Genomic deletions upstream of lamin B1 lead to atypical autosomal dominant leukodystrophy

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Abstract

Objective
Clinical, radiologic, and molecular analysis of patients with genomic deletions upstream of the LMNB1 gene.

Methods
Detailed neurologic, MRI examinations, custom array comparative genomic hybridization (aCGH) analysis, and expression analysis were performed in patients at different clinical centers. All procedures were approved by institutional review boards of the respective institutions.

Results
Five patients from 3 independent families presented at ages ranging from 32 to 52 years with neurologic symptoms that included progressive hypophonia, upper and lower limb weakness and spasticity, and cerebellar dysfunction and MRIs characterized by widespread white matter alterations. Patients had unique nonrecurrent deletions upstream of the LMNB1, varying in size from 250 kb to 670 kb. Deletion junctions were embedded in repetitive elements. Expression analysis revealed increased LMNB1 expression in patient cells.

Conclusions
Our findings confirmed the association between LMNB1 upstream deletions and leukodystrophy previously reported in a single family, expanding the phenotypic and molecular description of this condition. Although clinical and radiologic features overlapped with those of autosomal dominant leukodystrophy because of LMNB1 duplications, patients with deletions upstream of LMNB1 had an earlier age at symptom onset, lacked early dysautonomia, and appeared to have lesser involvement of the cerebellum and sparing of the spinal cord diameter on MRI. aCGH analysis defined a smaller minimal critical region required for disease causation and revealed that deletions occur at repetitive DNA genomic elements. Search for LMNB1 structural variants (duplications and upstream deletions) should be an integral part of the investigation of patients with autosomal dominant adult-onset leukodystrophy.

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Autosomal dominant leukodystrophy (ADLD, OMIM #169500) is a fatal, progressive neurologic disorder that presents in the 4th to 6th decade of life and primarily affects CNS myelin. Patients present with progressive autonomic dysfunction, followed by disturbance of motor control as a result of cerebellar deficits and spasticity; death occurs 10–20 years after the onset of symptoms. We have previously shown that ADLD is caused by a duplication of the lamin B1 gene (LMNB1, chr5q23.2), resulting in increased LMNB1 protein expression. Although only LMNB1 duplications have been definitively shown to cause ADLD, we recently identified a genomic deletion upstream of the LMNB1 gene in a single large pedigree (ADLD-1-TO) that resulted in a phenotype similar to ADLD caused by LMNB1 duplications. As the mutation was identified in only a single family, it was difficult to unequivocally confirm the link between the LMNB1-associated deletions and the leukodystrophy phenotype.

In this report, we present the analysis of 3 novel families with genomic deletions of varying sizes upstream of the LMNB1. The identification of a larger cohort of patients allows us to confirm the association between LMNB1 upstream deletions and disease, define a broader phenotypic spectrum associated with the mutation, and acquire mechanistic insights into the cause of this genomic rearrangement.

Methods

Five patients, belonging to 3 independent families, were examined because of adult-onset neurologic dysfunction. Array comparative genomic hybridization (aCGH) using a custom array, bioinformatics, and expression analysis was performed as described earlier. Histopathologic analysis and brief clinical and MRI findings from patient DEL2-1 have been described previously.

Standard protocol approvals, registrations, and patient consents

Clinical and radiologic evaluations took place under the guidelines of the respective institutional review boards, and all patients provided written informed consent.

Data availability statement

All data used in this study are included in this report or accompanying supplementary information.

Results

Clinical characteristics of the 5 patients are described in table 1 and e-supplementary clinical information (links.lww.com/NXG/A135). The age at onset of neurologic symptoms ranged from 32 to 52 years. Presenting symptoms included dysarthria and hypophonia (4/5), poor dexterity (4/5), imbalance (3/5), weakness of the extremities, including asymmetrical weakness (3/5), tremor (2/5), and painful leg spasms (1). Of note, early involvement of the autonomic nervous system was notable in only 1 patient with orthostatic intolerance and urinary urgency. In 2 patients, urinary urgency and incontinence were late features of the disorder, occurring only after development of severe lower limb spasticity. Two patients indicated significant propensity for worsening of symptoms in relation to elevated environmental heat and humidity.

Four patients underwent brain MRI (figure 1); all had a corticospinal tract involvement extending from the upper frontal lobes to the cerebral peduncles. Three patients (DEL1-1, DEL2-1, and DEL3-1) had extensive symmetrical white matter hyperintensities in all cerebral lobes with a less affected periventricular rim on T2-weighted spin-echo images. Patient DEL3-2 was unique, as she did not exhibit extensive lobar involvement compared with other patients. Of interest, the central parts of the pathologic areas in this patient showed a low signal intensity (SI) on T2-weighted fluid-attenuated inversion recovery images indicating high fluid content. The upper cervical spinal cord was seen in the sagittal brain images. The anteroposterior diameters at C II were below the normal range in all patients. MRI of the cervical and upper thoracic spine, obtained in DEL3-1 and DEL2-1, did not reveal atrophy at the lower levels, nor obvious SI alterations.

Patients were negative for mutations in known disease-causing genes including LMNB1 duplications (e-supplementary clinical information, links.lww.com/NXG/A135). Subsequent analysis using a custom aCGH assay allowed us to identify and map deletions upstream of the LMNB1 gene (figure 2). Deletions were unique to each family and ranged from ~670 kb to ~250 kb extending to within 88 kb–4.8 kb upstream of the LMNB1 start codon, respectively (table e-1). Analysis of the deletion boundaries (including those from the previously published ADLD-1-TO deletion) revealed that 3 of the 4 boundaries were in Alu repeats. The telomeric end of the DEL3 deletion was situated in a long interspersed nucleotide element (LINE) repeat element. A careful examination of the centromeric end of this deletion revealed a 20-bp region that had a high degree of homology to LINE elements. Thus, all the deletion boundaries appear to be embedded in some type of

Glossary

aCGH = array comparative genomic hybridization; ADLD = autosomal dominant leukodystrophy; LINE = long interspersed nucleotide element; SI = signal intensity; TAD = topologically associated domain.
repetitive DNA element. Expression analysis revealed increased levels LMNB1 mRNA in cells from one of the patients (Del1-1), consistent with the report on the ADLD-TO family (table e-2).6

### Table 1 Clinical data

| Patient | DEL1-1 | DEL2-1 | DEL3-1 | DEL3-2 | DEL3-3 |
|---------|--------|--------|--------|--------|--------|
| **Sex** | Male   | Male   | Female | Female | Male   |
| **Age at symptom onset (y)** | 34     | 52     | 37     | 34     | 32     |
| **Ethnicity** | East Asian | Northern European | Northern European/Native American | Northern European/Native American | Northern European/Native American |
| **Living status (age in years)** | Alive (40) | Deceased (59) | Alive (42) | Deceased (36) | Deceased (50) |
| **Presenting complaint at disease onset** | Right hand tremor and voice difficulties | Leg cramps and slowly progressive spasticity | Hypophonia, dysarthria, and hand incoordination | Soft/slurred speech and hand incoordination | Decreased dexterity of upper limb weakness and dysarthria |
| **Affect** | Normal | Chronic anxiety | Depressed mood | Normal | Pseudobulbar affect |
| **Oral motor control and articulation** | Soft “breathy” and halting voice and dysphagia | Speech was normal | Muffled voice, unilateral facial weakness, and drooling | Speech apraxia and dysphagia | Dysarthria |
| **Eye and extraocular movement examination** | Normal | Fine end-point nystagmus on lateral gaze. Interrupted saccades, in vertical and horizontal planes | Normal dilated examination, Saccadic intrusion | Optic disk pallor, limited up-gaze and saccadic intrusion | Normal |
| **Motor examination** | Head and hand tremor (asymmetrical), incoordination and spastic and ataxic gait, and weakness of lower extremities | Tremor of lower extremities, spasticity, and cerebellar deficits | Asymmetrical spastic weakness of upper and lower extremities | Asymmetrical (left > right) spastic weakness of upper and lower extremities | Decreased dexterity upper limb spasticity and asymmetrical weakness of both upper and lower limbs (right > left) |
| **DTRs/plantar responses** | Brisk with clonus/ extensor | Spastic in all limbs with lower limb clonus; positive Hoffman response | Lower extremity clonus/extensor | Brisk | Brisk/extensor |
| **Sensory examination** | Normal | Normal | Normal | Normal | Normal |
| **Autonomic nervous system** | Early orthostatic intolerance and urinary urgency | Late urinary urgency and incontinence | Normal by history and laboratory testinga | Normal by history | Late urinary urgency |
| **EMG/NCV** | NA | Normal | Normal | L peroneal entrapment neuropathy | NA |
| **Family history and inheritance patterns** | Negative | Positive/autosomal dominant | Positive/autosomal dominant | Positive/autosomal dominant | Positive/autosomal dominant |
| **Comorbidities** | Migraines, gout, and dyslipidemia | Hypertension, GERD, and DjD | None | None | None |
| **Other** | Neurologic deficits worsened with respiratory infection | Snout reflex | Neurologic deficits reported to be worsened by environmental heat | Dyspnea likely from neuromuscular weakness; L leg pain likely from peroneal neuropathy | Neurologic deficits reported to be worsened by environmental heat |

Abbreviations: DJD = degenerative joint disease; DTR = deep tendon reflex; GERD = gastroesophageal reflux disease; NA = not available; NCV = nerve conduction velocity.

a Included R-R variability, Tilt-table test, Valsalva maneuver, and sudomotor axon reflex test.

**Discussion**

Patients with deletions upstream of the LMNB1 gene had clinical and radiologic signs that exhibited both unique findings.
and partial overlap with ADLD due to LMNB1 duplications. Clinically, 4/5 patients presented at onset with speech symptoms including dysarthria and hypophonia. This has not been reported for ADLD with LMNB1 duplications, where the most common presenting feature was autonomic dysfunction. Onset of symptoms also appeared to be earlier in patients with deletions.

On MRI, cerebral white matter involvement was more extensive, especially in the temporal lobes in patients with deletions compared with those with duplications at the same ages. The periventricular rim was less severely affected than other pathologic lobar white matter in both patient groups. The corticospinal tracts were affected in both groups, but only in one of the deletion patients (DEL1-1) did this extend to the medulla oblongata. This was the only 1 of the 4 deletion patients having an abnormality in the cerebellar peduncles. This is in contrast to duplication patients who have early changes in the medulla oblongata and cerebellar peduncles. Cerebral atrophy is not a prominent feature in patients with duplications, but marked atrophy was found in 1 of our patients (DEL 2-1) and in the previously reported Italian ADLD-1-TO family. In patients with deletions, only the uppermost cervical spinal cord was atrophic, and in the 2 patients with a spinal MRI, no obvious SI changes were found in the rest of the cord. In LMNB1 duplication patients, the entire spinal cord is atrophic, and T2 signal in white matter is pathologic. This difference could explain the lack of early autonomic symptoms in patients with deletions, as it has been hypothesized that autonomic symptoms in ADLD with duplications are due to spinal cord involvement.

Analysis of the deletion events, which were clearly nonrecurrent, allowed us to define a minimal critical region of ~167 kb that is required for disease causation. Strikingly, this genomic region encompassed a boundary between 2 topologically associated
domains (TADs) and strengthens our original hypothesis that a disruption of the TAD boundary causes LMNB1 overexpression and in turn the disease. Sequencing the deletion junctions revealed the importance of repetitive elements (Alu, LINEs) in the genomic rearrangement. Their presence suggests that either a nonallelic homologous recombination mechanism or a microhomology-mediated break-induced repair type mechanism mediated by repeats is likely to cause the genomic deletions.

The identification of a larger cohort of patients confirms the pathogenic role of deletions upstream of LMNB1 in the leukodystrophy phenotype. Given that these mutations do not alter the coding sequence, our data also emphasize the importance of regulatory elements and the need for performing analyses for copy number variants that might be missed with the standard whole-exome sequencing, currently being used to identify mutations in patients with leukodystrophies.

**Author contributions**
C. Toro, A. Brusco, and Q.S. Padiath managed the project. A. Lehman, M.K. Koenig, R. Adejumo, M. Knight, R. Gavrilova, M. Alturkustani, M. Sharma, R. Hammond, W.A. Gahl, and C. Toro performed clinical and radiologic examinations. C. Toro and R. Raininko analyzed and compiled clinical and radiologic data. B. Nmezi and E. Giorgio performed aCGH experiments. B. Nmezi, E. Giorgio, M. Spielmann, A. Brusco, and Q.S. Padiath performed analysis of aCGH and genetic data. B. Nmezi, E. Giorgio, R. Raininko, C. Toro, A. Brusco, and Q.S. Padiath wrote the manuscript with inputs from all authors.

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**Disclosure**
B. Nmezi reports no disclosures. E. Giorgio holds patents for 3 siRNA sequences targeting a single allele of the human LamninB1 gene as therapeutic option for Autosomal Dominant Leukodystrophy. R. Raininko reports no disclosures. A. Lehman has received governmental research support from the Canadian Institutes for Health Research; has received academic research support from the Department of Medical Genetics at the University of British Columbia; and has received foundation/society research support from the Rare Disease Foundation and the British Columbia Clinical Genomics Network. M. Spielmann reports no disclosures. M.K. Koenig has served on the scientific advisory board of Novartis Pharmaceuticals; has received travel or speaker funding from Novartis Pharmaceuticals and Lundbeck; serves on the editorial board of the Journal of Child Neurology; holds a pending patent for a topical product for treatment of facial angiofibromas in Tuberous Sclerosis Complex; has served on speakers’ bureaus of Novartis Pharmaceuticals and Lundbeck; has received commercial research support from Novartis Pharmaceuticals, Reata Pharmaceuticals, EryDel S.p.A., Vtesse, Inc, Pfizer, Retrophin, Stealth, and Ultragenyx Pharmaceutical; has received governmental research support

**Figure 2** Pathogenic deletions upstream of LMNB1

Schematic diagram of the deletions upstream of the LMNB1 gene in the 3 reported families. Also shown is the deletion from the previously reported family (ADLD-1-TO) from Giorgio et al. (2015). A comparison between the genomic regions deleted in the different families allowed us to identify the minimal critical region required for the disease phenotype. Note that the critical region spans a 2 putative TADs (TD1 and TD2), and a deletion would result in the disruption of the TAD boundary (TDB). Putative forebrain enhancers are also indicated. TADs were characterized using the 3D Genome Browser (promoter.bx.psu.edu/hic) on fetal brain-derived data. ADLD = autosomal dominant leukodystrophy; TAD = topologically associated domain.
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