

Screening of novel restless legs syndrome–associated genes in French-Canadian families

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Abstract

Objective

To examine whether any rare, protein-altering variants could be identified across 13 recently identified restless legs syndrome (RLS) loci in familial French-Canadian cases.

Methods

Whole-exome sequences from 7 large French-Canadian families (4–8 affected per family for a total of 38 cases) were examined for variants in any genes located within 1 Mb on either side of each locus.

Results

Among the 43 rare protein-altering variants identified, none segregated with RLS in the families.

Conclusions

Our study does not support a role for causative protein-altering variants in the genes that are located either in the previously or newly identified RLS loci. It is therefore possible that noncoding regulatory variants within these loci or yet unidentified loci could be the cause of RLS in our families.

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Glossary

ExAC = exome aggregation consortium; RLS = restless legs syndrome; WES = whole-exome sequencing.

As much as 60% of patients with restless legs syndrome (RLS) have a positive family history,¹ with a heritability close to 20%.² Using a cohort of 671 cases (192 probands and 479 affected relatives), our team has previously reported that 77.1% of French-Canadian patients had a family history of RLS, suggesting an important contribution of genetic factors in this population.³ In an effort to identify coding variants in the 6 previously identified RLS loci (*MEIS1*, *BTBD9*, *PTPRD*, *MAP2K5/SKOR1*, *TOX3*, and rs6747972), we have previously examined 7 French-Canadian families with an autosomal dominant inheritance pattern using whole exome sequencing (WES). Variants were identified in *PTPRD* and *SKOR1*, but none of these segregated with the disease in the families studied.⁴ Recently, a large-scale meta-analysis confirmed the 6 loci known to be associated with RLS and identified 13 novel loci.² In the current study, we reanalyzed WES data from the 7 French-Canadian families to examine whether coding variants segregating with RLS could be identified in genes within 1Mb of all 19 loci.

Methods

Samples

Seven French-Canadian families consisting of 32 women (mean age \pm SD: 71.44 \pm 15.23 years) and 6 men (mean age \pm SD: 70.17 \pm 17.65 years) were examined using WES (female: male ratio of 5.33:1). All patients were diagnosed according to the International RLS Study Group criteria.⁵ Family pedigrees of probands are shown in figures 1–7.

Standard protocol approvals, registrations, and patient consents

All subjects provided informed consent, and the study was approved by the respective institutional review boards.

Whole exome sequencing

WES libraries were prepared using the Agilent SureSelect Human All Exon V4 (Agilent Technologies, Los Angeles, CA) capture kit and sequenced using an Illumina HiSeq2000 platform (100 base pair paired-end sequencing). Reads were aligned to the hg19 human reference genome using the Burrows-Wheeler Aligner tool.⁶ Variant calling was performed using the HaplotypeCaller tool from the Genome Analysis Toolkit v.3.5.^{7,8} Finally, variants were annotated for predicted protein alterations and population frequencies using annotate variation (ANNOVAR).⁹

Variant filtration and segregation analysis

Only variants that were predicted to be protein-altering (nonsynonymous, splicing, stop-gain) by ANNOVAR were included in the subsequent analysis. Variants were filtered by frequency using the Exome Aggregation Consortium (ExAC) browser, Cambridge, MA (exac.broadinstitute.org, accessed January 2018). Variants below a threshold of 0.05 allele frequency in the non-Finnish European population were included in the final results.

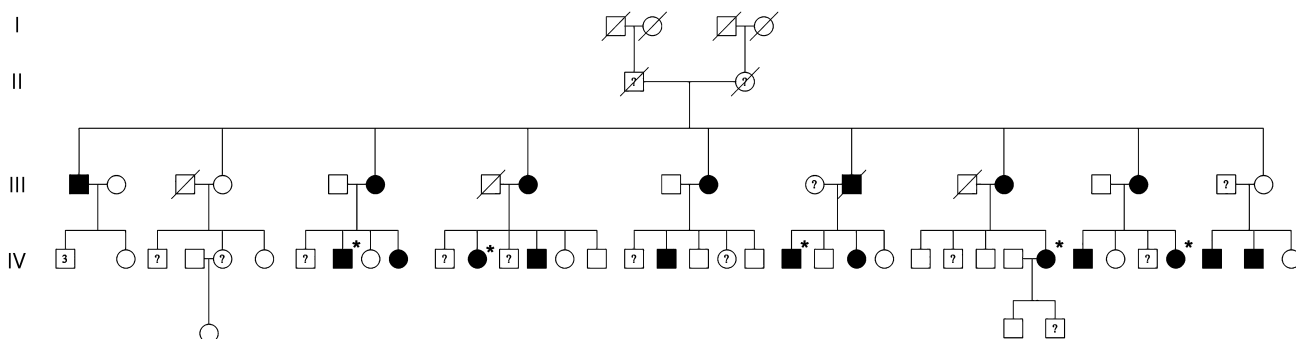
Data availability statement

The authors confirm that the data necessary for confirming the conclusions of this study are available within the article and its supplementary material. Raw whole exome sequencing data will be provided freely upon request.

Results

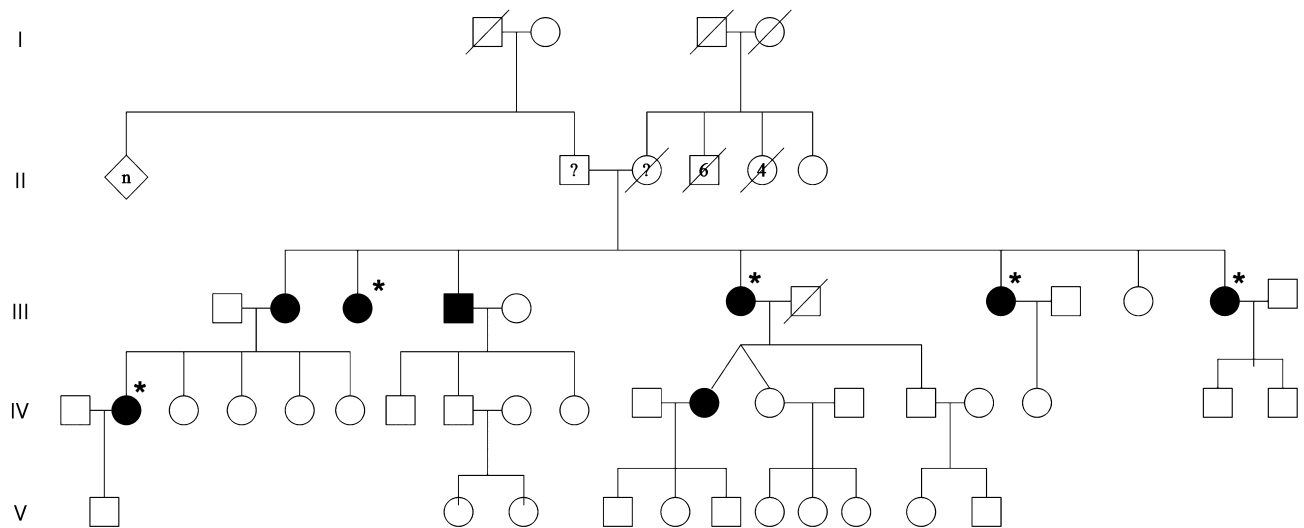
A total of 71 genes within 1Mb of the 19 loci were found to be screened in 38 affected individuals and a list of candidate variants was established (table e-1, links.lww.com/NXG/A131). The average and minimum coverage of genes screened were 87x

Figure 1 Pedigree of family 1



*Exome sequencing data available.

Figure 2 Pedigree of family 2



*Exome sequencing data available.

and 25x, respectively. A total of 43 variants were predicted to be protein-altering and had a population frequency less than 0.05. Among the variants identified, none of them segregated well with the disease in pedigrees, which suggests that they are not disease causing.

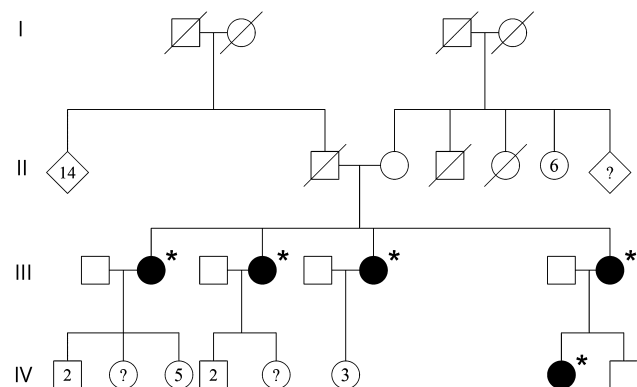
DNAH8 p.Val874Met (rs45529837, ExAC MAF = 0.03071) appeared to segregate well in one of the families, and as such, it might explain RLS in this family. However, this particular variant was also observed in another family (Family 5: IV-5,6,7 and V-8) where it did not segregate with the disease.

Discussion

Our results suggest that nonsynonymous variants within these loci do not explain RLS in these large families and that it is therefore likely that regulatory (coding or non-coding) variants

are associated with the risk of RLS. While p.Val874Met (rs45529837, ExAC MAF = 0.03071) in *DNAH8* (that encodes for an axonemal dynein involved in motility of cilia and flagella)¹⁰ segregated well in one of the families, it was also observed in another family (Family 5: IV-5,6,7 and V-8) where it did not segregate with the disease, therefore its segregation should be interpreted with caution. Rare causative variants, at much lower frequency than the associated common single nucleotide polymorphism (SNP), can create genome-wide associations even when they are megabases away from the common variants that tag them.¹¹ A WES approach, like the one used here, can enable the discovery of novel causative variants. The likelihood of achieving this increases with the size of the pedigrees and the penetrance of the condition examined. Although our study does not support a role of rare protein-altering variants in RLS-associated loci to be a cause of the disease, further studies in more pedigrees are required to determine whether there exist monogenic forms of RLS.

Figure 3 Pedigree of family 3



*Exome sequencing data available.

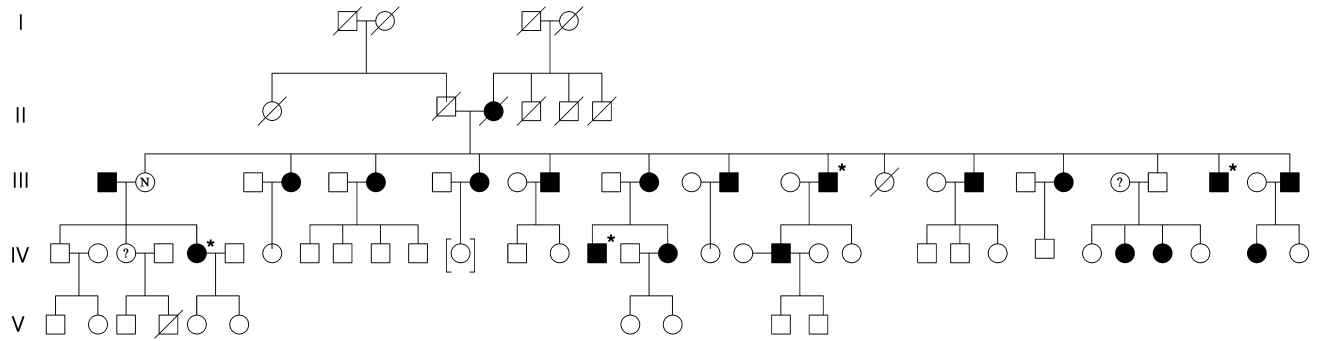
Author contributions

F. Akçimen: design and conceptualized the study; analysis and interpretation of the data; and drafting the manuscript for intellectual content. D. Spiegelman: analysis of the data. A. Dionne-Laporte: analysis of the data. Z. Gan-Or: drafting or revising the manuscript for intellectual content. P.A. Dion: design and conceptualized the study; interpretation of the data; and drafting or revising the manuscript for intellectual content. G.A. Rouleau: design and conceptualized the study; interpretation of the data; and drafting or revising the manuscript for intellectual content.

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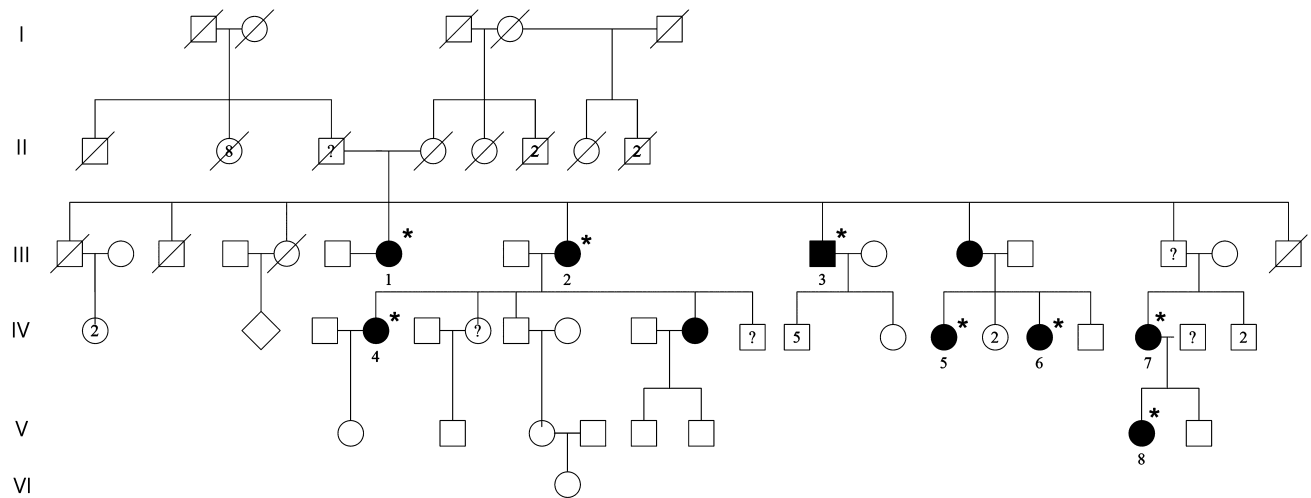
The authors thank the patients for their participation in the study. GAR holds a Canada Research Chair in Genetics of the

Figure 4 Pedigree of family 4



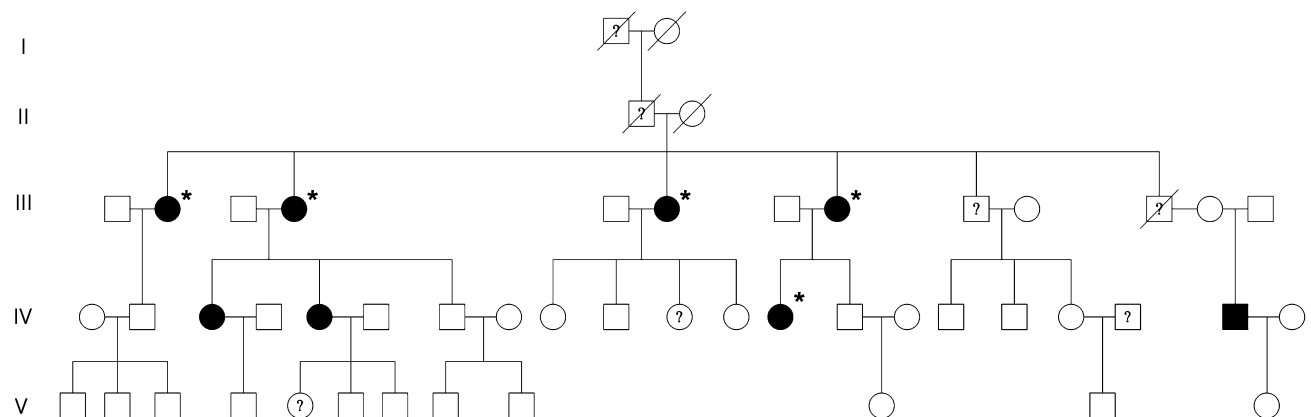
*Exome sequencing data available.

Figure 5 Pedigree of family 5



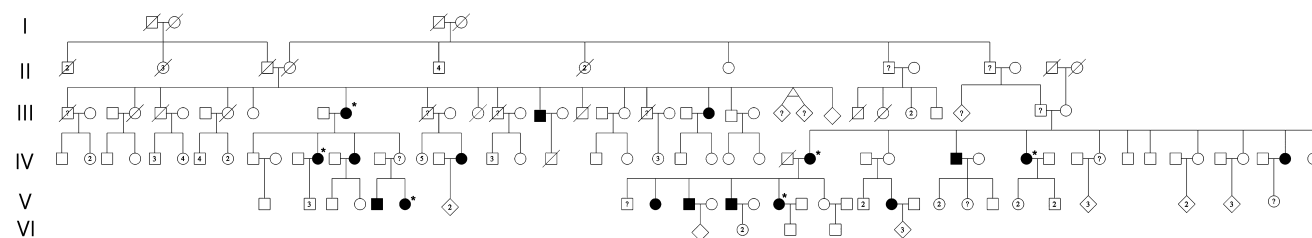
*Exome sequencing data available.

Figure 6 Pedigree of family 6



*Exome sequencing data available.

Figure 7 Pedigree of family 7



*Exome sequencing data available.

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References

1. Winkelmann J, Polo O, Provini F, et al. Genetics of restless legs syndrome (RLS): state-of-the-art and future directions. *Mov Disord* 2007;22(suppl 18): S449–S458.
2. Schormair B, Zhao C, Bell S, et al. Identification of novel risk loci for restless legs syndrome in genome-wide association studies in individuals of European ancestry: a meta-analysis. *Lancet Neurol* 2017;16:898–907.
3. Xiong L, Montplaisir J, Desautels A, et al. Family study of restless legs syndrome in Quebec, Canada: clinical characterization of 671 familial cases. *Arch Neurol* 2010;67: 617–622.
4. Gan-Or Z, Zhou S, Ambalavanan A, et al. Analysis of functional GLO1 variants in the BTBD9 locus and restless legs syndrome. *Sleep Med* 2015;16: 1151–1155.
5. Allen RP, Picchietti DL, Garcia-Borreguero D, et al. Restless legs syndrome/Willis-Ekbom disease diagnostic criteria: updated International Restless Legs Syndrome Study Group (IRLSSG) consensus criteria—history, rationale, description, and significance. *Sleep Med* 2014;15:860–873.
6. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25:1754–1760.
7. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011;43: 491–498.
8. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010; 20:1297–1303.
9. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010;38: e164.
10. Neesen J, Koehler MR, Kirschner R, et al. Identification of dynein heavy chain genes expressed in human and mouse testis: chromosomal localization of an axonemal dynein gene. *Gene* 1997;200:193–202.
11. Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nat Rev Genet* 2010;11: 415–425.