided us with the verdict, because we expected the 20 subjects carrying a known functional heterozygous mutation in \textit{LDLR} or \textit{APOB} to position in cluster 3. Indeed, 15 subjects fit this hypothesis and were located in cluster 3.
Then we came up with the question: “Can we get insight into whether a novel variant in LDL or APOB is the underlying cause for the severe hypercholesterolemia based on the metabolome profile?” Indeed, six out of eight carriers of a novel mutation fit in cluster 3, suggesting potential effects of these variants on LDLR-mediated uptake. This observation suggests that metabolic profiling is useful to delineate the subjects with a pathogenic mutation from those that do not carry any variant in either LDLR or APOB. However, not all subjects in cluster 3 carry a variant in LDLR or APOB. We realize that the pathway of LDLR-mediated endocytosis and intracellular cholesterol trafficking contains many more genes (26–28) than we have sequenced in our cohort. So expansion of the number of genes on the chip or choosing whole genome sequencing will ultimately improve the information on all genes involved in the LDLR pathway and may thus help to identify additional genetic variants underlying the pathobiology in the remaining 40 females in cluster 3. Meanwhile, we cannot exclude other processes underlying the hypercholesterolemia, such as epigenetic changes (12), lincRNA (29), microRNA (30), or combinations thereof.

Cluster 4 contained only one subject, and the individual had the highest large LDL particle concentration among the 119 hypercholesterolemic females. Interestingly, we did not identify any mutations in the sequenced genes, including LDLR, APOB, and PCSK9. This female subject was 28 years old with a BMI of 21.7 kg/m². Her waist circumference was 69 centimeters. When we compared her plasma metabolomics data to the other 118 hypercholesterolemic females, we identified 77 outlier variables [either below the first quantile (1.5 × interquartile range) or above the third quantile (1.5 × interquartile range); supplemental Table S3]. We noticed that this female had a high proportion of esterified cholesterol in VLDL and HDL particles compared with the remaining 118 subjects. Interestingly, the CETP/g−/apoCI−/− mouse model showed a very similar phenotype (31). APOC1 is an important regulator for CETP activity, which may partly underlie the observed phenotype (32). So far, no mutations in APOC1 have been described.

A recent study (33) showed that hypercholesterolemic subjects without any known genetic defect had lower levels of LDL cholesterol than those with a mutation. Therefore, we hypothesized that the origin of the hypercholesterolemia in cluster 1 may be either polygenic or due to lifestyle factors. After additional analysis of the relationships between the wGRS or lifestyle score and triglyceride or large LDL particle concentration, we observed that only genetic risk scores were negatively associated with triglyceride concentration (Kendall tau correlation coefficient −0.23, \( P = 0.04 \)). This observation suggests that this cluster of hypercholesterolemic subjects may be caused by less damaging mutations in genes involved in the LDLR pathway. The major observation in the subjects located in cluster 2 is that they had elevated plasma triglyceride. The genetic array used in the current study does not contain the genes involved in triglyceride metabolism. Our data suggest that generation of a triglyceride-specific gene array may generate interesting results in the subjects in this cluster.

In summary, this study shows that bioinformatic analysis of metabolomics data derived from hypercholesterolemic subjects generates interesting clusters of patients that may help to guide targeted genomics approaches for hypercholesterolemia.

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REFERENCES

1. Ference, B. A., H. N. Ginsberg, I. Graham, K. K. Ray, C. J. Packard, E. Bruckert, R. A. Hegele, R. M. Krauss, F. J. Raal, H. Schunkert, et al. 2017. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. a consensus statement from the European Atherosclerosis Society Consensus Panel. Eur. Heart J. 38: 2430–2450. 
2. Goldstein, J. I., and M. S. Brown. 2015. A century of cholesterol and coronaries: from plagues to genes to statins. Cell. 161: 161–172.
3. Packard, C. J., and J. Shepherd. 1997. Lipoprotein heterogeneity and apolipoprotein B metabolism. Arterioscler. Thromb. Vasc. Biol. 17: 3542–3556.
4. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. Science. 232: 54–47.
5. Sourat, A. K., and R. P. Naoumova. 2007. Mechanisms of disease: genetic causes of familial hypercholesterolemia. Nat. Clin. Pract. Cardiovasc. Med. 4: 214–225.
6. Taylor, A., D. Wang, K. Patel, R. Whitall, G. Wood, M. Farrier, R. D. G. Neely, S. Faugrieve, D. Nair, M. Barbar et al. 2010. Mutation detection rate and spectrum in familial hypercholesterolaemia patients in the UK pilot cascade project. Clin. Genet. 77: 572–580.
7. Khera, A. V., H. W. Won, G. M. Peloso, K. S. Lawson, T. M. Bartz, X. Deng, E. M. van Leeuwen, P. Natarajan, C. A. Emdin, A. G. Bick, et al. 2016. Diagnostic yield and clinical utility of sequencing familial hypercholesterolemia genes in patients with severe hypercholesterolemia. J. Am. Coll. Cardiol. 67: 2578–2589.
8. Wang, J., J. S. Dron, M. R. Ban, J. F. Robinson, A. D. McIntyre, M. Alazzam, P. J. Zhao, A. A. Dilliot, H. Cao, M. W. Huff, et al. 2016. Polygenic versus monogenic causes of hypercholesterolemia ascertained clinically. Arterioscler. Thromb. Vasc. Biol. 36: 2439–2445.
9. Futema, M., V. Plagnol, K. Li, R. A. Whitall, H. A. W. Neil, M. Seed, S. B. Consortium, S. Bertolini, S. Calandra, O. S. Descamps, et al. 2014. Whole exome sequencing of familial hypercholesterolaemia patients negative for LDLR/APOB/PCSΚ9 mutations. J. Med. Genet. 51: 537–544.
10. Balder, J., A. W. Rimbart, X. Zhang, M. Vel, R. Kanninga, F. van Dijk, P. Lansberg, R. Sinke, and J. A. Kuivenhoven. 2018. Genetics, lifestyle, and low-density lipoprotein cholesterol in young and apparently healthy women. Circulation. 137: 820–831.
11. Talnud, P. J., S. Shah, R. Whitall, M. Futema, P. Howard, J. A. Cooper, S. C. Harrison, K. Li, F. Drenos, F. Karpe, et al. 2015. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolemia: a case-control study. Lancet. 381: 1303–1309.
12. Dekkers, K. F., M. van Iterson, R. C. Bertolini, S. Calandra, O. S. Descamps, et al. 2014. Whole exome sequencing of familial hypercholesterolaemia patients negative for LDLR/APOB/PCSΚ9 mutations. J. Med. Genet. 51: 537–544.
13. Genome of the Netherlands Consortium. 2014. Whole-genome sequence variation, population structure and demographic history of the dutch population. Nat. Genet. 46: 818–825.
14. Johansson, L. F., F. van Dijk, E. N. de Boer, K. K. van Dijk-Bos, J. D. H. Jongbloed, A. H. van der Hout, H. Westers, R. J. Sinke, M. A. Swertz, R. H. Sijmons, et al. 2016. GoNaDING: single exon variation detection in targeted NGS data. Hum. Mutat. 37: 457–464.
15. Teslovich, T. M., K. Musunuru, A. V. Smith, A. C. Edmondson, I. M. Stylianou, M. Koski, J. P. Pirruccello, S. Ripatti, D. I. Chasman, C. J. Willer, et al. 2010. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 466: 707–713.
16. Talmud, P. J., F. Drenos, S. Shah, T. Shah, J. Palmen, C. Verzilli, T. R. Gaunt, J. Pallas, R. Lovering, K. Li, et al. 2009. Gene-centric association signals for lipids and apolipoproteins identified via the HumanCVD BeadChip. Am. J. Hum. Genet. 85: 628–642.

17. Khera, A. V., C. A. Emdin, I. Drake, P. Natarajan, A. G. Bick, N. R. Cook, D. I. Chasman, U. Baker, R. Mehran, D. J. Rader, et al. 2016. Genetic risk, adherence to a healthy lifestyle, and coronary disease. N. Engl. J. Med. 375: 2349–2358.

18. Fischer, K., J. Kettunen, P. Würtz, T. Haller, A. S. Havulinna, A. J. Kangas, P. Soininen, T. Esko, M-L. Tammesoo, R. Mägi, et al. 2014. Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. PLoS Med. 11: e1001606.

19. Soininen, P., A. J. Kangas, P. Würtz, T. Tynkkynen, D. Prieto-Merino, T. Tillin, A. Ghorbani, A. Artati, Q. Wang, M. Tiainen, et al. 2015. Metabolite profiling and cardiovascular event risk: A prospective study of 3 population-based cohorts. Circulation. 131: 774–785.

20. Holmes, M. V., I. Y. Millwood, C. Kartsonaki, M. R. Hill, D. A. Bennett, R. Boxall, Y. Guo, X. Xu, Z. Bian, R. Hu, et al.; China Kadoorie Biobank Collaborative Group. 2018. Lipids, lipoproteins, and metabolites of cholesterol homeostasis. J. Lipid Res. 59: 144–154.

21. Wüst, P., Q. Wang, P. Soininen, A. J. Kangas, G. Fatemifar, T. Tynkkynen, M. Tiainen, M. Perola, T. Tillin, A. D. Hughes, et al. 2016. Metabolomic profiling of statin use and genetic inhibition of Hmg-CoA reductase. J. Am. Coll. Cardiol. 67: 1200–1210.

22. Kettunen, J., M. V. Holmes, E. Allara, O. Anufrieva, P. Ohukainen, C. Oliver-Williams, T. Tillin, A. Hughes, M. Kahonen, T. Lehtimäki, et al. 2018. Lipoprotein signatures of cholesterol ester transfer protein and HMG-CoA reductase inhibition. Accessed April 5, 2018, at https://www.biorxiv.org/content/early/2018/04/05/295394.

23. Sliz, E., J. Kettunen, M. V. Holmes, C. Oliver-Williams, C. Boachie, Q. Wang, M. Mannikko, S. Sebert, R. Walters, K. Lin, et al. 2018. Metabolomic consequences of genetic inhibition of PCSK9 compared with statin treatment. Accessed March 14, 2018, at https://www.biorxiv.org/content/early/2018/03/14/278861.

24. Marques-Pinheiro, A., M. Marduel, J. P. Rabès, M. Devillers, L. Villéger, D. Allard, J. Weissbach, M. Guerin, Y. Zair, D. Erlich, et al. 2010. A fourth locus for autosomal dominant hypercholesterolemia maps at 16q22.1. Eur. J. Hum. Genet. 18: 1236–1242.

25. Bartuzi, P., D. D. Billadeau, R. Favier, S. Rong, D. Dekker, A. Fedosieienko, H. Fieten, M. Wijers, J. H. Levels, N. Huijkaas, et al. 2016. HMG-CoA reductase mediated endosomal sorting of LDL receptors is blunted in dyslipidemic patients with coronary artery disease. J. Lipid Res. 57: 1209–1209.

26. Paththinige, C. S., N. D. Sirisena, and V. Dissanayake. 2017. Genetic determinants of inherited susceptibility to hypercholesterolemia - a comprehensive literature review. Lipids Health Dis. 16: 103.

27. Hu, Y-W., J-Y. Yang, X. Ma, Z-P. Chen, Y-R. Hu, Y-J. Zhao, S-F. Li, Y-R. Qin, J-B. Lu, Y-C. Wang, et al. 2014. A lincRNA-DYNL1RB2-GPR119/GLP1R/ABCA1-dependent signal transduction pathway is essential for the regulation of cholesterol homeostasis. J. Lipid Res. 55: 681–697.

28. Irani, S., J. Iqbal, W. J. Antoni, L. Ijaz, and M. M. Hussain. 2018. MicroRNA-30c reduces plasma cholesterol in homozygous familial hypercholesterolemia and type 2 diabetic mouse models. J. Lipid Res. 59: 144–154.

29. Gautier, T., D. Masson, M. C. Jong, L. Duverneuil, N. Le Guern, V. Deckert, J.-P. Pais de Barros, L. Dumont, A. Bataille, Z. Zak, et al. 2002. Apolipoprotein C1 deficiency markedly augments plasma lipoprotein changes mediated by human cholesteryl ester transfer protein (CETP) in CETP transgenic/ApoC1-deficient mice. J. Biol. Chem. 277: 31354–31363.

30. Pillois, X., T. Gautier, B. Bouillet, J.-P. Pais de Barros, A. Jeannin, B. Vergès, J. Bonnet, and L. Lagrost. 2012. Constitutive inhibition of plasma CETP by apolipoprotein C1 is blunted in dyslipidemic patients with coronary artery disease. J. Lipid Res. 53: 1209–1209.

31. Lorenzo, A., J. D. L. da Silva, C. E. James, A. C. Pereira, and A. S. Moreira. 2018. Clinical, anthropometric and biochemical characteristics of patients with or without genetically confirmed familial hypercholesterolemia. Arq. Bras. Cardiol. 110: 119–123.