SUPPLEMENTAL MATERIAL

Supplemental Methods

Library construction and sequencing

The genomic DNA was sheared (sonication) with Covaris S2 to achieve a uniform distribution of fragments with a mean size of 200 bp. The sheared DNA was purified using Agencourt AMPure XP Solid Phase Reversible Immobilization paramagnetic bead (SPRI) and the quality of DNA was tested with the Agilent 2100 Bioanalyzer. The end repair was done by removing the 3′ overhangs followed by the addition of a single "A" base to the 3′ end of the DNA fragments using Klenow fragment (3′ to 5′ exo minus). Specialized adaptors that have a T-base overhang at their 3′ ends were ligated. Following ligation, the samples were purified (using SPRI beads), PCR amplified and the quality was checked by the Agilent 2100 Bioanalyzer.

After hybridization the captured DNA was purified and amplified. The quality of the library was evaluated using the Agilent Bioanalyzer. Finally the 100-bp single end sequencing was performed on the Illumina Hiseq2000 platform with one sample per lane.

Mutation validation

Sanger sequencing was used to confirm the presence and genotype of variants in the candidate genes identified via exome sequencing and to screen the variants in additional family members.

Cellular cholesterol efflux assays

Human skin fibroblasts were seeded in 12-well plates and at mid-confluence labelled with 2 μCi/ml [^{3}H]-cholesterol (Perkin-Elmer Life Sciences) for 48 hours. Cells were subsequently stimulated, or not, with 2.5 μg/ml 22(R)-hydroxycholesterol (22OH) and 10μM 9-cis-retinoic acid (9CRA) for 17 hours and then incubated, or not, with 15 μg/ml lipid-free apolipoproteinA-I (ApoA-I) (Meridian Life Sciences) for 5 hours. Radioactivity was counted in both the medium and the cells. Cellular cholesterol efflux was determined as follows: ^{3}H cpm in medium / (^{3}H cpm in medium + ^{3}H cpm in cells); the results were expressed as percentage of total radiolabeled cholesterol. For the cholesterol

efflux assays in the presence of 17β-estradiol (Sigma-Aldrich), fibroblasts were labeled with 2 μCi/ml [^{3}H]-cholesterol (Perkin-Elmer Life Sciences) for 24 hours, stimulated, or not, with 2.5 μg/ml 22OH and 10μM 9CRA for 17 hours and subsequently incubated, or not, with 15 μg/ml lipid-free apolipoproteinA-I (ApoA-I) (Meridian Life Sciences) for 4 hours. During the 17 hours incubation with 22OH/9CRA, cells were simultaneously treated with increasing concentrations of 17β-estradiol. As above, assays were performed in 22OH/9CRA stimulated fibroblasts (to induce ABCA1 expression), as well as in unstimulated cells, in the presence or absence of lipid free ApoA-I. Cellular cholesterol efflux was determined as described above, but in order to specifically assess the effect of estradiol on the ABCA1 variant, we adjusted for background basal conditions of passive diffusion of cellular cholesterol. Student t-test and non-parametric two sample Wilcoxon rank sum test were used to assess differences between cholesterol efflux of a S1731C male proband and a healthy male control. A non-parametric Spearman trend test in R was used in-order to test whether increasing concentrations of 17β-estradiol has a significant influence on the cholesterol efflux of the carrier proband and wild-type control. The triplicate data for each concentration was utilized by setting the number of observations per unit equal to 3. All functional experiments were performed three times independently, involving triplicate sample measurements from individual wells for each experiment. Figure 2A-B presents one such replicate, representative of all three experiments performed where values represent the mean ± S.D. from triplicate wells.

Parametric linkage analysis

Two-point parametric linkage analysis of the low HDL-C status was performed in the extended family using the 'Location-Score' option of the Mendel software.[1] We utilized an affecteds-only strategy, coding the family members as either "affected" or "unknown" based on the age- and sex-specific population 10th percentiles for HDL-C[2] to avoid problems of incomplete penetrance and ambiguity of the "unaffected" disease status. We used a dominant mode of inheritance, with gene frequencies set to 0.4% as described previously.[2] The genome scan was executed using 553 genome-wide microsatellite markers with an average density of 6 cM.[3] Genotyping and quality control

procedures of the microsatellite markers were explained in detailed previously.[3] The SLINK program[4] was utilized to approximate the maximum possible lod score of the extended family under the assumption of homogeneity within the pedigree. We used linkage parameters as given above and a marker with 4 alleles with equal frequencies. Based on 100 replicates the maximum lod score at θ=0.05 was 4.34.

Genotype by sex interaction

We included the extended family together with 10 additional families with previously identified mutations in ABCA1[5-7] in a gene-sex interaction analysis, comprising 200 individuals and 9 different mutations in ABCA1 (DelED1893, G616V, K776N, N1800H, Q2210H, R1851X, R2084X, R909X and S1731C). Genotype by sex interaction was tested by the SOLAR program[8] using variance-component analysis for discrete traits. We compared models with and without the gene-sex interaction term while keeping the ABCA1 genotypes in both the null and interaction model. We assumed a dominant genetic inheritance, classifying carriers of a mutation as 1 and 0 otherwise, and a multiplicative interaction term, multiplying the genotype score by sex (men=1 and women=0). We also coded a sex-interaction term in which men and post-menopausal women ($\geq$50 years) were coded as 1 and pre-menopausal women (<50 years) were coded as 0. Subjects with HDL-C levels < the age-sex specific 10th percentiles were classified as affected and subjects with HDL-C levels > the age-sex specific 20th percentiles as unaffected. P-values were generated by comparing the two models using a likelihood ratio statistic with one degree of freedom. Since the affection status is adjusted for gender, the inclusion of the main effect of sex in the model was no longer necessary. The binary HDL-C affection was tested because the variance of HDL-C levels in these ascertained families is reduced and thus limited for effective quantitative analysis.[9] SOLAR uses a liability threshold model in the variance-component analysis to handle discrete traits, assuming that the logarithm of the odds of being affected is a function of the effects of a major gene, polygenetic background, covariates, and residual environmental components.

Supplementary table 1. Lipid levels and other clinical characteristics of the 75 genotyped family members.

IND ID	ABCA1	LPL	TC	TG	HDL-C	%HDL	BMI	LDL-C	AGE	SEX
1	G/C	C/C	3.68	1.06	0.95	9	NA	2.24	88	F
3	C/C	C/T	2.58	6.36	0.36	<5	24.3	1.03	56	M
4	G/G	C/C	5.44	1.61	1.75	66	30.5	2.95	59	F
5	C/C	C/T	4.61	1.26	0.67	<5	22	3.36	66	M
6	G/G	C/C	6.04	1.04	2.04	84	30.7	3.53	68	F
7	G/C	C/C	3.57	1.8	0.85	<5	24.1	1.9	66	F
9	G/G	C/T	3.86	0.95	0.99	5	18.1	2.44	62	F
10	G/G	C/C	6.17	2.61	1.06	30	26.6	3.92	69	M
11	G/C	C/C	4.71	3.78	0.9	<5	23	1.92	64	F
12	G/G	C/C	3.65	2.11	0.87	11	23.4	1.82	75	M
13	C/C	C/C	4.68	0.73	1.16	15	24.7	NA	59	F
14	G/C	C/T	4.6	6.05	0.62	<5	25.8	NA	55	M
15	G/G	C/C	6.84	3.27	1.45	40	23.1	3.9	55	F
16	G/C	C/C	5.56	1.32	1.26	23	23	3.7	57	F
17	G/C	C/T	3.6	1.89	0.79	<5	21.9	1.94	31	F
18	G/C	C/T	3.74	4.8	0.72	<5	22.8	0	29	M
19	G/C	C/T	2.43	2.43	0.62	<5	27.2	0.7	21	M
20	G/C	C/C	8.7	1.62	0.77	<5	23.9	7.19	43	F
23	G/C	C/C	4.01	1.4	1.04	11	18.1	2.33	42	F
24	G/G	C/C	5.13	0.61	1.03	31	22.9	3.82	45	M
25	G/C	C/C	3.35	0.88	1.06	17	17.6	1.89	38	F
27	G/C	C/T	6.2	1.29	0.73	<5	20.2	4.88	35	F
29	G/C	C/C	4.95	1.16	0.8	<5	20.1	3.4	39	F
31	G/C	C/C	7.58	1.31	0.59	<5	21.1	5.97	45	F
32	G/G	C/C	4.68	1.94	1.22	59	22.7	2.58	48	M
34	G/G	C/C	6.61	0.84	1.6	92	26.9	4.62	45	M
35	G/G	C/C	4.45	3.29	0.54	<5	29.7	2.04	47	M
36	G/G	C/C	5.26	1.41	1.62	63	23.7	3	48	F
37	G/C	C/C	6.4	0.98	0.91	22	19.9	5.06	38	M
38	G/C	C/C	4.56	1.69	0.79	<5	26.4	2.83	45	F
40	G/G	C/C	4.54	1.9	0.64	<5	20.2	2.86	33	F
41	G/C	C/C	9.66	0.79	0.88	5	23.9	8.42	39	F
43	G/C	C/C	6.67	0.57	1.19	31	21.1	5.22	36	F
44	G/G	C/C	5.06	2.52	0.91	22	26.1	3	37	M
45	G/G	C/T	6.26	4.45	0.72	5	24.6	3.5	43	M
46	G/G	C/C	4.63	2.64	0.98	8	32.9	2.45	44	F
47	G/G	C/C	5.1	2.68	0.79	9	21.5	3.09	44	M
48	G/G	C/C	5.85	2.04	1.08	13	29.8	3.84	40	F
49	G/G	C/T	3.53	1.69	0.76	<5	20.8	1.75	43	F
50	G/G	C/C	5.58	1.41	1.19	61	28.1	3.75	43	M
51	G/G	C/C	5.37	2.31	0.82	13	24.2	3.49	37	M

52	G/G	C/C	4.79	0.8	1.82	85	18.8	2.61	37	F
55	G/G	C/C	3.24	2.46	1.07	39	19.3	1.04	27	M
56	G/G	C/T	2.84	0.83	0.85	12	18.8	1.61	24	M
57	G/G	C/C	2.72	1.03	1.1	49	24.4	1.15	43	M
58	G/C	C/C	3.92	1.37	0.67	<5	24.2	2.63	42	M
59	G/G	C/C	4.83	1.2	1.23	69	23	3.05	36	M
60	G/C	C/T	4.22	1.36	0.62	<5	28.2	2.62	35	F
61	G/G	C/C	4.56	3.44	1.35	52	20.4	1.65	29	F
62	G/G	C/C	6.11	3.02	1.46	64	25.2	3.28	24	F
63	G/C	C/C	6.51	0.7	0.98	6	17	5.21	13	F
64	G/C	C/C	4.57	1.66	1.24	39	19.5	2.58	21	F
65	G/C	C/C	5.16	1	1.41	59	17.6	3.3	19	F
66	G/G	C/C	3.22	0.74	1.26	43	17.1	1.62	16	F
67	C/C	C/C	2.85	0.71	0.73	<5	14.3	1.67	11	F
69	G/G	C/C	3.63	0.58	1.15	20	17.8	2.22	12	M
70	G/C	C/C	3.44	0.47	1.08	15	NA	2.15	10	M
71	G/G	C/C	3.51	1.23	1.31	48	18.4	1.64	21	F
73	G/C	C/C	2.71	1.01	0.75	<5	17.4	1.16	15	F
74	G/G	C/C	9.08	1.69	0.61	<5	28.1	7.29	25	M
75	G/G	C/C	6.18	1.59	1.4	58	21.5	4.06	23	F
76	G/G	C/C	4.23	0.77	1.36	80	20.3	2.52	20	M
77	G/G	C/C	5.57	1.66	0.68	<5	0	3.82	14	F
78	G/G	C/C	3.8	1.03	0.61	<5	22	2.28	15	F
79	G/C	C/C	3.18	0.72	0.83	<5	23.9	1.83	27	F
80	G/G	C/C	4.87	0.51	0.9	12	18.2	3.74	17	M
81	G/C	C/C	3.77	0.63	1.24	41	14.8	2.24	16	F
82	G/C	C/C	5.77	0.98	0.8	<5	16.5	4.52	10	F
87	G/G	C/C	3.86	0.91	1.07	36	23.1	2.38	21	M
89	G/G	C/T	3.81	2.19	0.7	<5	26.6	2.11	22	M
90	G/G	C/C	3.59	0.51	1.11	42	21.2	2.25	20	M
91	G/G	C/T	3.18	2.99	0.66	<5	23.5	0.94	16	M
92	G/G	C/C	4.04	0.55	1.39	79	18.4	2.4	16	M
93	G/G	C/C	3.98	0.87	0.93	15	19.7	2.65	15	M
94	G/G	C/C	4.21	0.42	1.43	53	17.6	2.59	13	M

The lipid levels are shown in millimoles per liter.

Supplementary table 2. List of 41 variants shared by the three exome sequenced individuals after filtering.

Chr no	Position no	rs number	Gene name	PolyPhen*	SIFT†
2	42990225	New	OXER1	Probably	Damaging
2	160993949	New	ITGB6	Stop	Stop
3	15477933	New	EAF1	Probably	Damaging
3	47047500	New	NBEAL2	Probably	Damaging
3	196529902	New	PAK2	Probably	Damaging
4	1388675	New	CRIPAK	Probably	Damaging
5	140784743	New	PCDHGA9	Probably	Tolerated
8	145094836	New	SPATC1	Possibly	Tolerated
8	145112971	New	OPLAH	Probably	Tolerated
9‡	**107558635**	**New**	**ABCA1**	**Probably**	**Damaging**
10	34606158	New	PARD3	Possibly	Tolerated
11	57076419	New	TNKS1BP1	Probably	Tolerated
12	124362332	New	DNAH10	Probably	Damaging
15	59139625	New	FAM63B	Benign	Damaging
16	2003016	New	RPL3L	Benign	Damaging
17	45234303	New	CDC27	Possibly	Tolerated
17	44144993	New	KIAA1267	Benign	Damaging
19	14675764	New	TECR	Probably	Damaging
22	45821982	New	RIBC2	Probably	Damaging
22	39069227	New	CBY1	Benign	Damaging
1	115537367	rs61730058	SYCP1	Probably	Tolerated
1	144852390	rs61804988	PDE4DIP	Stop	Stop
2	11943082	rs4669781	LPIN1	Possibly	Tolerated
3	49162583	rs35713889	LAMB2	Probably	Tolerated
5	35753763	rs79487218	SPEF2	Benign	Damaging
5	140255119	rs114654172	PCDHA12	Possibly	Tolerated
8‡	**19811790**	**rs118204060**	**LPL**	**Probably**	**Damaging**
8	144995494	rs76803079	PLEC	Probably	Damaging
10	43871158	rs41307500	FXYD4	Probably	Tolerated
10	127697954	rs1666	FANK1	Possibly	Damaging
11	68174189	rs4988321	LRP5	Probably	Tolerated
11	56310356	rs17547284	OR5M11	Stop	Stop
11	36458997	rs62621409	PRR5L	Probably	Damaging
15	45491082	rs80131405	SHF	Benign	Damaging
16	28488943	rs77595156	CLN3	Probably	Tolerated
16	1537693	rs61734779	PTX4	Possibly	Tolerated
17	37224211	rs75117355	PLXDC1	Probably	Tolerated
19	42341407	rs35476281	LYPD4	Probably	Tolerated
19	23545516	rs112713994	ZNF91	Probably	Tolerated
19	49445774	rs10423255	DHDH	Stop	Stop
19	41235167	rs112628847	ITPKC	Benign	Damaging

* PolyPhen-2 was used to predict the possible impact of an amino acid substitution on the structure and function of the protein. A score larger than 0.85 is considered as probably damaging, a score smaller than 0.15 as benign, and a score between 0.85 and 0.15 as possibly damaging, respectively.

† SIFT predicts the amino acid substitution to be damaging if the score is less than 0.05, and tolerated if the score is greater than 0.05.

‡ The ABCA1 (S1731C) and LPL (P234L) variants are highlighted in bold.

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