Additional file

Application of expanded genetic analysis in the diagnosis of familial hypercholesterolemia in patients with very early-onset coronary artery disease

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Table S1. Simon Broome diagnostic criteria for familial hypercholesterolemia

Diagnosis	**Criteria**
Definite FH	Cholesterol >7.5 mmol/L or LDL-cholesterol >4.9 mmol/L in adult
	Cholesterol >6.7 mmol/L or LDL-cholesterol >4.0 mmol/L in a child under 16 years of age
	PLUS
	Tendon xanthomas in patient or a 1st degree relative (parent, sibling, child), or in a 2nd degree relative (grand parent, uncle, aunt)
	OR
	DNA based evidence of a functional *LDLR, PCSK9* and *APOB* mutation
Probable FH	Cholesterol >7.5 mmol/L or LDL-cholesterol >4.9 mmol/L in adult
	Cholesterol >6.7 mmol/L or LDL-cholesterol >4.0 mmol/L in a child under 16 years of age
	PLUS
	Family History of myocardial infarction (MI) before 50 years of age in a 2nd degree relative or below age 60 in a 1st degree relative
	OR
	Family history of raised total cholesterol - >7.5 mmol/L in adult 1st or 2nd degree relative or >6.7 mmol/L in a child or sibling aged <16 years

Table S2. Dutch Lipid Clinic Network Clinical Criteria for familial hypercholesterolemia

Criteria	Points
Family history	
First-degree relative with known premature (men: <55 years; women: <60 years) coronary artery disease or vascular disease, or first-degree relative with known LDL-C level above the 95th percentile by age, gender for country	1
First-degree relative with tendinous xanthomata and/or arcus cornealis, or children aged less than 18 years with LDL-C level above the 95th percentile by age, gender for country	2
Clinical history	
Patient with premature (men: <55 years; women: <60 years) coronary artery disease	2
Patient with premature (men: <55 years; women: <60 years) cerebrovascular or peripheral vascular disease	1
Physical examination	
Tendinous xanthomata	6
Arcus cornealis prior to age 45 years	4
LDL-C levels	
LDL-C ≥8.5 mmol/l (~330 mg/dl)	8
LDL-C 6.5–8.4 mmol/l (~250–329 mg/dl)	5
LDL-C 5.0–6.4 mmol/l (~190–249 mg/dl)	3
LDL-C 4.0–4.9 mmol/l (~155–189 mg/dl)	1
DNA analysis	
Causative mutation in the *LDLR, ApoB* or *PCSK9* gene	8

>8 points Definite FH

6–8 points Probable FH

3–5 points Possible FH

0–2 points Unlikely FH

Table S3. Summary of pathogenic/likely pathogenic mutations in CAD patients

Gene	Nucleotide change	Effect on protein	PMID/Novel	No. of patient
LDLR	c.1158delC	p.Asp386fs	Novel	1
LDLR	c.1474G>A	p.Asp492Asn	9763532	1
LDLR	c.1724T>C	p.Leu575Pro	Novel	1
LDLR	c.1747C>T	p.His583Tyr	22698793	1
LDLR	c.1765G>A	p.Asp589Asn	16250003	1
LDLR	c.1A>T	p.Met1Leu	8831933	1
LDLR	c.2026G>C	p.Gly676Arg	26892515	1
LDLR	c.2389G>A	p.Val797Met	23375686	1
LDLR	c.313+3A>T	-	Novel	1
LDLR	c.510delC	p.Asp172Thrfs*34	22881376	1
LDLR	c.510delC	p.Asp170fs	Novel	1
LDLR	c.532G>T	p.Asp178Tyr	16389549	1
LDLR	c.670G>T	p.Asp224Tyr	Novel	1
LDLR	c.694+4T>G	-	Novel	1
LDLR	c.971G>A	p.Gly324Asp	Novel	1
LDLR homo	c.974G>A	p.Cys325Tyr	21865347	1
LDLR homo	c.1879G>A	p.Ala627Thr	23375686	1
LDLR homo	c.1448G>A	p.Trp483Ter	7903864	1
LDLR homo	c.1206delC	p.Phe403Serfs*10	15241806	1
APOB	c.1594C>T	p.Arg532Trp	Novel	1
APOB	c.6110T>C	p.Ile2037Thr	Novel	1
APOB	c.7223C>T	p.Ser2408Phe	Novel	1
APOB	c.8267G>T	p.Gly2756Val	Novel	1
APOB	c.8462C>T	p.Pro2821Leu	18710658	1
APOB	c.889C>T	p.Arg297Cys	Novel	1
APOB	c.9164A>G	p.Asn3055Ser	Novel	1
PCSK9	c.10G>A	p.Val4Ile	17316651	1
PCSK9	c.644G>A	p.Arg215His	24404629	1
STAP1	c.596A>G	p.Asn199Ser	Novel	1
LDLR, LDLR	c.665G>T; 2054C>T	p.Cys222Phe; p.Pro685Leu	25741868; 1830890	1
LDLR, LDLR	c.292G>A;c.1864G>A	p.Gly98Ser; p.Asp622Asn	16250003; 15701167	1
LDLR, LDLR	c.1432G>A; c.2054C>T	p.Gly478Arg; p.Pro685Leu	23064986; 23155708	1
LDLR, LDLR	c.665G>T; c.1747C>T	p.Cys222Phe; p.His583Thr	2574186; 7903864	1
LDLR, LDLR	c.769C>T; c.1765G>A	p.Arg257Trp; p.Asp589Asn	11462246; 16250003	1
LDLR, LDLR	c.1885_1886insCA; c.1889G>C	p.Phe629Serfs*37; p.Ser630Tyr	Novel; Novel	1
LDLR, LDLR	c.1884_1885insGA; c.1888_1889insAC	p.Phe629Aspfs*37; p.Ser630Asnfs*36	Novel; Novel	1

LDLR, LDLR	c.2336_2337delGA; c.2337_2338insTTTT;	p.Gly779Glufs*2; p.Gly779fs;	Novel; Novel	1
LDLR, APOB	*LDLR* c.682G>T; *APOB* c.2870T>C	*LDLR* p.Glu228Ter; *APOB* p.Ile957Thr	19843101; Novel	1
LDLR, APOB	*LDLR* c.670G>T; *APOB* c.10748A>T	*LDLR* p.Asp224Tyr; *APOB* p.His3583Leu	Novel; Novel	1
LDLR, *PCSK9*	*LDLR* c.1879G>A; *PCSK9* c.626C>T	*LDLR* p.Ala627Thr; *PCSK9* p.Pro209Leu	23375686; Novel	1

Table S4. Summary of variants of unknown significance in CAD patients.

Gene	Nucleotide change	Effect on protein
APOB	c.10835A>G	p.Ala3612Gly
APOB	c.12016G>A	p.Val4006Ile
APOB	c.1342G>A	p.Ala448Thr
APOB	c.13663G>A	p.Ala4555Thr
APOB	c.288G>T	p.Glyln96His
APOB	c.4163G>A	p.Arg1388His
APOB	c.4556A>G	p.Asn1519Ser
APOB	c.7331G>A	p.Arg2444His
APOB	c.7565G>A	p.Arg2522Gln
APOB	c.7724A>T	p.Lys2575Ile
APOB	c.7729A>C	p.Met2577Leu
PCSK9	c.1487G>A	p.Arg496Gln
PCSK9	c.1954A>G	p.Asn652Ala
APOE	c.149G>A	p.Arg50His

Table S5. Biochemical and clinical characteristics of patients in relation to DLCN Scores.

All (N=105)	**DLCN <3 N=28**	**DLCN3-5 N=49**	**DLCN 6-8 N=12**	**DLCN>8 N=16**	**P for trend**
Age, years	32.64 ± 3.08	33.14 ± 4.03	32.25 ± 3.62	25.13 ± 9.37	0.001
Male, n (%)	28 (100)	48 (98.0)	12 (100)	11 (68.8)	0.097
BMI, kg/(m^2)	34.87 ± 37.65	28.56 ± 3.87	26.76 ± 3.46	21.71±5.83	0.212
Family history of premature CAD, n (%)	0 (0)	10 (20.4)	6 (50.0)	4 (25.0)	0.002
History of MI, n (%)	17 (60.7)	23 (46.9)	6 (50.0)	6 (37.5)	0.483
Currently smoking, n (%)	21 (75.0)	38 (77.6)	7 (58.3)	4 (25.0)	0.001
Alcohol drinker, n (%)	13 (61.9)	17 (38.9)	3 (25.0)	3 (18.8)	0.038
Hypertension, n (%)	12 (42.9)	26 (51.3)	6 (50.0)	2 (12.5)	0.051
DM, n (%)	7 (25.0)	8 (16.3)	2 (16.7)	0 (0)	0.196
Statin, n (%)	21 (75.0)	39 (79.6)	9 (75.0)	12 (75.0)	0.958
TG, mmol/L	1.70 ± 0.68	2.03 ± 0.89	1.63 ± 0.56	1.47 ± 0.98	0.070
TC, mmol/L	3.97 ± 0.98	6.60 ± 10.29	6.22 ± 1.52	11.41 ± 3.95	0.016
HDL-C, mmol/L	0.95 ± 0.33	0.92 ± 0.20	0.87 ± 0.30	0.73 ± 0.23	0.053
LDL-C, mmol/L	3.66 ± 0.16	4.7 ± 0.68	6.78 ± 0.97	12.07±4.71	<0.001
Xanthoma, n (%)	0 (0)	0 (0)	0 (0)	12 (75)	<0.001
% of mutation	3 (10.7)	14 (28.6%)	11(91.7%)	9 (56.3)	
LDLR, n (%)	1 (3.6)	5 (10.2)	6 (50.0)	3 (18.8)	<0.001
APOB, n (%)	2 (7.1)	4 (8.2)	1 (8.3)	0 (0)	0.107
PCSK9, n (%)	0 (0)	1 (2.0)	1 (8.3)	0 (0)	0.001
STAP1, n (%)	0 (0)	1 (2.0)	0 (0)	0 (0)	0.001
LDLR Homozygote, n (%)	0 (0)	0 (0)	0 (0)	4 (25.0)	0.001
Two mutations, n (%)	0 (0)	3 (6.1)	3 (25.0)	5 (56.3)	0.001

Data are expressed as mean ± SD, or n (%). BMI: body mass index; CAD: coronary artery disease; MI: myocardial infarction; DM: diabetes mellitus; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; *LDLR*: low-density lipoprotein receptor; *APOB*: Apolipoprotein B; *PCSK9*: proprotein convertase subtilisin/Kexin type 9; *STAP1*: Signal-transducing adaptor protein 1.

Table S6. Plasma LDL-C levels stratified by genetic mutations in the patients with very early-onset CAD.

Mutation Type	**Number of patients**	**LDL-C, mmol/L**
All	105	5.77 ± 3.36
FH mutation-negative	65	4.60 ± 1.62
FH mutation-positive	40	7.65 ± 4.49
LDLR	15	7.46 ± 4.93
APOB	7	4.72 ± 1.05
PCSK9	2	5.71 ± 1.00
STAP1	1	4.67
LDLR Homozygote	4	13.88 ± 4.72
Two mutations	11	8.21 ± 3.23

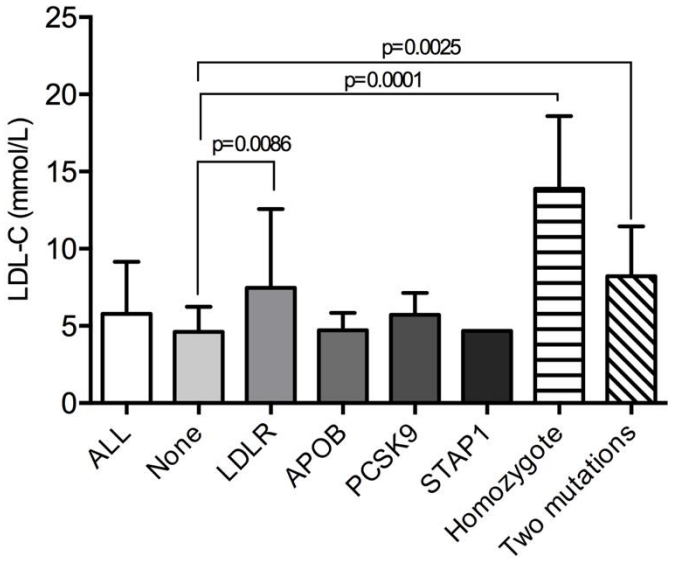
Data are expressed as mean ± SD, or n (%). LDL-C: low-density lipoprotein cholesterol; CAD: coronary artery disease;*LDLR*: low-density lipoprotein receptor; *APOB*: Apolipoprotein B; *PCSK9*: proprotein convertase subtilisin/Kexin type 9; *STAP1*: Signal-transducing adaptor protein 1.

Table S7. Percentage participants with early-onset CAD meeting clinical and genetic criteria for FH diagnosis based on different initial LDL-C levels.

LDL-C	No.	Simon Broome Criteria (Possible and Definite)	Dutch Lipid Clinic Criteria (Probable and Definite)	Genetic Study
≥ 4.9 (190 mg/dL)	43	18 (41.8%)	26 (60.5%)	27 (62.8%)
≥ 4.6 (178 mg/dL)	49	18 (36.7%)	27 (55.1%)	30 (61.2%)
≥ 4.4 (170 mg/dL)	58	18 (31.0%)	27 (46.6%)	31 (53.4%)
≥ 4.0 (155 mg/dL)	73	18 (24.7%)	28 (38.3%)	37 (50.7%)
≥ 3.8 (145 mg/dL)	77	18 (23.4%)	28 (36.4%)	38 (49.4%)
≥ 3.4 (130mg/dL)	105	18 (17.1%)	28 (26.7%)	40 (38.1%)

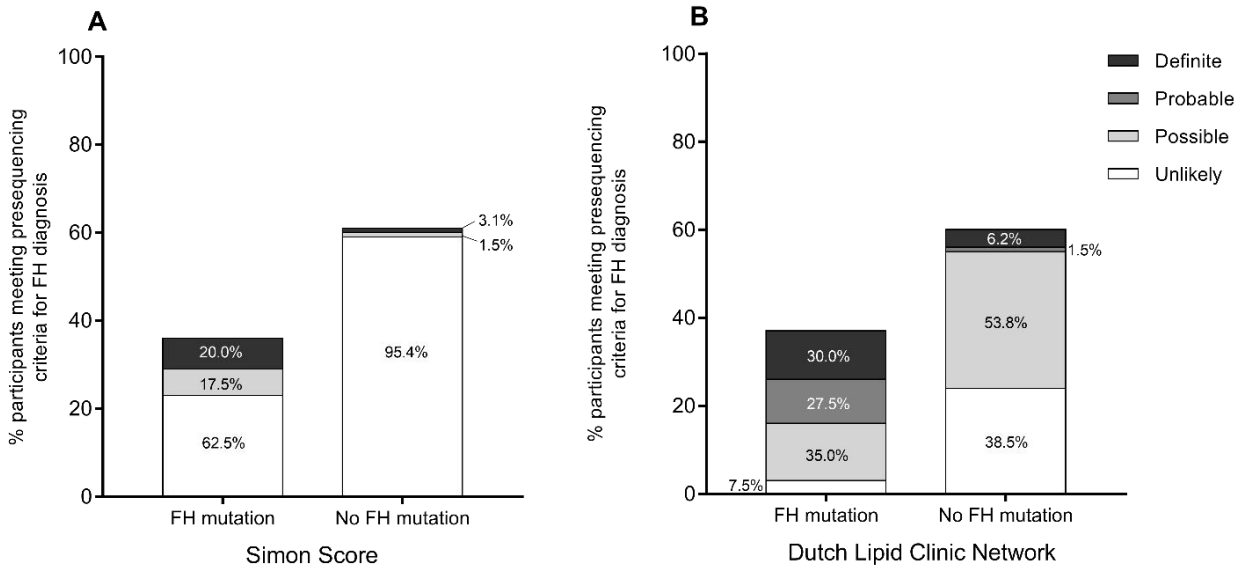
Data are expressed as n (%). LDL-C: low-density lipoprotein cholesterol.

Figure S1. Plasma LDL-C levels stratified by genetic mutations in the patients with very early-onset CAD.



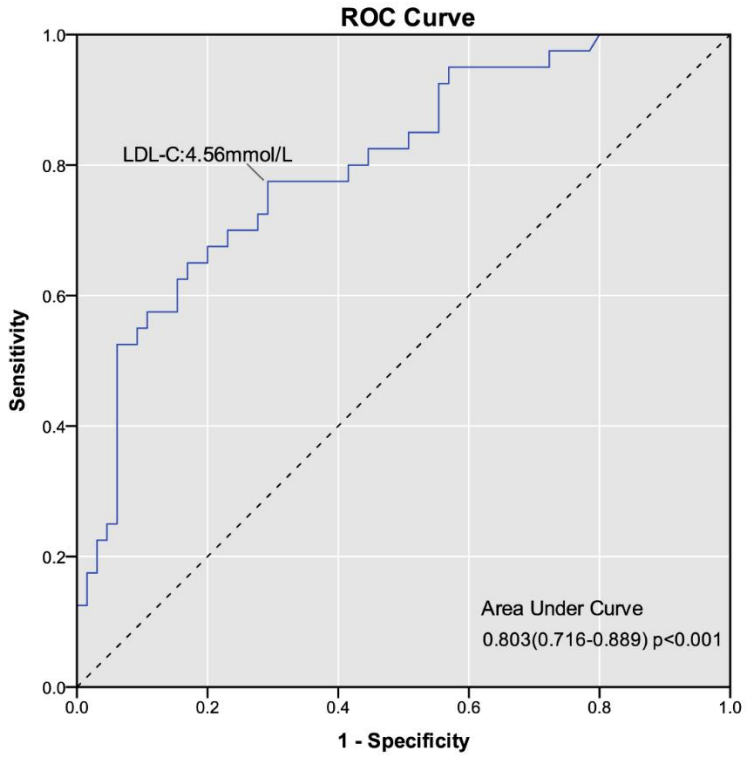
LDL-C: low-density lipoprotein cholesterol; CAD: coronary artery disease. *LDLR*: low-density lipoprotein receptor; *APOB*: Apolipoprotein B; *PCSK9*: proprotein convertase subtilisin/Kexin type 9; *STAP1*: Signal-transducing adaptor protein 1.

Figure S2. Percentage participants with CAD meeting clinical and genetic criteria for FH diagnosis.



CAD: coronary artery disease; FH: familial hypercholesterolemia.

Figure S3. Receiver operating characteristic curves of LDL-C (n=105).



LDL-C: low-density lipoprotein cholesterol. Sensitivity=0.775, Specificity=0.708.