Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Wald DS, Bestwick JP, Morris JK, Whyte K, Jenkins L, Wald NJ. Child–parent familial hypercholesterolemia screening in primary care. N Engl J Med 2016;375:1628-37. DOI: 10.1056/NEJMoa1602777

SUPPLEMENTARY APPENDIX

This appendix has been provided by the authors to give readers additional information about the study.

Table of Contents

Study Organisation

Steering Committee: David Wald, Jonathan Bestwick, Nicholas Wald, Joan Morris
Funding: Medical Research Council
Sponsor: Barts and The London School of Medicine, Queen Mary University of London

The General Practices that participated were part of the NIHR Primary Care Research Network. They were, in alphabetical order:

Albany House Medical Centre (Wellingborough)
Ambrose Avenue Surgery (Colchester)
Andaman Surgery (Lowestoft)
Ashfields Primary Care Centre (Cheshire)
Ashvale Health Centre (Surrey)
Axbridge Surgery (Somerset)
Beccles Medical Centre (Beccles)
Bradford-on-Avon Health Centre (Bradford-on-Avon)
Bridge Road Surgery (Lowestoft)
Brigstock Medical Practice (Surrey)
Broadway Medical Centre (Fleetwood)
Burns Medical Practice (Bennetthorpe)
Church View Surgery (Hedon)
Churchfields Surgery (Bromsgrove)
Claughton Medical Centre (Liverpool)
Crewkerne Health Centre (Crewkerne)
Cringleford Surgery (Norwich)
Cromer Group Practice (Norfolk)
Danetre Medical (Northants)
Desborough & Rothwell (Northants)
Dolphin House Surgery (Ware)
Drs Cloak, Choi and Milligan (Sunderland)
Dr Moss & Partners (Harrogate)
Duston Medical Centre/Harlestone Road (Northampton)
Enderley Medical Centre (Wealdstone)
Estuary View Medical Centre (Whitstable)
Failsworth Group Practice (Failsworth)
Frances Grove Surgery (London)
Garth Surgery (Guisborough)
Glastonbury Surgery (Glastonbury)
Granville Medical Centre (Ilford)
Green Bank Surgery (Warrington)
Greens Norton & Weedon (Northants)
Greensand Medical Practice (Potton)
Harborough Fields (Rushden)
Hendon Way Surgery (London)
Hillview Surgery (Perivale)
Ilford Lane Surgery (Ilford)
Keats Group Practice (London)
Kiltearn Medical Centre (Cheshire)
Kingswood Surgery (Turnbridge Wells)
Leicester Terrace Health Centre (Northampton)
Leighton Road Surgery (Luton)

Lower Clapton Health Centre (London)
Magdalen Medical Practice (Norwich)
Manor Surgery (Redruth)
Mathukia Surgery (Ilford)
Medwyn Surgery (Dorking)
Montpelier Health Centre (Bristol)
Mount Pleasant Health Centre (Exeter)
Mount View Practice (Fleetwood)
MSHC Hurstpierpoint Group Practice (West Sussex)
Northbourne Medical Centre (Shoreham-By-Sea)
Oakenhurst Practice (Blackburn)
Oak Lodge Medical Centre (Edgware)
Oak Tree Medical Practice (Ilford)
Oldfield Surgery (Bath)
Park Surgery (West Sussex)
Pound Hill Medical Group (Crawley)
Pullborough Medical Group Practice (Pullborough)
Queen Mary Practice (South Woodford)
Queen Square Surgery (Lancaster)
Richford Gate Medical Practice (London)
Rolle Medical Partnership (Exmouth)
Rosebank Medical Practice (Lancaster)
Rosedale Surgery (Lowestoft)
Ross Practice (Harlow)
Sedgefield Surgery/Harbinsion House (Sedgefield)
Shifa Surgery (Blackburn)
South Axholme Surgery (Epworth)
Southbourne Surgery (Emsworth)
St Andrews Group (Hull)
St James's Surgery (Bath)
St Katherines Surgery (Ledbury)
St. Mary's Surgery (Cambridgeshire)
St Stephen's Gate (Norwich)
Staploe Medical Centre (Ely)
Streatham Common Practice (Streatham)
The Bromley Common Practice (Bromley)
The Medical Centre/Lakeside (Northants)
The Park Medical Practice (Preston)
Trinity & Bowthorpe Medical Practice (Norwich)
Vine Surgery (Somerset)
Wallace House Surgery (Hertford)
Wandsworth Medical Centre (London)
Warders Medical Centre (Tonbridge)
West Malling Group Practice (West Malling)
Whitby Group Practice (Whitby)
Wood Lane Medical Centre (Ruislip)
Wrythe Green Surgery (Surrey)
Wyndmondham Medical Centre (Norfolk)
York Medical Group (Acomb)
York Road Surgery (Ilford)

Table S1: Familial Hypercholesterolemia (FH) mutations in FH48 panel

	FH48 Panel of Single Nucleotide Polymorphisms		Mutation type	Location
	DNA level coding	Protein level coding		
1	*APOB* c. 10580G>A	p.(Arg3527Gln)	Missense	*APOB* exon 26
2	*PCSK9*: c.1120G>T	p.(Asp374Tyr)	Missense	*PCSK9* exon 7
3	*LDLR*: c.313+1G>A	p.?	Splicing	Intron 3
4	*LDLR*: c.654_656delTGG	p.(Gly219del)	In-frame deletion	Exon 4
5	*LDLR*:c.2054C>T	p.(Pro685Leu)	Missense	Exon 14
6	*LDLR*: c.2061dup	p.(Asn688Glufs*29)	Frameshift	Exon 14
7	*LDLR*: c.6delG	p.(Trp4Glyfs*202)	Frameshift	Exon 1
8	*LDLR:* c.81C>G	p.(Cys27Trp)	Missense	Exon 2
9	*LDLR*: c.131G>A	p.(Trp44*)	Nonsense	Exon 2
10	*LDLR*: c.190+4A>T	p.?	Splicing	Intron 2
11	*LDLR:* c.259T>G	p.(Trp87Gly)	Missense	Exon 3
12	*LDLR:* c.266G>A	p.(Cys89Tyr)	Missense	Exon 3
13	*LDLR*: c.269A>G	p.(Asp90Gly)	Missense	Exon 3
14	*LDLR*: c.301G>A	p.(Glu101Lys)	Missense	Exon 3
15	*LDLR:* c.337G>A	p.(Glu113Lys)	Missense	Exon 4
16	*LDLR*: c.501C>A	p.(Cys167*)	Nonsense	Exon 4
17	*LDLR:* c.502G>A	p.(Asp168Asn)	Missense	Exon 4
18	*LDLR:* c.551G>A	p.(Cys184Tyr)	Missense	Exon 4
19	*LDLR*: c.564C>G	p.(Tyr188*)	Nonsense	Exon 4
20	*LDLR:* c.662A>G	p.(Asp221Gly)	Missense	Exon 4
21	*LDLR*: c.680_681del	p.(Asp227Glyfs*12)	Frameshift	Exon 4
22	*LDLR*: c.681C>A	p.(Asp227Glu)	Missense	Exon 4
23	*LDLR:* c.682G>T	p.(Glu228*)	Nonsense	Exon 4
24	*LDLR*: c.798T>A	p.(Asp266Glu)	Missense	Exon 5
25	*LDLR*: c.858C>A	p.(Ser286Arg)	Missense	Exon 6
26	*LDLR*: c.933del	p.(Glu312Serfs*58)	Frameshift	Exon 6
27	*LDLR*: c.939C>A	p.(Cys313*)	Nonsense	Exon 6
28	*LDLR:* c. 1027G>A	p.(Gly343Ser)	Missense	Exon 7
29	*LDLR*: c.1048C>T	p.(Arg350*)	Nonsense	Exon 7
30	*LDLR*: c.1150C>T	p.(Gln384*)	Nonsense	Exon 8
31	*LDLR*: c.938G>A	p.(Cys313Tyr)	Missense	Exon 6
32	*LDLR*: c.1222G>A	p.(Glu408Lys)	Missense	Exon 9
33	*LDLR*: c.1238C>T	p.(Thr413Met)	Missense	Exon 9
34	*LDLR*: c.1246C>T	p.(Arg416Trp)	Missense	Exon 9
35	*LDLR*: c.1285G>A	p.(Val429Met)	Missense	Exon 9
36	*LDLR*: c.1436 T>C	p.(Leu479Pro)	Missense	Exon 10
37	*LDLR*: c.1444G>A	p.(Asp482Asn)	Missense	Exon 10
38	*LDLR*: c.1444G>C	p.(Asp482His)	Missense	Exon 10
39	*LDLR*: c.1447T>C	p.(Trp483Arg)	Missense	Exon 10
40	*LDLR*: c.1474G>A	p.(Asp492Asn)	Missense	Exon 10
41	*LDLR*: c.1646G>A	p.(Gly549Asp)	Missense	Exon 11
42	*LDLR*: c.1033C>T	p.(Gln345*)	Nonsense	Exon 10
43	*LDLR*: c.1745T>C	p.(Leu582Pro)	Missense	Exon 16
44	*LDLR*: c.1196C>A	p.(Ala399Asp)	Missense	Exon 9
45	*LDLR*: c.1897C>T	p.(Arg633Cys)	Missense	Exon 13
46	*LDLR*: c.2389+2T>G	p.?	Splicing	Intron 16
47	*LDLR*: c.2389G>A	p.(Val797Met)	Missense	Exon 16
48	*LDLR*: c.1747C>T	p.(His583Tyr)	Missense	Exon 12

The 48 most common mutations in the *LDLR* gene, *APOB* gene and *PCSK9* gene identified by North East Thames Regional Genetics Lab over a 10 year period (2001-2010), in patients with suspected FH undergoing DNA analysis for an FH mutation [13] (www.ucl.ac.uk/ldlr/Current/index.php?select_db=LDLR). Mutation nomenclature is in accordance with Human Genome Variation Society (HGVS) guidelines (http://www.hgvs.org/mutnomen/). Reference sequences: *LDLR*: NM_000527.4, *APOB*: NM_000384.2; *PCSK9*: NM_174936.2
https://portal.biobase-international.com/hgmd/pro/mut.php?accession=CM970879

Table S2: Familial hypercholesterolemia (FH) mutations ranked by child's total cholesterol, and LDL and total cholesterol levels in child and parent with the FH mutation.

		Mutation found by		Cholesterol (mg/dL)			
				Child◆		Parent with mutation	
	Mutation	FH48 panel	DNA sequencing	Total	LDL	Total	LDL
1	MLPA heterozygote deletion of exon probes 13-14		X	282	142	237	159
2	*LDLR* heterozygote deletion of two probes in promotor and exon 1		X	273	218	264	174
3	*APOB* c.10580G>A heterozygote	X		273	203	168	73
4	*APOB* c.10580G>A heterozygote	X		266	225	229	142
5	*LDLR* c.1444G>A heterozygote	X		264	200	209	149
6	*LDLR* c.1705+1G>A heterozygote		X	264	199	286	224
7	*APOB* c.10580G>A heterozygote	X		260	203	198	135
8	*APOB* (FDB) c.10580G>A heterozygote	X		257	194	360	291
9	*APOB* c.10580G>A heterozygote	X		256	189	227	174
10	*LDLR* c.2093_2094dup heterozygote		X	246	181	248	191
11	*LDLR* c.551G>A heterozygote	X		245	183	251	173
12	*LDLR* heterozygote for exon 15 probes		X	243	158	226	118
13	*LDLR* c.542C>G heterozygote		X	242	174	207	113
14	*LDLR* c.1027G>A heterozygote	X		241	171	200	103
15	*LDLR* c.2483A>C heterozygote		X	240	210	265	191
16	*APOB* c.10580G>A heterozygote	X		239	177	279	177
17	*APOB* c.10580G>A heterozygote	X		239	172	197	136
18	*APOB* c.10580G>A heterozygote	X		235	172	246	165
19	*LDLR* c.337G>A heterozygote	X		232	151	174	108
20	*APOB* c.10580G>A p.Arg3527Gln	X		231	163	360	291
21	*APOB* c.10579C>T heterozygote[§]	X		227	170	276	213
22	*APOB* c.10580G>A heterozygote	X		221	127	316	-
23	*LDLR* c.682G>A heterozygote	X		220	159	298	241
24	*LDLR* c.259T>G heterozygote	X		220	152	204	134
25	*LDLR* c.662A>G, p. (Asp221Gly)	X		217	138	*	
26	*APOB* c.10580G>A heterozygote	X		206	157	280	172
27	*APOB* c.10580G>A heterozygote	X		204	133	225	163
28	*LDLR* c.266G>A heterozygote	X		203	145	280	217
29	*APOB* c.10580G>A heterozygote	X		193	126	223	128
30	*LDLR* c.502G>A heterozygote	X		184	111	184	125
31	*APOB* c.10580G>A heterozygote	X		175	87	229	163
32	*LDLR* c.1027G>A heterozygote	X		172	111	168	102
33	*APOB* c.10672C>T heterozygote	X		148	90	174	125
34	*APOB* c.10580G>A heterozygote	X		143	82	168	118
35	*LDLR* c.337G>A heterozygote	X		141	67	132	48
36	*LDLR* c.1238C>T heterozygote	X		131	99	174	120
37	*LDLR* c.81C>G heterozygote	X		100	61	**	

◆ 1 to 20 total cholesterol≥1.53MoM, 21-37 total cholesterol <1.53MoM

* father declined testing and mother had no mutation ** father unavailable and mother had no FH mutation

[§]non-target SNP identified in off-cluster data for the *APOB* c.10580G>A probe and confirmed by sequencing.

To convert mg/dL to mmol/L divide by 38.67

Table S3: Detection rate (DR), false positive rate (FPR) and Odds of being affected given a positive result (OAPR) according to specified outcome

Outcome	Children with total cholesterol ≥1.53 MoM				
	DR (sensitivity)		FPR (100%-specificity)		
	n	% (95% CI)	n	% (95% CI)	OAPR (95% CI)**
FH48 mutation present	13/30	43% (25%-63%)	79/10,065	0.8% (0.6%-1.0%)	1:6 (1:3-1:12)
FH48 mutation or mutation present found through sequencing	20/37	54% (37%-71%)	72/10,058	0.7% (0.6%-0.9%)	1:4 (1:2-1:6)
FH48 mutation or mutation present found through sequencing or a first and second cholesterol ≥1.53 MoM	28/45	62% (47%-76%)	64/10,050	0.6% (0.5%-0.8%)	1:2 (1:1-1:4)
FH48 mutation or mutation present found through sequencing allowing for limited sequencing*	20/43	47% (31%-62%)	72/10,052	0.7% (0.6%-0.9%)	1:4 (1:2-1:6)
FH48 mutation or mutation found through sequencing or repeat cholesterol ≥1.53 MoM allowing for limited sequencing*	28/51	55% (40%-69%)	64/10,044	0.6% (0.5%-0.8%)	1:2 (1:1-1:4)

*Since sequencing was only performed in children with total cholesterol ≥1.53 MoM without an FH48 mutation, a further 7/20×17=6 FH mutations would be expected if sequencing had been done in children with a cholesterol<1.53 MoM

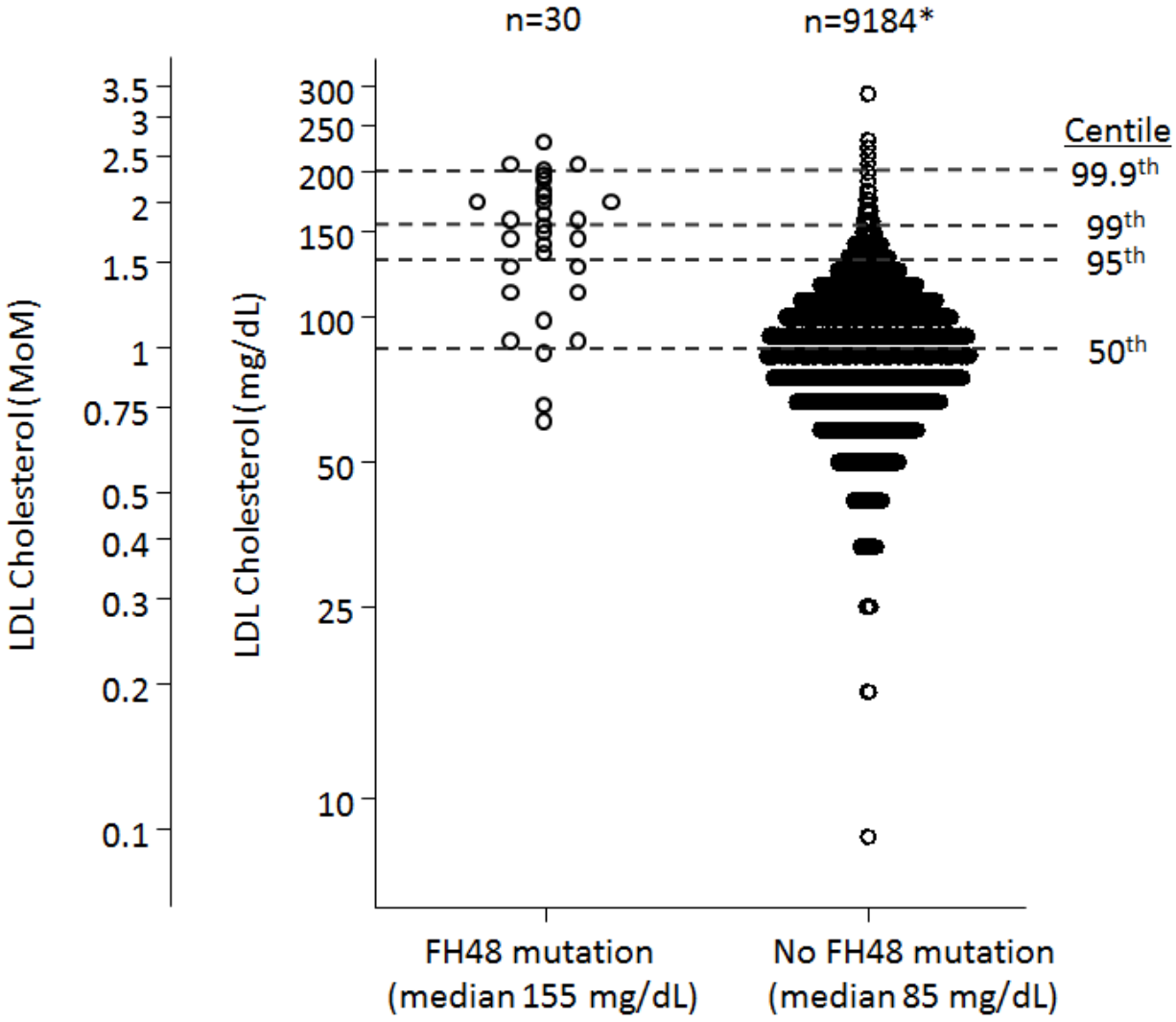
**OAPR = positive predictive value expressed as an odds

Table S4: Parent's cholesterol according to child's cholesterol (≥1.53 MoM [99.2nd centile] or ≥1.35 MoM [95th centile]) and according to presence/absence of FH mutation or higher/lower cholesterol in absence of mutation

		Parents' cholesterol							
		FH Mutation				No FH Mutation			
Child's total cholesterol		Total cholesterol		LDL cholesterol		Total cholesterol		LDL cholesterol	
≥1.53 MoM	≥1.35 MoM	mg/dL	MoM	mg/dL	MoM	mg/dL†	MoM	mg/dL†	MoM
With FH mutation									
Yes	Yes	360	2.16	291	2.87	139	0.84	63	0.62
Yes	Yes	360	2.16	291	2.87	139	0.84	n/a*	n/a
No	Yes	316	1.90	n/a*	n/a	128	0.77	68	0.67
No	Yes	298	1.79	241	2.37	168	1.01	101	1.00
Yes	Yes	286	1.72	224	2.21	n/a**	n/a	n/a**	n/a
No	Yes	280	1.68	172	1.70	188	1.13	122	1.20
No	Yes	280	1.68	217	2.14	191	1.15	84	0.83
Yes	Yes	279	1.67	177	1.75	202	1.21	108	1.06
No	Yes	276	1.66	213	2.10	160	0.96	73	0.72
Yes	Yes	265	1.59	191	1.88	155	0.93	92	0.90
Yes	Yes	264	1.59	174	1.72	234	1.41	161	1.59
Yes	Yes	251	1.51	173	1.71	163	0.98	90	0.89
Yes	Yes	248	1.49	191	1.89	169	1.02	69	0.68
Yes	Yes	246	1.48	165	1.63	184	1.11	113	1.12
Yes	Yes	237	1.42	159	1.56	113	0.68	54	0.53
No	No	229	1.38	163	1.61	155	0.93	89	0.88
Yes	Yes	229	1.38	142	1.40	162	0.97	80	0.79
Yes	Yes	227	1.36	174	1.71	129	0.78	n/a*	n/a
Yes	Yes	226	1.36	118	1.16	205	1.23	106	1.05
No	Yes	225	1.35	163	1.61	161	0.97	53	0.53
No	No	223	1.34	128	1.27	162	0.97	80	0.79
No	No	213	1.28	120	1.18	149	0.89	82	0.81
Yes	Yes	209	1.25	149	1.47	n/a**	n/a	n/a**	n/a
Yes	Yes	207	1.25	113	1.11	139	0.84	85	0.84
No	Yes	204	1.22	134	1.32	172	1.04	106	1.05
Yes	Yes	200	1.20	103	1.02	235	1.41	n/a**	n/a
Yes	Yes	198	1.19	135	1.34	148	0.89	102	1.01
Yes	Yes	197	1.18	136	1.34	165	0.99	118	1.17
No	No	184	1.11	125	1.23	189	1.13	128	1.27
Yes	Yes	174	1.05	n/a*	n/a	155	0.93	99	0.97
No	No	174	1.04	n/a*	n/a	165	0.99	108	1.06
No	No	168	1.01	73	0.73	192	1.15	125	1.23
Yes	Yes	168	1.01	118	1.16	192	1.15	101	1.00
No	No	168	1.01	102	1.00	n/a**	n/a	n/a**	n/a
No	No	132	0.79	48	0.48	215	1.29	118	1.16
No	No	n/a**	n/a	n/a**	n/a	151	0.91	51	0.50
No	Yes	n/a**	n/a	n/a**	n/a	101	0.60	70	0.69
With high repeat cholesterol		Higher total cholesterol		Higher LDL cholesterol		Lower total cholesterol		Lower LDL cholesterol	
Yes	Yes	263	1.58	176	1.74	169	1.02	101	1.00
Yes	Yes	255	1.53	n/a*	n/a	254	1.53	162	1.60
Yes	Yes	241	1.44	129	1.27	179	1.08	102	1.00
Yes	Yes	238	1.43	143	1.41	100	0.60	n/a*	n/a
Yes	Yes	231	1.39	174	1.71	222	1.34	145	1.43
Yes	Yes	230	1.38	123	1.21	203	1.22	104	1.02
Yes	Yes	217	1.31	152	1.50	206	1.24	122	1.20
Yes	Yes	171	1.03	104	1.03	169	1.02	104	1.03
Overall median		229	1.38	152	1.50	167	1.00	101	1.00

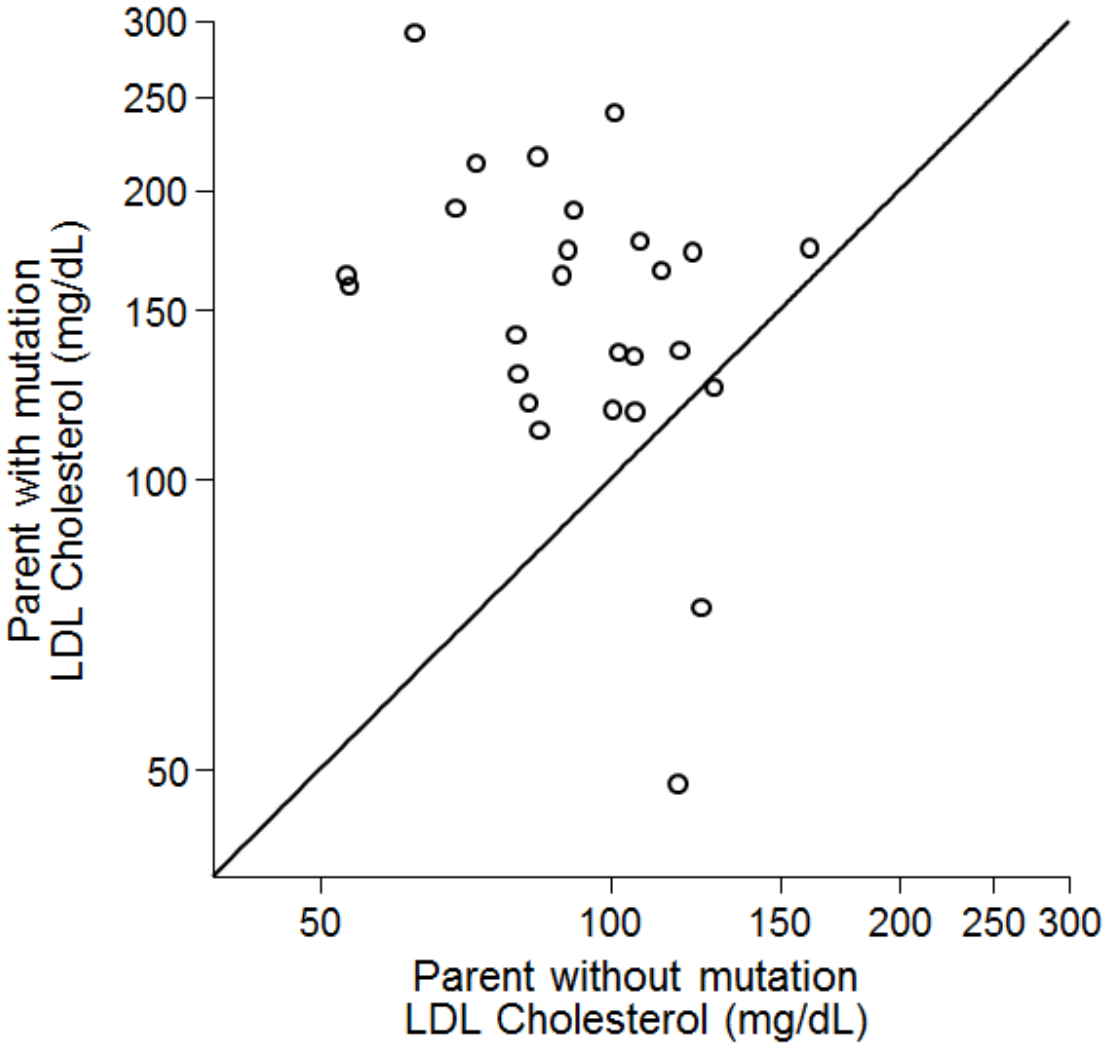
* LDL not reliably calculated (triglycerides or HDL out of range) **father unavailable or declined testing.
†values used to calculate MoMs. To convert mg/dL to mmol/L divide by 38.67

Figure S1: LDL-cholesterol levels in children with and without an FH48 mutation



*n is less than in Figure 2 for total cholesterol because LDL-cholesterol could not be reliably calculated (triglycerides or HDL out of range). To convert mg/dL to mmol/L divide by 38.67

Figure S2: Plot of LDL-cholesterol and pairs of parents with and without FH mutation



To convert mg/dL to mmol/L divide by 38.67