MACROPHAGES OF GENETICALLY CHARACTERIZED FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS SHOW UPREGULATION OF LDL-RECEPTOR RELATED PROTEINS

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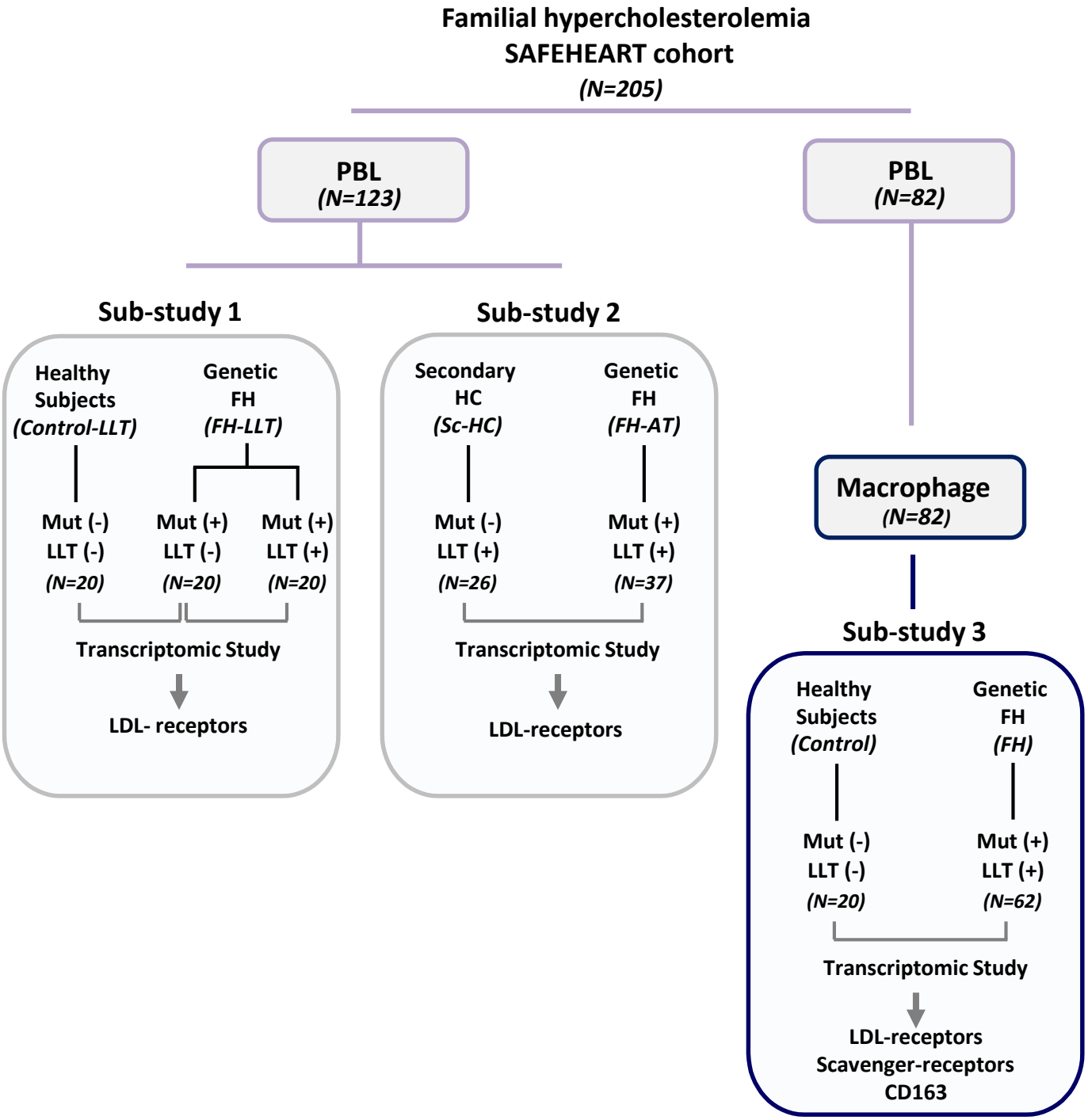
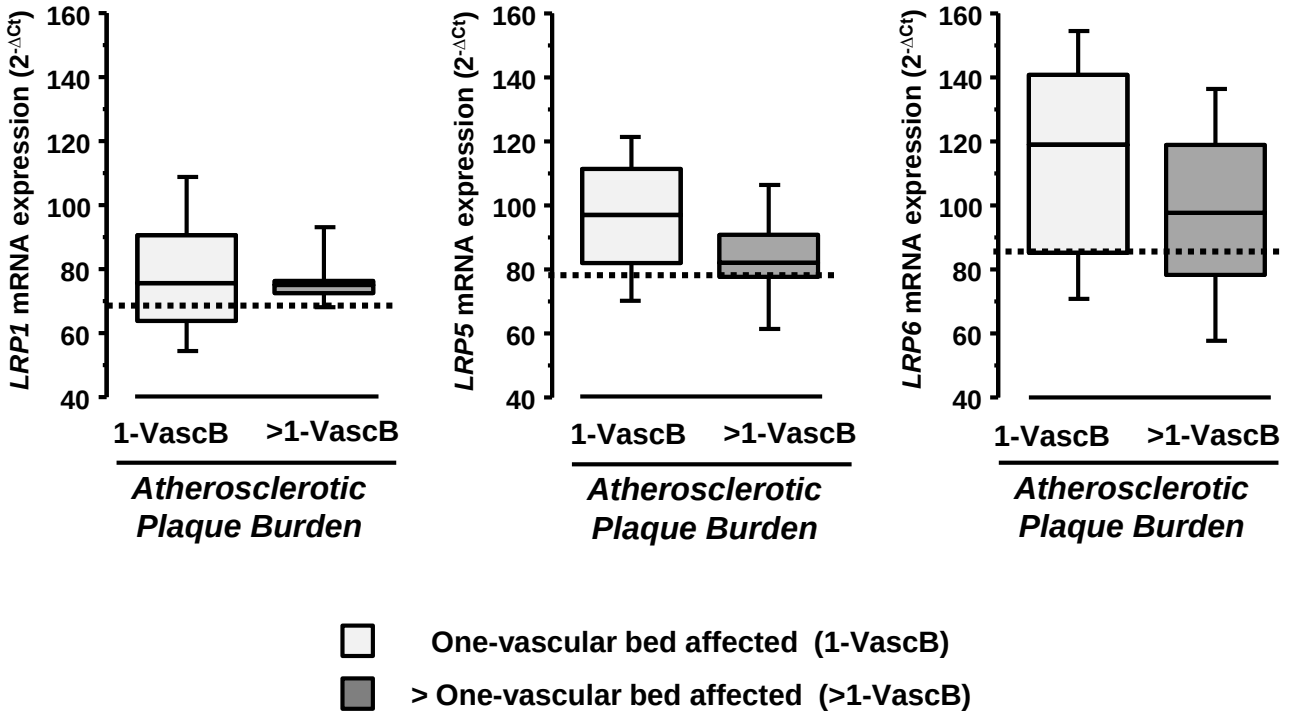
Supporting Information: 4 Supplementary Figures and 4 Supplementary Tables



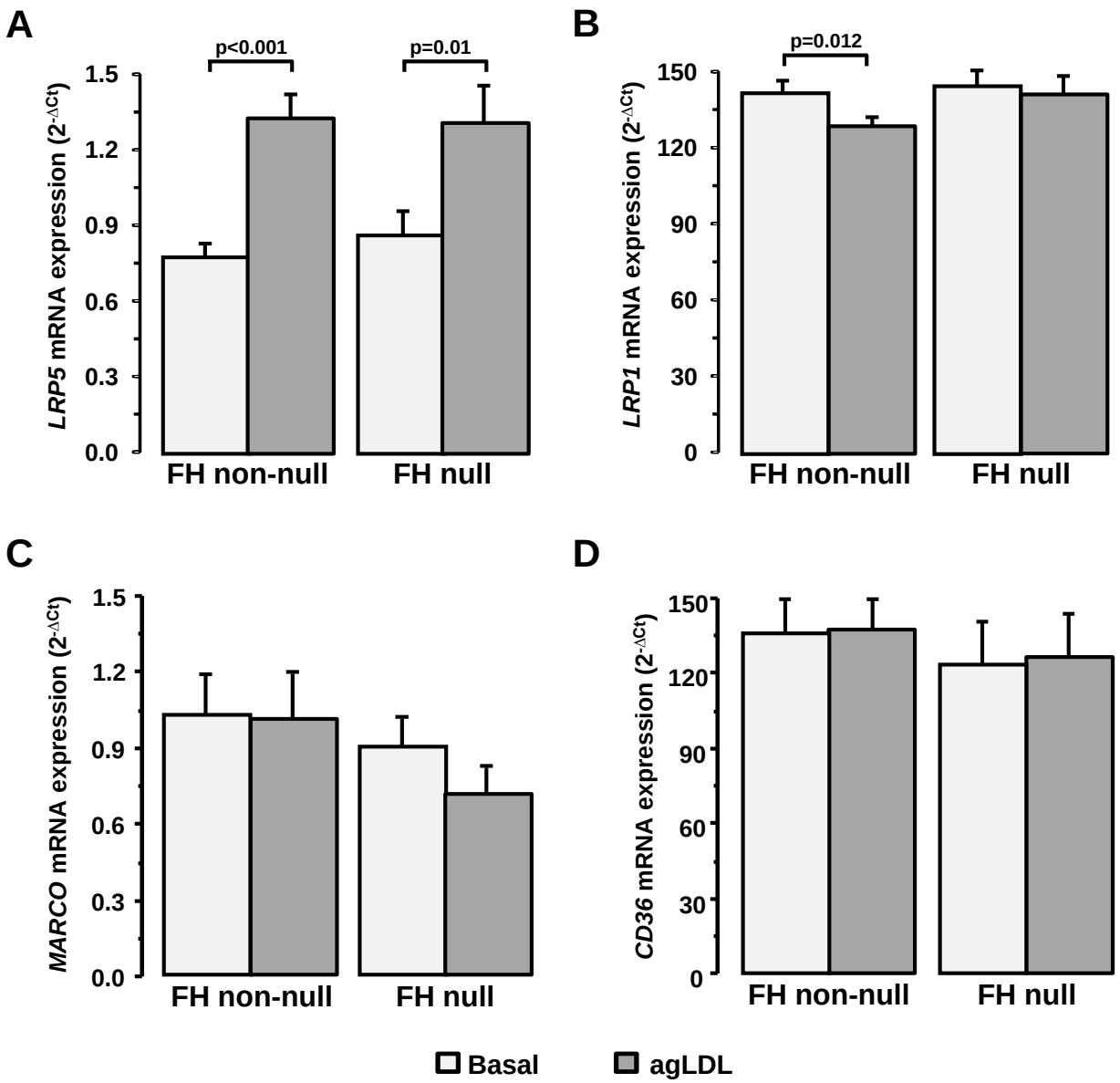
Supplementary Figure 1. Schematic diagram representing the study design with 205 subjects of the SAFEHEART Cohort

FH: Familial hypercholesterolemia; HC: hypercholesterolemia; Mut (+): With LDLR mutation, Mut (-): Without LDLR mutation; LLT (+): with lipid-lowering treatment; LLT (-): without lipid-lowering treatment. Secondary HC is shown in manuscript as sc-HC.

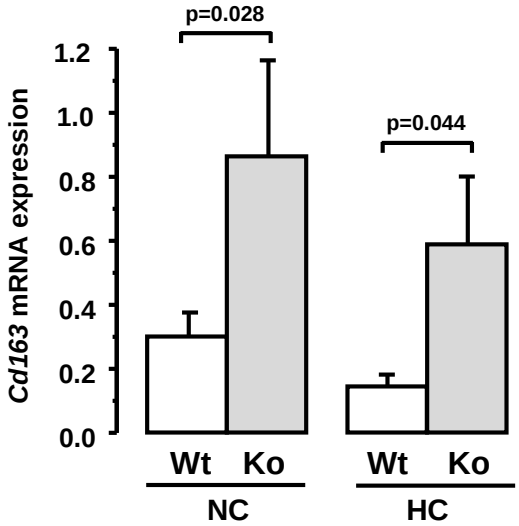


Supplementary Figure 2. LRP5 expression in PBL from FH-AT patients

Box and whisker plot of LRP1, LRP5 and LRP6 mRNA expression associated to atherosclerotic plaque burden in FH-patients. Horizontal doted lines indicate median levels of mRNA expression ($2^{-\Delta Ct}$) of the control sc-HC group for LRP1 (68), LRP5 (79), and LRP6 (84). Thirty FH-AT patients only had one vascular bed affected (aortic or carotid) and 7 patients had atherosclerotic burden in the aortic and carotid beds. Values are give as median and interquatile range. Differences between groups are not statistically significant (U-Mann Whitney).



Supplementary Figure 3. mRNA expression levels of LRPs and scavenger receptors in macrophages of FH-patients according their genetically characterized ldlr-mutation.
Macrophage from LDLR-non-null (FH non-null, N=40) and LDLR-null (FH null, N=22) FH-patients were incubated with 100μg/mL agLDL for 24 hours. mRNA expression was quantified by real time PCR in sample duplicates. Bars represent mean±SEM. Only p-values <0.05 are shown.



Supplementary Figure 4. *Cd163* expression is upregulated in PBLs of *Lrp5*$^{-/-}$ mice.
Differential expression of *Cd163* in PBLs of wild type (Wt) and *Lrp5*$^{-/-}$ (Ko) mice fed hypercholesterolemic (HC) and normocholesterolemic (NC) diet. Real-time PCR in duplicates and normalized to 18S (N= 7 mice/group). Bars represent mean±SEM. Only p-values <0.05 are shown.

Supplementary Table 1. Clinical characteristics of Control-LLT$^-$, FH-LLT$^+$ and FH-LLT$^-$ donors for studies on peripheral blood leukocytes (PBL).

	Control-LLT$^-$ *n=20*	FH-LLT$^+$ *n* =20	FH-LLT$^-$ *n* =20	Statistics
Age (years, mean ± SEM)	35 ± 2	37 ± 2	34 ± 1	p=0.501
Male/Female, *n*	10/10	10/10	10/10	-
Body mass index (Kg/m^2, mean ± SEM)	23.3 ± 0.9	24.7 ± 1.1	24.6 ± 0.9	p=0.646
Waist to height ratio (mean ± SEM)	0.5 ± 0.3	0.48 ± 0.2	0.47 ± 0.1	p=0.973
Risk Factors, *n* (%)				
Diabetes mellitus	0 (0%)	0 (0%)	0 (0%)	-
Systemic hypertension	0 (0%)	0 (0%)	0 (0%)	-
Obesity (BMI>30)	1 (5%)	3 (30%)	2 (10%)	p=0.574
Current tobacco consumption	5 (25%)	9 (45%)	9 (45%)	p=0.324
Lipid-lowering therapy, *n* (%)				
Statins	0 (0%)	20 (100%)	0 (0%)	p<0.001
Ezetimibe	0 (0%)	0 (0%)	0 (0%)	
Statin treatment time (years, mean ± SEM)	0	15 ± 1	0	p<0.001
Clinical data (mean ± SEM)				
Total cholesterol (mg/dL)	167.9 ± 4.2	296.9 ± 7.5[a]	302.9 ± 6.4[a]	p<0.001
LDL-cholesterol (mg/dL)	92.7 ± 3.4	222.7 ± 6[a]	231.3 ± 6.9[a]	p<0.001
HDL-cholesterol (mg/dL)	60.1 ± 4.3	55.9 ± 3.4	53.6 ± 2.4[a]	p=0.749
Non-HDL-cholesterol (mg/dL)	107.8 ± 3.3	240.9 ± 7.1[a]	249.3 ± 6.4[a]	p<0.001
TC/HDL-cholesterol ratio	2.8 ± 0.1	5.6 ± 0.3[a]	5.9 ± 0.3[a]	p<0.001
Triglyceride (mg/dL)	75.1 ± 8.1	90.5 ± 10.1	87.2 ± 8.6	p=0.251
Hight-sensitive CRP (mg/dL)	1.7 ± 0.4	2.8 ± 0.9	1.6 ± 0.3	p=0.701
Fasting plasma glucose (mg/dL)	82.4 ± 2.4	77.9 ± 2.1	79.1 ± 1.9	p=0.385

Control-LLT$^-$ group: healthy subjects without lipid-lowering therapy; FH-LLT$^+$ and FH-LLT$^-$ group: FH-patients with or without LLT; BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TC: total cholesterol and CRP: C-reactive protein. [a] p-values respect to Control-LLT$^-$ group.

Supplementary Table 2. Clinical characteristics of sc-HC and FH-AT donors for studies on peripheral blood leukocytes (PBL).

	sc-HC *n* =26	FH-AT *n* =37	Statistics
Age (years, mean ± SEM)	56 ± 3	44 ± 2	p=0.001
Male/Female, *n*	10/16	19/18	p=0.312
Body mass index (Kg/m^2, mean ± SEM)	28.2 ± 1.3	26 ± 0.7	p=0.138
Waist to height ratio (mean ± SEM)	0.57 ± 0.2	0.50 ± 0.01	p=0.004
Risk Factors, *n* (%)			
Diabetes mellitus	2 (8%)	0 (0%)	p=0.086
Systemic hypertension	5 (19%)	4 (11%)	p=0.347
Obesity (BMI>30)	10 (38%)	3 (8%)	p=0.003
Current tobacco consumption	7 (27%)	13 (35%)	p=0.491
Lipid-lowering therapy, *n* (%)			
Statins	26 (100%)	37 (100%)	-
Ezetimibe	2 (8%)	13 (35%)	p=0.012
Statin treatment time (years, mean ± SEM)	12 ± 1	11 ± 1	p=0.626
Clinical data (mean ± SEM)			
Total cholesterol (mg/dL)	212.5 ± 7.5	232.9 ± 12.6	p=0.418
LDL-cholesterol (mg/dL)	136.2 ± 6.8	160.1 ± 10.7	p=0.133
HDL-cholesterol (mg/dL)	56.4 ± 2.8	49.6 ± 2.1	p=0.077
Non-HDL-cholesterol (mg/dL)	156 ± 7.2	182 ± 12.2	p=0.176
TC/HDL-cholesterol ratio	3.9 ± 0.2	4.9 ± 0.3	p=0.036
Triglyceride (mg/dL)	99.2 ± 9.5	39 ± 6.4	p<0.001
Hight-sensitive CRP (mg/dL)	3.1 ± 0.9	2.9 ± 0.6	p=0.469
Fasting plasma glucose (mg/dL)	92.3 ± 3.8	87.4 ± 3.2	p=0.459

Sc-HC group: Subjects with secondary hypercholesterolemia and FH-AT group: FH-patients with atherosclerotic lesion analyzed with magnetic resonance image; BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TC: total cholesterol and CRP: C-reactive protein.

Supplementary Table 3. Clinical characteristics of control and FH donors for studies on monocyte differentiation to macrophage.

	Control *n* =20	FH *n* =62	Statistics
Age (years, mean ± SEM)	47 ± 3	46 ± 2	p=0.888
Male/Female, *n*	11/9	31/31	p=0.697
Body mass index (Kg/m^2, mean ± SEM)	25.6 ± 1.1	26.9 ± 0.7	p=0.483
Waist to height ratio (mean ± SEM)	0.53 ± 0.02	0.56 ± 0.01	p=0.329
Risk Factors, *n* (%)			
Diabetes mellitus	0 (0%)	3 (4.8%)	p=0.316
Systemic hypertension	1 (5%)	6 (9.7%)	p=0.515
Obesity (BMI>30)	4 (20%)	13 (21%)	p=0.325
Current tobacco consumption	8 (40%)	19 (30.6%)	p=0.438
Lipid-lowering therapy, *n* (%)			
Statins	9 (45%)	62 (100%)	p<0.001
Ezetimibe	0 (0%)	2 (1.2%)	p=0.416
Statin treatment time (years, mean ± SEM)	3 ± 1	15 ± 1	p<0.001
Clinical data (mean ± SEM)			
Total cholesterol (mg/dL)	188.3 ± 4.6	243.6 ± 7.4	p<0.001
LDL-cholesterol (mg/dL)	117.6 ± 4	176.7 ± 6.8	p<0.001
HDL-cholesterol (mg/dL)	47.2 ± 2.6	49 ± 1.3	p=0.496
Non-HDL-cholesterol (mg/dL)	141.1 ± 4	195 ± 7.4	p<0.001
TC/HDL-cholesterol ratio	4.2 ± 0.2	5.2 ± 0.2	p=0.003
Triglyceride (mg/dL)	135.9 ± 23.6	89.3 ± 6.5	p=0.063
Hight-sensitive CRP (mg/dL)	2.1 ± 0.4	1.5 ± 0.2	p=0.457
Fasting plasma glucose (mg/dL)	80.2 ± 2.4	81.4 ± 1.7	p=0.756

Control group (C-MAC): healthy subjects with or without lipid-lowering therapy (LLT); FH group (FH-MAC): FH patients with LLT; BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TC: total cholesterol and CRP: C-reactive protein.

Supplementary Table 4. FH genotypes according to location of LDLR mutation

FH group	Location	Predicted effect	N=62
null	Ex 15	Shift reading frame	10
null	Ex 7		
null	Ex 6		
null	Ex 4	Stop codon	12
null	Ex 9		
null	Ex 2		
non-null	Prom	Regulation of expression	4
non-null			
non-null	Ex 17	Change amino acid	15
non-null	Ex 3		
non-null	Ex 4		
non-null	Ex 7		
non-null	Ex 14		
non-null	Ex 8		
non-null	Int 3	Splicing	9
non-null	Int 16		
non-null	Int 9		
non-null	Int 12		
non-null	Ex 3 + Int 3	Others	12
non-null	Ex 13		
non-null	Ex 11 + Ex 17		
non-null	Ex 16		

Stop codon and shift reading frame mutations were classified as FH null (N=22) while the other mutations were classified as FH non-null (N=40). Ex: exon; Int: intron; Prom: promoter; N: number of patients for each predicted effect of LDLR mutation.