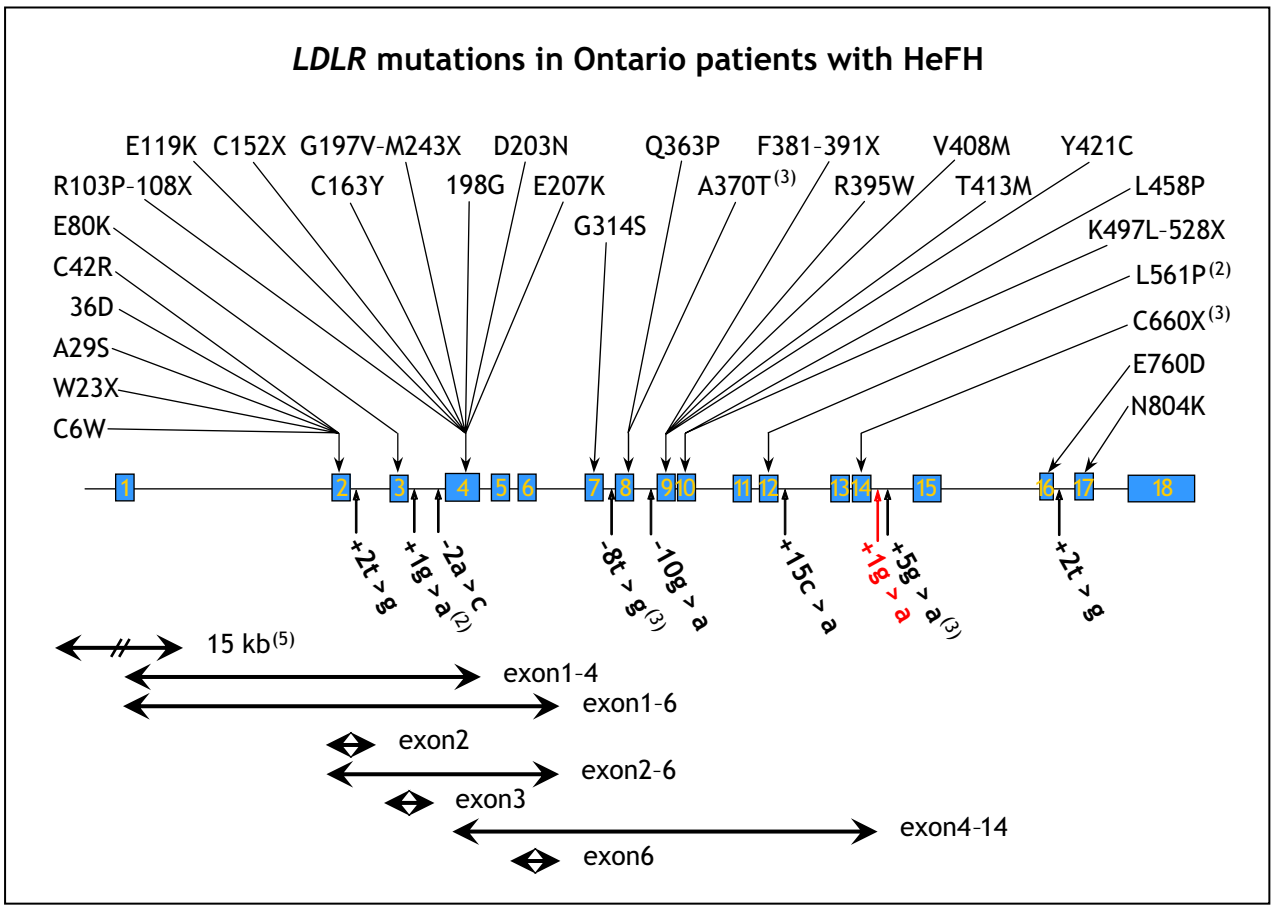
Appendix 1: Genetic maps of *LDLR* mutations in heterozygous familial hypercholesterolemia (HeFH)

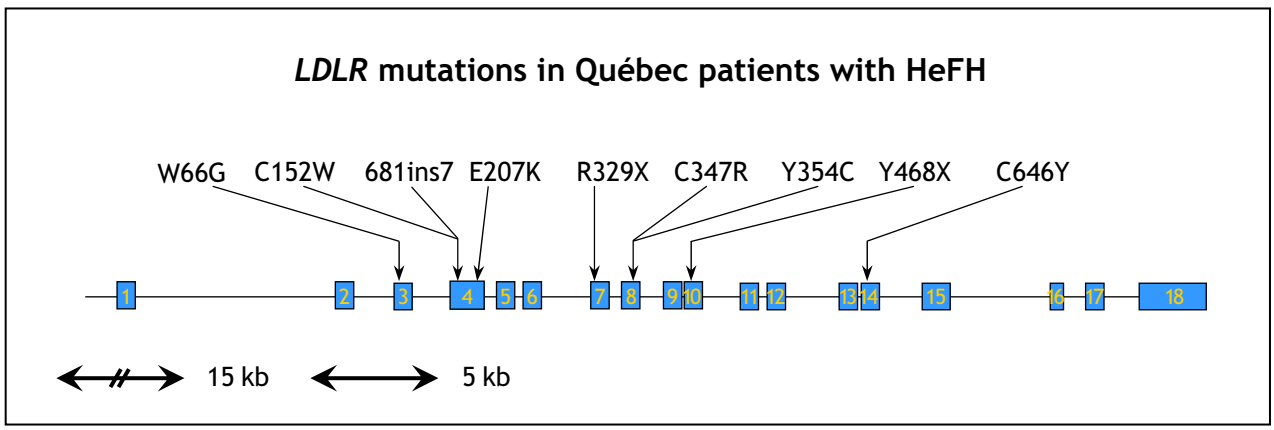
The first 80 of 189 unrelated probands with a clinical diagnosis of definite or probable HeFH based on non-molecular criteria (see Table 1 of the article), including the proband of the current report, had genomic DNA nucleotide sequence analyses of the known causative genes for HeFH, namely *LDLR*, *APOB*, *ARH* and *PCSK9*, as previously reported.[1,2] The *LDLR* gene of subjects whose genomes showed no abnormalities by DNA sequencing was examined using multiplex ligation-dependent probe amplification (MLPA) to detect possible larger insertions or deletions (also called copy-number polymorphisms or copy-number variations).[3] All mutations detected were confirmed by replication of results on 2 different days, sequencing in 2 directions with 2 independent analyses, or both.

Each of the 2 linear maps shows the genomic structure of the *LDLR* gene in the middle of each panel. Blue boxes represent exons; arrows point to mutation positions. Above the map are single-nucleotide and small insertion–deletion (indel) mutations. Single-letter amino codes are shown, along with the mutated codon number. Directly under the linear map are splicing mutations, indicated with diagonal labels. The position relative to the intron–exon boundary is shown numerically; the mutated nucleotide is shown in lower case. Larger deletions are shown as horizontal bidirectional arrows further below the linear map, with the span of the deleted region indicated by text label as well as by arrow length.



Among 80 patients with HeFH in Ontario who underwent molecular study, we found 58 with a heterozygous abnormality in the *LDLR* gene and another 5 with one in the *APOB* gene (R3500Q mutations). None of the 80 patients had a mutation in the *ARH* or *PCSK9* genes. Thus, 17 people had no abnormality detected in any gene. Among the 58 patients with *LDLR* mutations, 40 had single-nucleotide mutations and 18, indel mutations. There were 45 unique *LDLR* gene mutations in total, with no predominant recurring mutation. (On the gene map, when a mutation occurred in 2 or more unrelated proband patients, the number of recurrences is indicated within superscripted parentheses.)

Among the 31 single-nucleotide mutations, 19 were missense mutations (C6W, A29S, C42R, E80K, E119K, C163Y, D203N, E207K, G314S, Q363P, A370T [3 probands], R395W, V408M, T413M, Y421C, L458P, L561P [2 probands], E760D and N804K); 3 were nonsense mutations (W23X, C152X and C660X [2 probands]); and 9 were splicing mutations (intron 2 +2t > g; intron 3 +1g > a [2 probands] and –2a > c; intron 7 –8t > g [3 probands]; intron 8 –10g > a; intron 12 +15c > a; intron 14 +1g > a [current proband's mutation, boxed] and +5g > a [3 times]; and intron 16 +2t > g). Among the 14 unique indel mutations, 2 were inframe deletions detected by nucleotide sequence analysis (36D and 198G); 4 were small-frameshift deletions causing premature truncations (R103P–108X; G197V–M243X; F381–391X and K497L–528X); and 8 were larger deletions of about 300–15 000 nucleotide base pairs detected only by MLPA analysis, with the span of the deleted region shown by bidirectional arrows: 15 kb including exon 1 (5 times; also very common among Québec patients with HeFH [see linear map below); exon1–4; exon1–6; exon2; exon2–6; exon3; exon4–14; and exon6. Each mutation was clearly heterozygous by DNA sequencing or MLPA, and absent from the genomes of 100 normolipidemic subjects. The mutation in the proband of the current report (**+1g > a**, in red) was previously reported in patients from Utah who were of northern European descent, but has not previously been found in any Canadian with HeFH. The loss of the splice donor resulted in the recognition of another donor site, 214 bp further downstream, with inclusion of the 214-bp intron sequence into the mutant mRNA.[4] This mutant sequence predicted 70 mutant amino acids followed by a premature stop codon and severely truncated protein.



Reports of large clinical cohorts have consistently shown that > 90% of patients in Québec with HeFH have 1 of 11 *LDLR* mutations, most of which are highly recurrent in the population. Among the 8 mutations involving single nucleotides, 6 were missense mutations (W66G, C152W, E207K [also found in Ontario], C347R, Y354C and C646Y) and 2 were nonsense mutations (R329X and Y468X). Among the 3 indel mutations, 1 was a small frameshift deletion with premature truncation (681ins7) and 2 were larger deletions (15 kb including exon 1 [also found in Ontario patients] and 5 kb).

References

1. Wang J, Huff E, Janecka L, et al. Low density lipoprotein receptor (LDLR) gene mutations in Canadian subjects with familial hypercholesterolemia, but not of French descent. *Hum Mutat* 2001;18:359.
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3. Sharp AJ, Locke DP, McGrath SD, et al. Segmental duplications and copy-number variation in the human genome. *Am J Hum Genet* 2005;77:78-88.
4. Takada D, Emi M, Ezura Y, et al. Interaction between the LDL-receptor gene bearing a novel mutation and a variant in the apolipoprotein A-II promoter: molecular study in a 1135-member familial hypercholesterolemia kindred. *J Hum Genet* 2002;47:656-64.