A Novel Duplication Confirms the Involvement of 5q23.2 in Autosomal Dominant Leukodystrophy

Inge A. Meijer, PhD; Ana A. Simoes-Lopes, MD; Sandra Laurent; Tanya Katz, BSc; Judith St-Onge, DEC; Dominique J. Verlaan, PhD; Nicolas Dupré, MD, MSc; Manon Thibault, MD; Johanne Mathurin, MD; Jean-Pierre Bouchard, MD; Guy A. Rouleau, MD, PhD

Objective: To identify the underlying locus and disease-causing mutation for adult-onset autosomal dominant leukodystrophy (ADLD).

Design: Previously, an adult-onset ADLD locus on chromosome 5q23 was mapped between markers D5S1495 and CTT/CCT15. This region contains 13 known and putative candidate genes. A 2-point linkage analysis confirmed linkage of a large multigenerational French Canadian family to chromosome 5q23. In addition, screening of the 13 genes within the candidate interval as well as 5 neighboring genes was completed, followed by comparative genomic hybridization.

Subjects: A multigenerational French Canadian family with ADLD mimicking progressive multiple sclerosis was identified and studied. Eight affected family members were available for the study and presented with autonomic dysfunction as well as upper motorneuron signs affecting gait.

Results: The thorough candidate gene approach did not identify any mutation. Consequently, a whole-chromosome comparative genomic hybridization for chromosome 5 identified a 280-kilobase duplication within the chromosomal band 5q23.2 in 2 affected individuals. This duplication contains 3 genes: LMNB1, FLJ36242, and MARCH3.

Conclusion: We have identified a novel duplication on chromosomal band 5q23.2 in a French Canadian family with ADLD that supports the implication of duplicated LMNB1 as the disease-causing mutation. However, additional functional studies of lamin B1 overexpression are necessary to elucidate the involvement of lamin B1 in myelination and in degenerative disorders such as ADLD and multiple sclerosis.

Arch Neurol. 2008;65(11):1496-1501
During a candidate gene analysis, Padiath et al identified 3 tandem duplications in the ADLD candidate region by means of single-nucleotide polymorphism (SNP) sequence-peak comparisons. In the study, they presented 4 families including 2 Irish American families with a common ancestor and 2 Japanese families. The borders of the 3 duplications were determined; they spanned approximately 341 kilobase pairs (kb) and 169 kb, respectively. The duplicated regions consistently contained 3 genes: LMNB1, FLJ36242, and MARCH3.

Lamin B1 (LMNB1) belongs to the family of laminas (LMNA, LMNB1, and LMNB2), which are type V intermediate filaments in the nuclear lamina. However, the function of LMNB1 has not been well characterized. Mouse deficient in LMNB1 die shortly after birth and display bone and lung abnormalities. On a molecular level, the nuclear membrane of the deficient mouse embryonic fibroblasts showed nuclear blebs possibly due to impaired cellular differentiation. Overexpression of the fly ortholog, lam- min DM, as well as a human LMNB1 transgene in a Drosophila melanogaster model using different drivers showed increased lethality, most notably in the glial-specific model. Mutations have also been described in other lamins; for example, mutations in LMNA cause different types of muscular dystrophies, lipodystrophy, and premature aging diseases. Similar phenotypes were also found in LMNA-null mice. Interestingly, LMNB1 is ubiquitously expressed throughout development, whereas LMNA is only expressed in differentiated cells. Mutations in LMNB2 are associated with acquired partial lipodystrophy. In addition, the autosomal dominant Pelger-Huet anomaly is caused by reduced expression of the LMNB receptor, which affects neutrophil nuclear shape and chromatin distribution in a dose-dependent manner. These diseases are called laminopathies and are caused by mutations in the nuclear lamins. In this study we present a French Canadian family with ADLD, a likely laminopathy, in which an approximately 280-kb duplication on chromosome 5q23 has been identified.

### METHODS

#### SUBJECTS

Informed written consent was obtained from subjects to participate in the study, which was approved by the local ethics review board of the Centre Hospitalier de Charlevoix, where all of the subjects were recruited. Peripheral blood samples were obtained from 21 individuals including 8 affected family members. The DNA was extracted by standard methods and lymphoblastoid cell lines were established. Individuals were recruited by a neurologist (J.-P.B.), and the diagnosis was based on a complete neurological examination and imaging of the brain by magnetic resonance imaging or computed tomographic scan. Five patients had complementary procedures such as electroencephalogram, electromyogram and nerve conduction studies as well as laboratory testing of blood and cerebrospinal fluid.

#### GENOTYPING

Polymorphic markers were amplified by polymerase chain reaction, incorporating radiolabeled sulfur 35 deoxyadenosine triphosphate into the product. The products were separated on 6% denaturing polyacrylamide gels and visualized on autoradiographic film. Key markers were initially genotyped in and around the ADLD locus, followed by more extensive fine mapping. Linkage analysis was performed using the MLINK program of the LINKMAP software package (National Institutes of Health, Bethesda, Maryland) assuming equal recombination frequency; a disease frequency of 1/1000 persons; age liability classes of 0-46 years, 50% penetrance; older than 47 years, 90% penetrance; and a phenocopy rate of 0. Haplotype construction assuming minimal recombination was performed manually and ordered according to the goldenpath physical map (UCSC Genome Browser; http://www.genome.ucsc.edu/).

### RESULTS

#### CLINICAL DATA

Figure 1 shows the multigenerational French Canadian family with ADLD with autosomal dominant segregation in 16 affected members over 3 generations. Interestingly, an affected branch of the family also carries a disease allele for the sacsin-encoding gene; therefore individual IV:1 was affected with both ADLD and autosomal recessive spastic ataxia of Charlevoix-Saguenay (OMIM 270550). Autosomal recessive spastic ataxia of Charlevoix-Saguenay is a young-onset spastic ataxia with a high carrier prevalence in the Charlevoix-Saguenay-Lac-Saint-Jean region of Quebec. Eight affected individuals were followed up by the same neurologist (J.-P.B.) while the clinical details of older family members were obtained through oral personal accounts. The disease status of each affected member was confirmed by imaging. The fifth generation was not included because of age-dependent reduced penetrance.

The symptoms in this family usually presented early in their fifth decade of life and consisted of bladder and/or bowel disturbances, lower limb weakness, orthostatic hy-
potension, and/or impotence. The disease progresses slowly and sometimes seems to remit (as is seen in MS). The severity of the disease also varies within the family; the most severe outcomes were observed in individuals who had the longest disease duration. The 2 affected subjects in the second generation (II:2 and II:4) were said to be “crawling on the floor” in their fifties. One individual (III:10) became bedridden (tetraplegic and anarthric) at 72 years of age, but survived until 78 years.

The signs and symptoms of the family are similar to those described by Eldridge et al, with a few exceptions. The lower limbs show marked hyperreflexia in most individuals with occasional involvement of the upper limbs. The patients display a spastic gait and have frequent falls. The Babinski sign is usually present or indifferent. There are less overt cerebellar signs with slightly affected rapid alternating movements and finger-to-nose tests. The sensory system is intact, with no hearing or visual loss noted. No dementia was reported in this family either. The nerve conduction studies were normal and the electroencephalogram did not show any epileptic discharges, but did show a diffuse slowing of electrical activity. Some autonomic features including impotence, bladder incontinence, constipation or fecal incontinence, decreased sweating, and orthostatic hypotension were eventually present in all affected individuals. One patient (IV:13) was most severely dysautonomic and had severe episodic temperature dysregulation leading to drops in temperature to as low as 32°C, which may ultimately have caused his death. Other causes of death in the family were neoplasms and infections, with an average age at death of 62 years.

Figure 1. The family tree of adult-onset autosomal dominant leukodystrophy (ADLD)-1. Pedigree showing autosomal dominant segregation ADLD in a large French Canadian family. Individual IV:1 (black filled square with smaller white square) was also affected with autosomal recessive spastic ataxia of Charlevoix-Saguenay. Roman numerals are general patient identifiers; numbers beginning with R are laboratory identifiers (DNA is available for these individuals).

Figure 2

Figure 3

LINKAGE AND HAPLOTYPE ANALYSIS

Two-point linkage analysis using an age-based liability class showed a Zmax of 4.03 (θ=0) for marker D5S2039. The markers D5S2055 and D5S649 showed a log of odds score of less than –2 and therefore excluded the regions outside of these markers. Fine mapping ensued and extensive genotyping of more than 35 markers in the region determined the critical segregating region between markers D5S2055 and D5S649 that spans 13 Mb or 7.74 cM. This candidate region overlapped the locus reported by Coffeen et al, and the overlap contained the aforementioned 18 candidate genes (Table). Meanwhile, Marklund et al reported a smaller locus flanked by markers D5S1495 and CTT/CCTT which only contained 13 genes.

After an optimal marker density was achieved, a candidate gene screen followed. The 18 candidate genes were thoroughly screened and no mutation was identified. The absence of a point mutation led to the Nimblegen comparative genomic hybridization analysis, which identified a large duplication spanning approximately 280 kb (Figure 3). The duplicated region contains 2 complete
genes, LMNB1 and FLJ36242, as well as the 5′ end of the MARCH3 gene. Sequencing of additional SNPs in the region as previously described by Padiath et al further confirmed the duplication. There were neither known copy-number variants nor insertions and/or deletions within and near the duplication.

This study identified a novel duplication on chromosome 5q23 in a large French Canadian family affected with ADLD. The finding confirms the results from Padiath et al that the cause of ADLD is a duplication containing 3 genes: MARCH3 (partially), FLJ36242, and LMNB1. The implication of a duplication in a demyelinating, highly penetrant, Mendelian disorder demonstrates the importance of including a copy-number variant analysis in standard mutation screens. Furthermore, sequence analysis should be programmed to recognize differences in peak heights that are suggestive of a duplication or a deletion. Hybrid cells containing only the affected allele did not aid in detecting the duplication and are more useful in detecting deletions.

Of the 3 genes found within the duplication, LMNB1 is the only complete gene known to be expressed in the brain, with particularly high expression in the cerebellum. Further analysis of the LMNB1 gene in the patients' brain samples showed that there were higher levels of LMNB1 mRNA and protein. Taken with the fact that duplications have been identified in 4 families with ADLD without other mutations in the candidate interval, overexpression of LMNB1 is the most likely cause of ADLD. However, overexpression studies in a cell model and eventually in a second organism will be necessary to better understand the role of LMNB1 in ADLD. In addition, the question remains whether point mutations in LMNB1 can also lead to this phenotype.

The duplication was identified by comparative genomic hybridization analysis in 2 affected individuals and verified by SNP peak analysis. Although the exact borders of the duplication were not determined because the region contained the same 3 genes as the ones identified by Padiath et al, the duplication was found to be of a different size and was estimated to span 280 kb. The fact that 3 similar duplications were found in 3 ethnic groups suggests that there might be a sequence motif predisposing...
Recent studies have marked the protein sirtuin 2 as a potential therapeutic avenue for demyelinating disease specific to oligodendrocytes because it was shown that the levels of sirtuin 2 transport are reduced in PLP-knock-out mice. In addition, lamins are early targets for caspase degradation. These interesting therapeutic avenues, together with a shift in focus to overexpression of LMNB1, might lead the way in the development of potential therapies for myelin disorders.

Accepted for Publication: January 8, 2008.

Correspondence: Guy A. Rouleau, MD, PhD, Centre de Recherche du Centre Hospitalier de l’Université de Montréal, Hôpital Notre Dame, Université de Montréal, Bâtiment De la Chute, 1560, rue Sherbrooke Est, Montréal, QC, Canada H2L 4M1 (guy.rouleau@umontreal.ca).

Author Contributions: Study concept and design: Meijer, Katz, Bouchard, and Rouleau. Acquisition of data: Meijer, Simoes-Lopes, Laurent, Katz, St-Onge, Dupré, Thibault, Mathurin, and Bouchard. Analysis and interpretation of data: Meijer, Simoes-Lopes, Laurent, Verlaan, Mathurin, Bouchard, and Rouleau. Drafting of the manuscript: Meijer, St-Onge, Mathurin, and Bouchard.
Critical revision of the manuscript for important intellectual content: Simoes-Lopes, Katz, Verlaan, Duprée, Thibault, Bouchard, and Rouleau. Statistical analysis: Verlaan. Obtained funding: Meijer and Rouleau. Administrative, technical, and material support: Meijer, Laurent, Katz, St-Onge, Mathurin, Bouchard, and Rouleau. Study supervision: Mathurin, Bouchard, and Rouleau.

Financial Disclosure: None reported.

Funding/Support: This study was supported by the Canadian Institute of Health Research (Dr Rouleau) and the Fonds de la recherche en santé du Québec (Dr Meijer).

Additional Contributions: We would like to thank the family for their participation and Johanne Simard, RN, coordinator of the Neuromuscular Disorder Clinic in Charlevoix, for her dedicated assistance.

REFERENCES


