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Case Report

# Rapid DNA Diagnosis of Familial Hypercholesterolemia Due to the *LDLR* 15.8-Kilobase Deletion

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**Heterozygous familial hypercholesterolemia (HeFH) affects 1 in 250-300 Canadians and usually is caused by a pathogenic DNA variant in the *LDLR* gene, resulting in impaired catabolism of low-density lipoprotein (LDL) and an increased risk of atherosclerotic cardiovascular disease.[1] Because > 3000 causal variants are reported, molecular diagnosis requires next-generation DNA sequencing (NGS), which is accurate, but it also requires a relatively long turnaround time.[2] We saw a patient with suspected HeFH who required an expedited molecular diagnosis to meet a deadline for clinical trial enrollment. This need was met using a rapid allele-specific polymerase chain reaction (PCR)-based detection method that was subsequently confirmed by NGS.**

An asymptomatic 16 year-old female patient was referred by her family doctor for assessment of suspected HeFH and potential enrollment in a clinical trial of an investigational treatment. Her 42-year-old mother had an elevated cholesterol level but no cardiovascular history, and she had been taking rosuvastatin 40 mg, and ezetimibe 10 mg, daily for almost 2 decades. None of the patient's 4 siblings had lipid abnormalities. The patient's maternal grandfather was a Franco-Ontarian who had suffered a fatal myocardial infarction in his sixties. On examination, the patient's blood pressure was 106/68 mm Hg, and her pulse was 70 beats per minute. She had no physical findings suggestive of hypercholesterolemia, and her cardiovascular examination was unremarkable. When she was age 15 years, her LDL cholesterol was 6.4 mmol/L, and subsequently she was prescribed rosuvastatin 10 mg daily, to which her compliance was poor. She took no other medications.

The patient's lipid profile at the time of assessment showed total, LDL, and high-density lipoprotein cholesterol levels of 7.1, 5.0, and 1.7 mmol/L, respectively, with a triglyceride level of 0.9 mmol/L. No clinical or biochemical abnormalities suggesting an untreated secondary cause of dyslipidemia were present. The 12-lead electrocardiogram was normal. Her Dutch Lipid Clinic Network score of 4 was consistent with "possible HeFH," consistent with scoring using the Canadian familial hypercholesterolemia (FH) algorithm.[3]

The patient and her parents were interested in participating in a clinical trial, depending on a definite HeFH diagnosis. They consented to DNA analysis, approval for which was covered by Western University Institutional Review Board protocol 0379E. A potential complicating issue was the imminent closure date for screening for and enrollment in the trial, which fell 7 days after her clinic appointment. A "stat," targeted NGS analysis for HeFH requires 4 weeks; nevertheless, her DNA was sent for this testing. But in the meantime, we considered alternative DNA diagnostic methods to provide a timelier answer for the family.

Because of her maternal Francophone ancestry, we reasoned that our patient might have inherited one of the handful of distinct "French Canadian" pathogenic *LDLR* variants. The most prevalent of these is the large 15.8-kb deletion encompassing the 5′-untranslated region and intron 1 of the *LDLR* gene (sometimes called "French Canadian 1," and here abbreviated as "15.8-kb del"), which is a null allele from which no functional receptor protein is produced.[4] Previously, we used dedicated PCR reagents specifically designed to rapidly detect this particular *LDLR* variant.[5] We applied this procedure and reagents to determine whether the 15.8-kb del variant was present in our patient.

Genomic DNA was extracted from blood and saliva. We included DNA from a known NGS-proven positive control for the heterozygous *LDLR* 15.8-kb del variant and from a known negative control with NGS-proven biallelic normal *LDLR* sequence. A DNA-free blank also was included. We amplified DNA samples using PCR conditions and reagents as described previously (details available upon request from R.A.H.).[5] This testing was completed within 48 hours and showed definitively that the patient was heterozygous for the *LDLR* 15.8-kb del variant (Fig. 1A-C). With these new data, her revised Dutch Lipid Clinic Network score increased to 12, consistent with "definite HeFH," which was confirmed by the Canadian FH algorithm also.[3] The patient and her family elected to pursue enrollment in the clinical trial, and she completed screening before the trial's closing date.

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**Novel Teaching Points**

- Next-generation DNA sequencing is the current recommended method to diagnose FH, but turnaround times are relatively protracted.
- A large deletion of 15.8 kilobases of DNA in the 5' region of the *LDLR* gene is the most commonly encountered pathogenic variant in both Quebec and Ontario.
- Older diagnostic methods utilizing PCR can rapidly detect certain pathogenic variants, such as the *LDLR* 15.8-kb del.
- We present a case study in which we detected the *LDLR* 15.8-kb del variant within 48 hours, using a dedicated rapid-diagnostic technique.

After the PCR-based diagnosis, results of confirmatory laboratory procedures were reported. First, after 7 days, Sanger sequencing clearly showed the deletion breakpoint, thus confirming the deletion of the intervening 15.8 kb of genomic DNA sequence at the 5′-end of the *LDLR* gene (Fig. 1D). In addition, NGS complemented by copy number−variation analysis independently confirmed heterozygosity for the *LDLR* 15.8-kb del variant (data not shown). Furthermore, no other pathogenic variants in *LDLR* or other causal genes for FH were detected. Although the NGS results were confirmatory, they became available only 21 days after the closure date for the clinical trial, and thus, they would not have allowed a definite diagnosis before the study closure date.

## Discussion

We describe expedited DNA diagnosis of an *LDLR* 15.8-kb del pathogenic variant also known as the "French Canadian-1 FH mutation," and variably, in the past, as "> 10 kb del" and "> 15 kb del."[4] The dedicated PCR-based method yielded a definitive diagnosis within 48 hours, compared with a 28-day turnaround time for the NGS method commonly used in diagnostic facilities across Canada. Also, confirmatory definitive results were obtained within 7 days using a third method, namely Sanger-based DNA sequencing. The circumstances were somewhat unusual for our patient, given the time pressure for a DNA diagnosis, owing to a clinical trial closure date. Pragmatically, the patient integrated the genetic information into her personal decision-making process. Had only NGS technology been available, molecular diagnosis would have been delayed, possibly leading to a different outcome. Although the PCR-based method described enabled a rapid diagnosis, it was limited to detection of this variant allele only; thus, a negative result would not have excluded a different pathogenic variant for FH in our patient. The variant also was confirmed by Sanger sequencing and NGS, with no other mutations detected. Because the NGS method was targeted to dyslipidemia, an incidental finding unrelated to lipid disorders had an essentially zero likelihood.

We have observed the *LDLR* 15.8-kb del variant recurrently in our Ontario FH registry: specifically, it is present in 26 of 339 Ontario-born adults (7.7%) who were referred with a diagnosis of possible or probable HeFH, most of whom have reported having a Francophone heritage. In fact, the 15.8-kb del is the most common single FH-causing variant of the *LDLR* gene in our patient archive. This variant is also the most common pathogenic variant for FH in Quebec.[4,6]

The colonization of nouvelle France by 8500 settlers between 1608 and 1759 has given rise to 22 Mendelian diseases that present at high rates in French Canadian individuals, one of which is HeFH.[6] Genealogical reconstructions identified 14 French founders of this deletion from the 17th century, 7 of whom resided near Saumur, France, which appears to be the likely geographic origin locus for the *LDLR* 15.8-kb del variant.[6] The 15.8-kb del variant accounts for ∼56% of the total pathogenic *LDLR* alleles in Quebecois, supporting a founder effect.[6] Although the population was established in Lower Canada (present-day Quebec), French explorers traversed the regions of Canada, including Upper Canada (present-day Ontario).

During the 17th and 18th centuries, several regions of present-day Ontario, including the North (eg, the Sudbury region), the Southwest (eg, the Windsor-Chatham region), and the Ottawa Valley were settled by individuals who had relocated from present-day Quebec. The geographic distribution of the *LDLR* 15.8-kb del variant highlights the close historical and geographic relationship between the Ontario and Quebec populations. A finding that would be of interest is determination of the prevalence of the *LDLR* 15.8-kb del variant in other regions of Canada that have subpopulations with historical Francophone ancestry, such as Manitoba and the Prairie provinces, as well as the Maritime provinces. Also, evidence of a founder effect for HeFH has been found among Franco-Americans, reflecting the historical migration of French Canadians to New England, Louisiana, and other regions in the US.[7]

A notable point from our patient's experience is that an older, low-tech method for this specific variant yielded a diagnostic result more rapidly than the current gold-standard, recommended methodology. However, the turnaround times for molecular diagnosis with NGS are improving. Furthermore, although the cost of the PCR-based method is ∼15% of the cost of NGS, the results are restricted only to this particular deletion variant; this screening method is thus very limited for the general population. With technological progress however, in the future, a patient faced with a scenario similar to the one reported here might receive an expedited, in-time diagnosis with NGS, likely at lower cost.

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## Ethics Statement

The research reported has adhered to tricouncil ethical guidelines, approval for which was covered by Western University Institutional Review Board protocol 0379E.

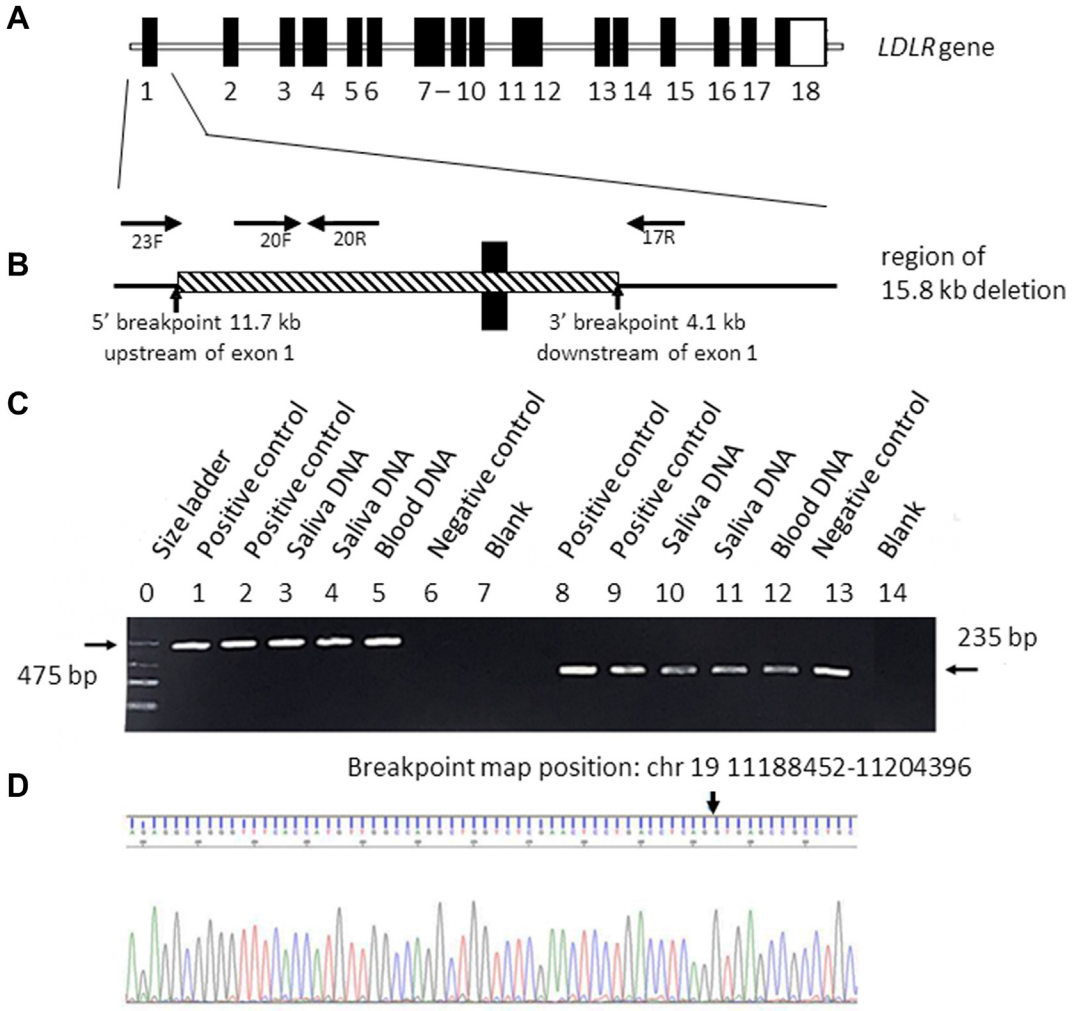


**Figure 1.** Rapid polymerase chain reaction (PCR)-based detection of the French Canadian *LDLR* 15.8-kb deletion variant (a 15.8-kilobase deletion encompassing the 5′-untranslated region and intron 1 of the *LDLR* gene). (**A**) Shown is the genomic structure of the *LDLR* gene, which is composed of 18 coding exons, numbered as indicated. (**B**) Shown is a closeup of the 5′-end of *LDLR* showing the region harboring the 15.8-kb deletion variant. The deleted sequence is indicated by the **hashed box**. Diagnostic PCR primers 23F and 17R anneal to DNA sequences outside of exon 1—5′ untranslated region (UTR) and intron 1—and will not produce an amplified fragment when the deletion is absent, but they will yield an abnormal 475—base pair (bp) fragment when the deletion is present. In contrast, diagnostic primers 20F and 20R anneal to a normal DNA sequence and will not produce a fragment when the deletion is present, but they will produce a 235-bp fragment when the deletion is absent. (**C**) Shown are diagnostic amplified fragments visualized using 1.5% agarose gel electrophoresis. Lane 0 contains a DNA sizing ladder. Lanes 1-7 show amplification products from primers 23F and 17R. Lanes 1 and 2 come from positive controls known to be heterozygous for the *LDLR* 15.8-kb del variant showing the abnormal 475-bp fragment. Lanes 3 and 4 come from amplification of the patient's salivary DNA showing the abnormal 475-bp fragment characteristic of the *LDLR* 15.8-kb del variant. Lane 5 comes from amplification of the patient's blood DNA and also shows the diagnostic 475-bp fragment. Lane 6 comes from an individual with 2 normal copies of the *LDLR* gene, confirming absence of the abnormal fragment. Lane 7 was derived from amplification of a blank sample containing no DNA. Lanes 8-14 were derived using amplification primers 20F and 20R specific for the normal *LDLR* allele. The order of samples parallels that in lanes 1-7. Presence of the normal 235-bp DNA fragment in all samples confirms that each had at least one copy of normal *LDLR* sequence, including lanes 8 and 9 from proven heterozygous familial hypercholesterolemia patients, and lanes 10-12 from the patient. Because these 3 individuals also had the abnormal 475-bp fragment (see lanes 1-5), heterozygosity was confirmed. Lane 13 came from an individual with 2 normal copies of the *LDLR* gene; the presence of the normal 235-bp band, together with the absence of the abnormal fragment in lane 6, confirmed homozygosity for the normal *LDLR* sequence. Lane 14 came from amplification of a blank sample containing no DNA. Overall results confirm that our patient was heterozygous for the *LDLR* 15.8-kb deletion. (**D**) Shown are a Sanger sequencing tracing of the patient's DNA, highlighting the critical deletion breakpoint depicted from (**B**), with the position and genomic map coordinates of the deletion breakpoint on chromosome (chr) 19.

## Patient Consent

The authors confirm that a patient consent form has been obtained for this article.

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## Disclosures

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