DATA REPORT

A novel TUBB4A mutation G96R identified in a patient with hypomyelinating leukodystrophy onset beyond adolescence

Yongping Lu1, Yumiko Ondo1, Keiko Shimojima1, Hitoshi Osaka2 and Toshiyuki Yamamoto1

The tubulin beta-4A gene (TUBB4A) is associated with two different clinical conditions, dystonia type 4 (DYT4) and hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC). We identified a novel TUBB4A mutation, c.286G > A (p.G96R), in an adult male patient who suffered neurological symptoms beyond adolescence. This patient shows intermediate clinical features between DYT4 and H-ABC, suggesting that the TUBB4A disorder would constitute a spectrum disorder.

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H-ABC (MIM#612438 (hypomyelinating leukodystrophy-6; HLD6)) is a rare leukodystrophy caused by mutations in the TUBB4A.1,2 H-ABC is characterized by clinical features associated with hypomyelination, i.e., delayed motor development, extrapyramidal movement disorders (dystonia, choreothetosis, rigidity, opisthotonus and oculogyric crises), ataxia, progressive spastic tetraplegia, and in some cases, seizures. Patients showed clinical onset before 3 years of age.1,3 The final diagnosis of H-ABC is based on distinctive magnetic resonance imaging (MRI) findings, including the combination of hypomyelination, cerebellar atrophy and disappearance of the putamen.

TUBB4A mutations were also associated with autosomal dominant torsion DYT4 (MIM#128101).4 Patients with DYT4 exhibit normal MRI findings, but have a 'whispering' dysphonia, generalized dystonia and gait ataxia. Clinical onsets of DYT4 patients are in second to third decade of life. Although TUBB4A mutations have been identified in familial cases with DYT4, the severity of the clinical symptoms in H-ABC patients precludes offspring and all identified TUBB4A mutations in H-ABC patients were de novo, except for a definite mosaic inheritance.3

A 21-year-old Japanese male patient showed an unremarkable family history. His abnormal gait was first observed beyond teen age although definite onset time is ambiguous. The patient was referred to a hospital when he was 17 years old. Upon physical examination, he showed no abnormalities or hepatosplenomegaly. However, neurological examination showed mild spasticity in his lower extremities. An ophthalmological examination showed a cherry-red spot that suggests atrophy of the optic nerve but no nystagmus. Brain MRI showed an abnormal intensity in the white matter (diffuse high intensity by T2 and fluid attenuated inversion recovery images; Figure 1a), but no atrophic findings in the basal ganglia and cerebellum were observed. An auditory brain response test showed a delayed peak of the wave V in both sides (left: 7.1 msec, right: 6.8 msec). Finally, visual-evoked potential and somatosensory-evoked potential tests of the upper extremities also showed delayed peaks.

When he was 19 years old, this patient started to show mildly dystonic movements. There was no dysphonia and dysarthria. His intelligence quotient was evaluated as 74 (language: 84, performance: 68) by the Wechsler Adult Intelligence Scale-III. Although the Mini-Mental State Examination showed a normal score of 27/30, the ‘Kana-hiroi’ test showed significantly lower scores in comparison to the age-matched mean score.5 Finally, a test for stabilometry revealed a horizontal postural sway with and without opening of the eyes. There was no remarkable abnormality in laboratory examination. At present, he still can play table tennis.

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Tokyo Women’s Medical University. After receiving written informed consent, next-generation sequencing using the TruSight One v1.0 sequencing panel (Illumina, San Diego, CA, USA) was performed, as described.6 However, no possible candidate variants were detected. Since TUBB4A was not included in the panel, Sanger sequencing for all TUBB4A exons was then performed as described.7 As a result, NM_006087.3(TUBB4A):c.286G > A [p.Gly(G)96Arg(R)] was detected in the conserved region (Figures 1b and c). This was considered as de novo mutation because both parents did not carry this mutation. The prediction scores of this mutation, which was evaluated by wANNOVAR (http://wannovar.usc.edu/), suggested this mutation was ‘damaging’ (Supplementary Table S1). This mutation was not registered in the database including Human Genetic Variation Database (http://www.hgvdb.genome.med.kyoto-u.ac.jp/), the Genome Aggregation Database (http://gnomad.broadinstitute.org/) and the Exome Aggregation Consortium (http://exac.broadinstitute.org/). Based on these findings, the c.286G > A [p.G96R] mutation was considered to be disease-causing.

The previously reported mutations in the coding regions of TUBB4A are summarized in Supplementary Table S2. A total of 36 mutations have been reported and did not include the mutation identified in this study, indicating that p.G96R is a novel disease-causing mutation. Furthermore, the ages of onset for the reported patients are also listed in Supplementary Table S2. Based on these data, the present patient was the oldest patient among those with hypomyelinating leukodystrophy.

Mutations in TUBB4A have been reported in association with two different disorders including DYT4, which is characterized by later adult-onset dystonia with normal brain MRI findings,8 and H-ABC, which is characterized by neonatal or earlier onset and
traits of hypomyelination, cerebellar atrophy and progressive degeneration of the putamen and the caudate nucleus.\textsuperscript{1,2} Since both DYT4 and H-ABC are due to the same disease-causing gene and result in the neurological impairments, it remains unclear whether these two diseases are allelic diseases or two ends of a clinical spectrum.\textsuperscript{8} Indeed, severe generalized dystonia with subtle neurological impairment, the clinical features and the amino acid change of this mutation expand our current understanding of the neurological phenotype, the clinical features and the amino acid alterations at the same residue.

Intriguingly, the mutations located on the outside of the αβ-tubulin heterodimer, which is distant from the guanosine triphosphate domain, are likely to result in milder phenotypes without atrophy of the basal ganglia and cerebellum. For example, the p.E410K mutation was reported in patients with spastic paraparesis and segmental dystonia,\textsuperscript{21} and the p.V180G mutation was related to isolated hypomyelination.\textsuperscript{9,22} One exception is a heterozygous mutation at residue p.R2. The mutation p.R2Q is associated with DYT4 phenotypes, whereas p.R2W result in H-ABC phenotypes. The possible explanation for the different clinical outcome arisen by the different amino acid alteration at the same residue.

In conclusion, the c.286G>A (p.G96R) mutation identified in this study is a novel mutation located on the outside of the αβ-tubulin heterodimer. The patient exhibited hypomyelinating leukodystrophy that was associated with milder phenotypic features and a later age of onset. These findings are consistent with the proposed genotype-phenotype correlation. Overall, the neurological phenotype, the clinical features and the amino acid change of this mutation expand our current understanding of the clinical spectrum for TUBB4A disorder.

**HGV DATABASE**

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.1396.

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**COMPETING INTERESTS**

The authors declare no conflict of interest.
Supplementary Information for this article can be found on the Human Genome Variation website (http://www.nature.com/hgv)