HHS Public Access

Author manuscript

J Clin Lipidol. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

J Clin Lipidol. 2018 ; 12(1): 110–115. doi:10.1016/j.jacl.2017.10.021.

Novel Polymorphisms Associated with Hyperalphalipoproteinemia (HALP) and Apparent Cardioprotection

Connor Oates, BA, **Darya Koenig, MD**, **Jeffrey Rhyne, BA**, **Nikolay Bogush, MD**, **Jeffrey O'Connell, PhD**, **Braxton D. Mitchell, PhD**, and **Michael Miller, MD***

Department of Medicine, Divisions of Cardiovascular Medicine and Endocrinology, University of Maryland School of Medicine and the Veterans Affairs Medical Center, Baltimore MD 21201

Abstract

Background—Hyperalphalipoproteinemia (HALP) is inversely correlated with coronary heart disease (CHD) although genetic variants associated with high serum levels of HDL-C have not been shown to be cardioprotective.

Objective—To uncover novel genetic variants associated with HALP and possibly with reduced risk of CHD.

Methods—Exome sequencing data, HDL-C and triglyceride (TG) levels were analyzed in 1645 subjects. They included the University of Maryland outpatients with high HDL-C (n=12), Cardiovascular Health Study (CHS) (n=210), Jackson Heart Study (JHS) (n=402), Multi-Ethnic Study of Atherosclerosis (MESA) (n=404), Framingham Heart Study (FHS) (n=463) and old Order Amish (n=154).

Results—Novel nonsynonymous SNPs (nsSNPs) were identified in men and women with primary HALP (mean HDL-C, 145 +/− 30 mg/dL). Using PolyPhen-2 and Combined Annotation Dependent Depletion (CADD) to estimate the predictive effect of each nsSNP on the gene product, rare, deleterious polymorphisms in *UGT1A3, PLLP, PLEKHH1, ANK2, DIS3L, ACACB* and *LRP4* were identified in 16 subjects with HALP but not in any tested subject with low HDL-C (< 40 mg/dL). In addition, a single novel polymorphism, rs376849274, was found in *OSBPL1A*. The majority of these candidate genes have been implicated in fat and lipid metabolism and none of these subjects has a history of CHD despite 75% of subjects having risk factors for CHD. Overall, the probability of finding these nsSNPs in a non-high HDL-C population ranges from 1×10^{-17} to 1×10^{-25}.

*Author of correspondence: 110 S Paca St, Rm 7-124, Baltimore, MD 21201, mmiller@som.umaryland.edu, Work: 410 328-6299, Fax: 410 328-1048.

Financial Disclosure

The authors have no financial relationships to disclose as relevant to the contents of the manuscript.

Conclusion—Novel functional polymorphisms in 8 candidate genes are associated with HALP in the absence of CHD. Future study is required to examine the extent to which these genes may affect HDL function and serve as potential therapeutic targets for CHD risk reduction.

Introduction

For the past several decades, an inverse association between high-density lipoprotein cholesterol (HDL-C) and coronary heart disease (CHD) has been well recognized (1–3). In addition to its role in reverse cholesterol transport, HDL possesses antioxidant and anti-inflammatory properties that are believed to contribute to its cardioprotective role (4). However, while family-based and cross-sectional studies have suggested that primary hyperalphalipoproteinemia (HALP) is associated with longevity (5–6), HDL-C-raising variants in the established gene candidates linked to HALP, cholesteryl ester transfer protein (*CETP*), hepatic lipase (*HL*), and endothelial lipase (*LIPG*), have not resulted in cardioprotection or longevity (7–10). Moreover, a rare variant in scavenger receptor class B type 1 (*SRB1*) that raises HDL-C has been associated with elevated CHD risk (11), and randomized human outcome trials have failed to demonstrate clinical benefit following pharmacologically-mediated CETP inhibition (12–15). Therefore, the goal of the present study was to identify novel gene candidates associated with HALP and reduced CHD risk.

Methods

We identified 62 subjects with HALP (defined as 80 mg/dL or greater) (16–17) from 5 different sources with whole exome sequencing to identify novel nonsynonymous SNPs (nsSNPs) associated with the high HDL-C phenotype. The five population HALP sources were the University of Maryland Preventive Cardiology clinic (Baltimore, MD) (n = 12), the Cardiovascular Health Study (CHS) (n = 8), Jackson Heart Study (JHS) (n = 9), Multi-Ethnic Study of Atherosclerosis (MESA) (n = 22), the Framingham Heart Study (FHS) (n = 15), and the Old Order Amish population of Lancaster, PA (n =6) (18–22).

HALP subjects were initially selected from the University of Maryland Preventive Cardiology clinic and derived from 2 families (pedigree #1, pedigree #2) and 6 biologically unrelated subjects with HALP. Whole exome sequencing was performed using the Illumina Genome Analyzer platform methodology as previously described (23). Mutation analysis included all coding, intron-exon regions and promoter regions; questionable readings were verified using the Broad Institute's Integrative Genomics Viewer (IGV). Genetic variants were confirmed within the NHLBI GO Exome Sequencing Project's exome variant server (EVS) and the University of Michigan's BRAVO server. Allele frequencies were identified using the Broad Institute's Exome Aggregation Consortium browser using sequencing data from over 60,000 subjects. lntronic or synonymous mutations were excluded from analysis. All study procedures were approved by the Institutional Review Board of the University of Maryland School of Medicine.

We then analyzed the whole exome sequencing data from our subjects in conjunction with complete exome sequencing of HALP subjects from the CHS, JHS, MESA, FHS and the Old Order Amish population of Lancaster, PA. As part of the NHLBI GO Exome

Sequencing Project, subjects from JHS, MESA, FHS and FHS had undergone whole exome sequencing completed using Illumina Genome Analyzer IIX or Illumina HiSeq 2000. Similarly, the Old Order Amish population of Lancaster, PA had undergone whole genome sequencing using Illumina HiSeq X Ten as part of the Amish Complex Disease Research Program at the University of Maryland. This available data were readily used to identify rare nsSNPs that were shared by members of the four families as well as individuals from the large population based studies.

The nsSNPs identified in the 62 HALP subjects were then compared with complete exome sequencing data from 1573 subjects without HALP from CHS (n=202), JHS (n=393), MESA (n=382), FHS (n=448) and Old Order Amish (n=148). Of these, 324 subjects exhibited low HDL-C (< 40 mg/dL); nsSNPs shared with HALP and low HDL-C subjects were excluded from further analysis.

We then used two widely utilized in silico functional and ensemble algorithms to determine the predicted functional significance of an amino acid change on each gene product and select for deleterious nsSNPs (24). PolyPhen-2 is a bioinformatics system that integrates features from eight protein sequences and three protein structures to generate a numerical score (0–1) of nsSNP deleteriousness (25–26). PolyPhen-2 defines ranges of nsSNP gene product as “possibly-damaging” (>0.45 and </= 0.95) or “probably-damaging” (>0.95) compared to those viewed as “benign” (</= 0.45). Combined Annotation-Dependent Depletion (CADD) is a framework that integrates 63 distinct measures of nsSNP deleteriousness into scaled C-scores ranging from 1 to 99 based expected outcome of each variant (27). A CADD score >15 considers the nsSNP to be deleterious to the gene product.

Results

Complete exome sequencing was available in 1645 subjects with 62 (3.8%) exhibiting the HALP phenotype (Table 1). We identified 8 novel candidate genes not previously known to affect HDL-C metabolism (Table 2). Each of these genes, *UGT1A3, PLLP, PLEKHH1, ANK2, DIS3L, ACACB* and *LRP4*, displayed nsSNPs with very rare allele frequencies (< 0.0009) that were identified as deleterious by PolyPhen-2 and the CADD score. A novel polymorphism, rs376849274, was also identified in *OSBPL1A*, a gene previously shown to affect HDL-C metabolism (28). Importantly, these nsSNPs were observed only in subjects with HALP and not among any of the 324 low HDL-C subjects with complete exome sequencing.

Cardiovascular and metabolic health data (Table 3) demonstrated several paradoxical findings in the very high HDL-C cohort, inasmuch as 25% were obese, 64% were hypertensive and 56% smoked cigarettes. Yet, despite the considerable risk imposed by the high prevalence of factors known to promote atherothrombosis, none of the HALP subjects had a history of CHD and there was a history of familial longevity (2 or more family members living to at least age 90).

Discussion

Integrating our familial studies with large population based studies, we identified rare nsSNPs in 3 novel gene candidates, *UGT1A3, PLLP* and *PLEKHH1*, associated with the HALP phenotype. The presence of identical, exceedingly rare nsSNPs that are likely deleterious to their gene product in siblings with HALP suggests a heritable gene product involved with cholesterol metabolism. These data were corroborated by identifying additional unrelated subjects from large population based studies who have likely deleterious nsSNPs within the same gene and the HALP phenotype. For example, p(R45W) within UGT1A3 was identified in siblings with HDL-C of 116 (male) and 158 (female) as well as a woman from MESA with an HDL-C of 84. Subsequently, this nsSNP was not shared with any individual within the low HDL-C control group. The allele frequencies of rs45625338 (UGT1A3), rs200119528 (PLEKHH1) and rs143221173 (PLLP) are likely even more rare than recorded given that sibling recurrence-risk ratio for a phenotype such as hyperalphalipoproteinemia will be greater than genotype relative risk within the general population. PolyPhen-2 and CADD have also identified this nsSNP as likely damaging to the gene product. While *UGT1A3, PLLP* and *PLEKHH1* have not previously associated with HALP, *PLEKHH1* has been suggested to play a role in cholesterol and fatty acid metabolism (29).

We also identified 4 novel genes, *ANK2, DIS3L, ACACB* and *LRP4*, associated with the HALP phenotype amongst unrelated subjects from the large population based studies. While no rare deleterious nsSNPs in these genes were identified within our related individuals, multiple rare deleterious nsSNPs within each of these genes that was only observed in subjects with HALP supports the notion that these were not random occurrences. Furthermore, none of these nsSNPs were identified in any individuals with low HDL-C. For instance, deleterious nsSNPs in ANK2 were identified within three unrelated individuals with HALP. Two of the three nsSNPs were identified in an American male of European ancestry (CHS) with an HDL-C of 115 mg/dL and African American female (JHS) with an HDL-C of 121 mg/dL. Interestingly, the *LRP4* locus was previously found to be associated with HDL-C (30), supporting the notion that common as well as rare SNPs (identified in the present study) contribute to the high HDL-C phenotype. Finally, *ACACB* has been linked to obesity and diabetes mellitus (31), pathological processes that may adversely influence HDL-C metabolism and functionality (32).

One additional nsSNP was discovered within the *OSBPL1A* gene, but it remains noteworthy due to the gene's known association with HDL-C metabolism (28). Direct measurement of cholesterol efflux in fibroblasts with this mutation and localization to apoA-1 containing hepatocytes suggests that OSBPL1A interacts specifically with the ABCA1 mediated pathway of cholesterol metabolism, possibly through direct interaction with apoA-1.

There are several limitations inherent to our study design that restrict the precision and power of our findings. Specifically, our data originate from several different protocols that utilized three distinct Illumina sequencing platforms. Consequently, the reproducibility of exome sequencing across platforms, though anticipated to be high, is not certain.

Furthermore, like many genetic studies, our findings are also limited by an inherent non-randomized generation of data.

Recent results from Mendelian randomization studies have demonstrated that HDL-C-raising alleles at multiple loci encoding cholesterol metabolism genes do not protect against CHD (8). These findings have been disappointing in view of the wealth of epidemiologic data supporting HALP phenotype as a cardioprotective phenotype. Moreover, pharmacological interventions designed to raise HDL-C have also not translated into reduced CHD risk (33). However, failure to demonstrate the link between raising levels of HDL-C through one mechanism (i.e., CETP inhibition) does not necessarily rule out the possibility that other pathways exist enabling HDL to exert cardioprotective effects based on its inherent anti-inflammatory, anti-oxidant and cholesterol efflux properties (32). Using a hybrid model incorporating family and population-based studies, the present study identifies eight candidate genes associated with significantly elevated serum HDL-C in subjects without a history of CHD. While the extent to which possible confounders (e.g., LDL-C, triglycerides), might have favorably influenced these subjects' apparent cardioprotection, these newly uncovered polymorphisms present an opportunity to further explore the complex molecular underpinnings of HDL-C and its putative role in reverse cholesterol transport and cardiovascular health.

Acknowledgments

This study was funded by NIH grants HL094980 and HL61369. Authors' contributions: J.R., J.O., B.M. and M.M. contributed to the design of the study. C.O., D.M., N.B., J.R., J.O. performed data collection and genetic analysis. C.O., J.R., M.M. and B.M. contributed to data interpretation. All authors contributed to the drafting, editing and final approval of the manuscript.

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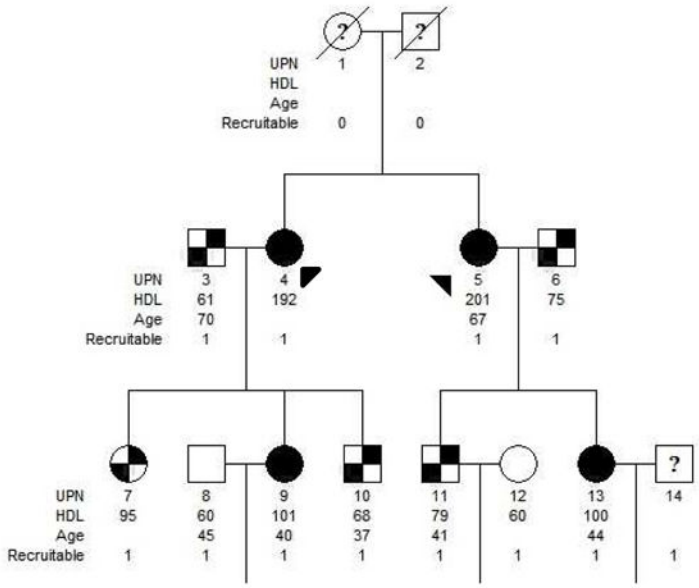
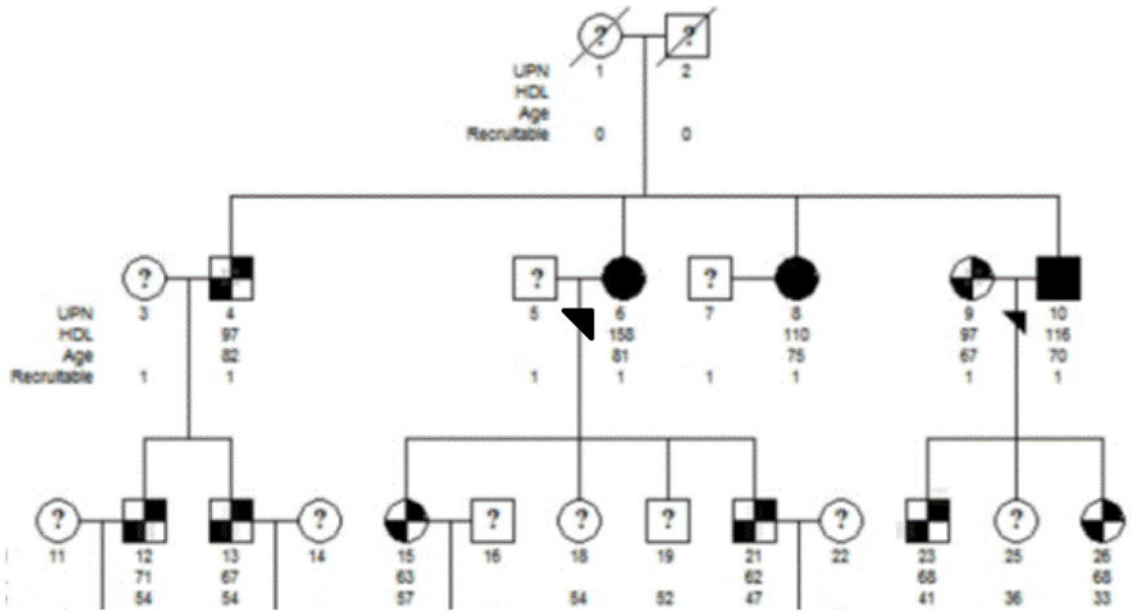
Highlights

- Exome sequencing data were analyzed in 1645 subjects from diverse populations.
- Functional polymorphisms were identified in genes not previously associated with HDL.
- Novel candidate genes may contribute to the hyperalpha (HALP) phenotype.



Pedigree 1.

◥ Siblings sharing nsSNP at rs143221173 (PLLP)
O Females
□ Males
● Females with HALP and exome sequencing
■ Males with HALP and exome sequencing
◓ Females excluded from exome sequencing
◪ Males excluded from exome sequencing
? Subjects without genetic relationships to probands



Pedigree 2.

◥ Siblings sharing nsSNPs at rs45625338 (UGT1A3) and rs200119528 (PLEKHH1)
O Females
□ Males
● Females with HALP and exome sequencing
■ Males with HALP and exome sequencing
◓ Females excluded from exome sequencing
◪ Males excluded from exome sequencing
? Subjects without genetic relationships to probands

Figure.
Two Families with the HALP Phenotype

Table 1

Prevalence of HALP in Subjects with Whole Exome Sequencing

Study	Total Subjects	Subjects with HALP*	Prevalence of HALP
Cardiovascular Health Study	210	8	3.8%
Jackson Heart Study	402	9	2.2%
Multi - Ethnic Study of Atherosclerosis	404	22	5.5%
Framingham Heart Study	463	15	3.2%
UMD Preventive Cardiology Clinic	12	12	N/A
Old Order Amish	154	**6**	**3.9%**
Total	**1645**	**62**	**3.8%**

*Subjects with HDL-C >/= 80 mg/dL

Table 2

Novel Candidate Genes Associated with the HALP Phenotype

Chromosome	Gene name	Number of novel nsSNPs	Number of subjects	Average Allele Frequency	Average HDL (mg/dL)	Average Triglycerides (mg/dL)
2	**UGT1A3**	1	3*	0.004267	**117**	59
14	**PLEKHH1**	2	3*	0.000231263	**120**	66
16	**PLLP**	3	4^	0.000097737	**147**	63
4	**ANK2**	3	3	0.00016578	**111**	84
15	**DIS3L**	2	2	0.00004945	**96**	56
12	**ACACB**	3	3	0.00024454	**92**	76
11	**LRP4**	2	2	0.00007419	**86**	341
1	**OSBPL1A**	1	1	0.000008269	**92**	35

*includes siblings from pedigree 1

^includes siblings from pedigree 2

J Clin Lipidol. Author manuscript; available in PMC 2019 January 01.

Table 3

nsSNPs Associated with the HALP Phenotype

Gene	Amino Acid	rsID	Study	Allele Frequency	HDL-C	Sex	Race	PolyPhen-2 Score	CADDS Score
UGT1A3	p.(R45W)	rs45625338	MESA	0.004267	84	F	EA	probably-damaging: 0.998	19.2
	p.(R45W)	rs45625338	UMD	0.004267	116	M*	EA	probably-damaging:0.998	19.2
	p.(R45W)	rs45625338	UMD	0.004267	158	F*	EA	probably-damaging: 0.998	19.2
PLEKHH1	p.(K1311Q)	rs371360639	MESA	0.00005799	93	F	AA	probably-damaging: 1.0	28.4
	p.(R1179C)	rs200119528	UMD	0.0003179	116	M*	EA	probably-damaging:1.0	20.8
	p.(R1179C)	rs200119528	UMD	0.0003179	158	F*	EA	probably-damaging: 1.0	20.8
PLLP	p.(A53V)	rs149486153	MESA	0.0001155	101	F	EA	probably-damaging: 1.0	23.6
	p.(L114R)		MESA	0.00003305	92	M	EA	probably-damaging: 1.0	n/a
	p.(A141T)	rs143221173	UMD	0.0001212	192	F^	EA	probably-damaging: 0.958	24.8
	p.(A141T)	rs143221173	UMD	0.0001212	201	F^	EA	probably-damaging: 0.958	24.8
ANK2	p.(T527A)		MESA	0.0000247	97	F	EA	possibly-damaging: 0.804	n/a
	p.(E1458G)	rs72544141	CHS	0.0004222	115	M	EA	possibly-damaging: 0.812	23.7
	p.(T2242M)	rs37648404	JHS	0.00005044	121	F	AA	probably-damaging: 1.0	30
DIS3L	p.(E130A)	rs3677091	JHS	0.00008241	90	F	AA	probably-damaging: 0.981	19.91
	p.(R928Q)		MESA	0.00001649	102	F	AA	probably-damaging: 0.993	34
ACACB	p.(G646D)	rs372168822	FHS	0.000008251	92	F	EA	probably-damaging: 1.0	29.2
	p.(R1119C)	rs150478780	CHS	0.00001647	83	F	EA	probably-damaging: 1.0	35
	p.(R1586H)	rs142393083	CHS	0.0007089	100	M	AA	probably-damaging: 1.0	26.6
LRP4	p.(R457C)	rs148856658	FHS	0.00006591	80	M	EA	probably-damaging:0.999	35
	p.(A1130V)	rs138418874	CHS	0.00008247	91	F	AA	possibly-damaging: 0.937	22.8
OSBPL1A	p.(R14G)	rs376849274	FHS	0.000008269	92	F	EA	probably-damaging: 0.991	26.4

*siblings from pedigree 1

^siblings from pedigree 2

CHS: Cardiovascular Health Study

FHS: Framingham Heart Study

JHS: Jackson Heart Study

MESA: Multi-Ethnic Study of Atherosclerosis

EA: European American

AA: African American

Table 4

Cardiovascular and Metabolic Health Data in HALP subjects with Novel nsSNPs

Gene	AA	HDL	Sex	Race	Age	Lipid Med	LDL	TG	BMI	Waist	DM med	DM	Sys/Dia BP	HTN med	HTN	MI	Fm Hx Stroke	Smoke
UGT1A3	**p.(R45W)**	**84**	**F**	**EA**	**48**	**0**	**56**	**49**	**N/A**	**97**	0	0	106/73	0	0	0	0	1
	p.(R45W)	**116**	**M***	**EA**	**70**	**0**	**72**	**48**	**N/A**	**N/A**	0	0	N/A	0	0	0	0	0
	p.(R45W)	**152**	**F***	**EA**	**81**	**0**	**116**	**80**	**N/A**	**N/A**	0	0	N/A	0	0	0	0	0
PLEKHH1	**p.(K1311Q)**	**93**	**F**	**AA**	**65**	**0**	**125**	**71**	**35.3**	**113**	0	0	143/73	1	1	0	0	1
	p.(R1179C)	**116**	**M***	**EA**	**70**	**0**	**72**	**48**	**N/A**	**N/A**	0	0	N/A	0	0	0	0	0
	p.(R1179C)	**152**	**F***	**EA**	**81**	**0**	**116**	**80**	**N/A**	**N/A**	0	0	N/A	0	0	0	0	0
PLLP	**p.(A53V)**	**101**	**F**	**EA**	**63**	**0**	**117**	**86**	**23.3**	**84**	0	0	103/67	1	1	0	1	0
	p.(L114R)	**92**	**M**	**EA**	**58**	**0**	**136**	**47**	**24.7**	**88**	0	0	124/78	0	0	0	1	0
	p.(A141T)	**192**	**F^**	**EA**	**69**	**0**	**125**	**71**	**N/A**	**N/A**	0	0	N/A	0	0	0	0	0
	p.(A141T)	**201**	**F^**	**EA**	**67**	**0**	**130**	**48**	**N/A**	**N/A**	0	0	N/A	0	0	0	0	0
ANK2	**p.(T527A)**	**97**	**F**	**EA**	**52**	**0**	**78**	**137**	**23.3**	**86**	0	0	104/69	0	0	0	0	0
	p.(E1458G)	**115**	**M**	**EA**	**73**	**0**	**79**	**69**	**21.6**	**76**	0	0	167/78	0	1	0	0	1
	p.(T2242M)	**121**	**F**	**AA**	**44**	**0**	**154**	**47**	**26.3**	**88**	0	0	97/63	1	1	0	0	1
DIS3L	**p.(E130A)**	**90**	**F**	**AA**	**48**	**1**	**90**	**68**	**50.9**	**123**	0	1	136/84	1	1	0	1	0
	p.(R928Q)	**102**	**F**	**AA**	**68**	**0**	**60**	**45**	**21.4**	**78**	0	0	97/61	0	0	0	0	1
ACACB	**p.(G646D)**	**92**	**F**	**EA**	**45**	**0**	**123**	**34**	**20.3**	**60**	0	0	172/110	0	1	0	0	0
	p.(R1119C)	**83**	**F**	**EA**	**69**	**0**	**42**	**81**	**25.3**	**77**	0	0	132/70	0	0	0	0	0
	p.(R1586H)	**100**	**M**	**AA**	**79**	**0**	**130**	**112**	**25.3**	**97**	0	0	126/67	0	1	0	0	1
LRP4	**p.(R457C)**	**80**	**M**	**EA**	**52**	**1**	**N/A**	**513**	**23.9**	**96**	0	0	154/99	1	1	0	0	1
	p.(A1130V)	**91**	**F**	**AA**	**75**	**0**	**44**	**169**	**25.4**	**80**	0	0	173/59	1	1	0	1	1
OSBPL1A	**p.(R14G)**	**92**	**F**	**EA**	**45**	**0**	**123**	**34**	**20.3**	**60**	0	0	172/110	0	1	0	0	0

*siblings from pedigree 1

^siblings from pedigree 2

N/A: not available