Abstract

Autosomal dominant leukodystrophy is an adult onset neurodegenerative disorder presenting with progressive symptoms of ataxia and autonomic dysfunction in fourth or fifth decade in life. It has clinical similarity with multiple sclerosis, but shows characteristic magnetic resonance imaging findings of diffuse bilaterally symmetrical leukodystrophy which can distinguish this disorder. It is a rare disorder with no known treatment till date, and has never been described from the Indian subcontinent. We present an Indian family with autosomal dominant adult-onset demyelinating leukodystrophy with multiple members affected over four generations, and demonstrate a cheap and accurate molecular method of real-time polymerase chain reaction to detect the LMNB1 gene duplication, which is the genetic basis of this devastating disorder.

Keywords: ADLD, adult-onset leukodystrophy, autonomic dysfunction, lamin B, LMNB1

INTRODUCTION

Autosomal dominant adult-onset demyelinating leukodystrophy (ADLD) is characterized by progressive loss of white matter within the central nervous system (CNS). Clinical presentations of ADLD include autonomic dysfunction, ataxia and cognitive impairment, which manifest around 40–50 years of age. Magnetic resonance imaging (MRI) of the brain in patients with ADLD typically reveals demyelination in white matter of the brain and spinal cord. Heterozygous duplications of the lamin B1 gene (LMNB1, located at chromosome 5q23.2) are observed in patients with ADLD. The disease is rare and to our knowledge has not yet been described from the Indian subcontinent.

We report a family of LMNB1 duplication mediated ADLD. The proband had a strong familial history of the disease, gene copy number of his father had been analyzed. Gene duplication in the proband was evaluated by a cost-effective, rapid and reliable quantitative real-time PCR (qPCR) technique which opened up newer vistas in the molecular assessment of ADLD patients.

CASE REPORT

The proband was a 45-year-old male, presenting with easy fatigability for 1.5–2 years. There was difficulty in rising up from a sitting posture, climbing stairs, and gripping slippers while walking. The proband did not experience weakness or abnormal sensation in the upper limbs. Minor mental exercise such as calculations led to confusion and anxiety. Additionally, he complained of constipation and urgency in micturition for the last 2 years. He had an emotional lability, asthenia, anhedonia, and had difficulty in controlling emotions. His sleep pattern was disturbed and erratic. In the family history, his father had developed similar symptoms at the age of 56 years and gradually progressed to a bed-ridden state at age 65, and died at 71 years of age. Proband’s grandmother and other relatives were also affected with similar illness (Figure 1).

Genetic testing for leukodystrophy was performed in proband’s father in United States of America, by whole exome sequencing and copy number variation analysis, which revealed a full gene duplication of LMNB1 gene.

On examination, the proband manifested with fine tremors of hands, no facial weakness, and had normal gait, mildly decreased muscle tone, and negative Gower sign. His speech was normal and there was no nystagmus. Although he had tremors in all the four limbs, he was able to move the limbs through a complete range of motion, against gravity and on full resistance (power 5/5). His reflexes were brisk in all the four limbs. He exhibited normal position sense and sensations. No cerebellar sign was observed except a mildly positive past pointing sign.

Clinical investigations demonstrated a normal blood count, thyroid, and lipid profiles. Hepatic and renal function tests...
were also normal. Serum vitamin B₁₂ level was normal although the level of vitamin D₃ was insufficient at 18 ng/ml. Serum creatine phospho-kinase (CK) level was 113 U/L (normal male range 22–198 U/L) that reduced the probability of muscle involvement.

MRI of the brain was obtained which revealed diffuse symmetrical T2W and FLAIR hyperintense signals. Areas of brain involved included periventricular and subcortical white matter in bilateral, fronto-parietal and occipital lobes, genu and body of corpus callosum, deep cerebellar white matter, middle cerebellar peduncles, pons, and cortico-spinal tracts in internal capsule along the bilateral posterior limb [Figure 2a-f].

In view of the strong family history, the proband’s test for LMNB1 gene was directly carried out starting with whole genome microarray based hybridization on AGILENT catalogue 4 × 180 K (CGH+SNP) array slide, followed by software utilizing UCSC build 37 (Hg19) for analysis. Since the microarray could not detect the duplication reliably, analysis was then performed by quantitative real-time PCR (qPCR) sybergreen assay. The procedure of qPCR was performed using modified protocols described previously [3, personal communication Dr Padiath QS]. Briefly, the qPCR was performed using an ABI Step OnePlus TM real-time thermal cycler. The melting curve analysis ensured correct determination of homogeneity of the PCR products including primer–dimers that confirmed the specificity of the qPCR reaction. The control gene Albumin was used as the reference. LMNB1 gene copy number was finally computed using the conventional 2-ΔΔct method. Our result indicates 1.47-fold higher value of LMNB1 gene in the proband compared to normal, confirming whole gene duplication of LMNB1 gene [Figure 3].

**DISCUSSION**

LMNB1 belongs to the intermediate filament protein superfamily playing a crucial structural function of forming the lamina of inner nuclear membrane maintaining nuclear integrity. *In vitro* studies indicate that an overexpression of LMNB1 protein increases nuclear rigidity in ADLD.⁴ Thus, clinical symptoms of leukodystrophy are because of the duplication of the lamin B1 protein producing gene, leading to overexpression.

LMNB1 duplications have been documented in varied genotypic populations such as American,⁴ Japanese, and French-Canadian. It has also been identified in people of Indian, Chinese, Irish, German, Swedish, and Israeli origins.⁵ Although described in the Indian population, the disorder has not been identified yet from India.

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**Discuss**

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Patients with ADLD typically present in the fifth or sixth decade of life with autonomic symptoms coinciding with pyramidal signs and ataxia. Finnsson et al. performed a natural history of clinical and radiological course of this disease in 23 patients from 2 families over the course of two decades. A comparison of our case with the cohort of Finnsson et al. is presented in Table 1. Autonomic symptoms seem to predominate in the early phase, followed by other neuroradiological deterioration over next decade or so. Clinical spastic para or quadriplegia sets in correlating with radiological worsening, eventually leading to cognitive involvement, pseudobulbar palsy and death after two decades of onset.\[^{6}\]\[6\] Periods of pseudoexacerbations are known in ADLD, making it difficult to differentiate from multiple sclerosis, at times.\[^{6}\]\[6\] Thus, a family history and genetic testing become crucial in diagnosis of ADLD.

The precise mechanisms of \textit{LMNB1} duplications causing ADLD are unclear. Multiple pathways have been suggested that include lamin B1 mediated downregulation of the proteolipid protein through regulated binding of Yin-Yang 1 transcription factor,\[^{7}\]\[7\] altered microRNA regulation of \textit{LMNB1}, and reduced lipid synthesis mediated by epigenetic modifications.\[^{8}\]\[8\] Newer technologies such as CRISPR have opened up new vistas for treatment of genetic disorders such as ADLD. A recent study on lung carcinogenesis through CRISPR/Cas9-mediated lamin B1 gene targeting has suggested invaluable potential therapeutic harnessing of the RET/p38 signaling pathway as treatment of ADLD.\[^{9}\]\[9\]

Copy number variants are not reliably identified by next-generation sequencing or chromosomal microarray analysis, especially if not large enough. Therefore, if ADLD is suspected clinically, specific testing for \textit{LMNB1} duplications must be performed.\[^{10}\]\[10\] Our study embellishes the molecular assessment of ADLD by qPCR in Indian context. The technique of real-time PCR allows the quantitation of the fragment of DNA which is of interest (in our case – \textit{LMNB1} gene) to provide a copy number of the gene. Instead of the usual two copies (one inherited from each parent), the patients have three copies (one copy is duplicated). In this q-PCR technique, gene copy number is assessed by the fold change difference relative to the normal subjects (with two copies of gene). Such a robust and rapid technique would lead to improved diagnosis and management in patients presenting with ADLD.

In conclusion, we evaluated gene duplication in the proband by a cost-effective, high-throughput, robust, rapid and reliable qPCR technique which is expected to facilitate the molecular assessment of ADLD patients in India.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.

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**Conflicts of interest**

None declared.

| Functional impairment                                      | Number of cases in literature\[^{6}\]\[6\] | Present case         |
|------------------------------------------------------------|------------------------------------------|----------------------|
| Autonomic (%)                                              | 22 (100%)                                | Yes                  |
| Bladder dysfunction and/or constipation/obstipation         | 22 (100%)                                | Yes                  |
| Orthostatic hypotension                                    | 17 (77%)                                 | No                   |
| Erectile dysfunction as early symptom                       | 4 (40%)                                  | Not known            |
| Other                                                       | -                                        | Anxiety, depression, emotional lability |
| Pyramidal signs                                            |                                          |                      |
| Including: lower limbs, lower and upper limbs, pseudobulbar| 20 (91%)                                 | Yes                  |
| Ataxia                                                     |                                          |                      |
| Including: spectrum of imbalance of gait, ataxia in upper   | 20 (91%)                                 | Yes                  |
|   limbs, truncal ataxia                                     |                                          |                      |
| Tremor                                                     | 10 (45%)                                 | Yes                  |
| Sensory deficits in lower limbs                             | 7 (32%)                                  | No                   |

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**Figure 3:** Diagrammatic representation to show fold change of gene copy number of patient relative to normal. Data presented as mean ± SD.
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