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Research Letter

Bilateral striatal necrosis and dyschromatosis symmetrica hereditaria: A-I editing efficiency of *ADAR1* mutants and phenotype expression

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***Short title*:** DSH and BSN in bi-allelic *ADAR1* mutation

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Main Text

ADAR1 mutations cause an autosomal dominant pigmentary disorder, dyschromatosis symmetrica hereditaria (DSH)[1], and bilateral striatal necrosis (BSN) affecting the central nervous system (CNS).[2] Here we report gene analysis of an Indian girl with dyschromatosis and dystonia[3] and diagnose her as having DSH with BSN. No cases of DSH with BSN harboring *ADAR1* bi-allelic mutations have been reported previously.

A 10-year-old Indian girl had been suffering from abnormal body movement and difficulty in walking since infancy. She was quite well up to 10 months of age, after which gradually progressive developmental delay was recognized. She had feeble vocalization and dystonia of the limbs (Fig. 1b). No history of fever or convulsion was reported.

Physical examination revealed freckle-like macules on her face and dyschromatosis on the extremities (Fig. 1c-d). Her mother had faint skin lesions (Fig. 1e).

A computed tomography (CT) of the brain showed no calcification. Magnetic resonance

imaging (MRI) of the brain revealed bilateral linear hyperintensities in the posterior putamen (Fig. 1f).

We diagnosed this patient clinically as having DSH and BSN.

The local institutional review board approved this study, and all the participants provided written informed consent. Mutation analysis as previously reported[4] identified the compound heterozygous *ADAR1* mutations p.Pro193Ala (paternal) and p.Cys1036Ser (maternal) (Fig. 1g). Both were repetitive.[5,6]

To date, eight BSN patients with *ADAR1* mutations have been reported.[2] Though a single BSN case was reported to show freckle-like skin changes without any photograph[7], no BSN patient has shown DSH skin manifestations. Aicardi-Goutières syndrome 6 (AGS6) was also known to be caused by *ADAR1* mutations.[6] AGS6 also affects the CNS.

The ADAR1 mutation p.Pro193Ala detected in the present study has been frequently seen in both the BSN and AGS cohorts. Indeed, 11 of the 13 cases of BSN or AGS6 with compound heterozygous *ADAR1* mutations that have been reported to date had p.Pro193Ala.[2,4,6] The p.Pro193Ala mutation has been recorded in 41 out of 6,553 individuals annotated in the Exome Variant Server database. The p.Pro193Ala mutation was also found in Southern Asian populations with an allele frequency of 0.0001 in the 1000 Genomes Project data. The allele frequency of p.Pro193Ala is relatively higher than those of other *ADAR1* mutations in the general population. However, p.Pro193Ala has never been reported in the DSH cohort. Thus, the p.Pro193Ala mutation might be a unique mutation that may cause BSN or AGS in the compound heterozygous state, but that may not cause DSH in the heterozygous state. The findings in the present family support this hypothesis. In the present family, the proband's mother, with p.Cys1036Ser, showed skin symptoms, but the proband's father, carrying p.Pro193Ala, had no eruptions.

As shown in Supplementary Figure 1a (all supplementary information is available on direct request to the corresponding author), p.Pro193Ala, which was previously found in AGS and BSN cohorts, is a p150 isoform-specific *ADAR1* mutation. ADAR1 products derived from the p.Pro193Ala mutant allele include the wild-type p110 isoform. To date, several p150 isoform-specific *ADAR1* mutations have been found in DSH patients with typical skin manifestations. All the p150 isoform-specific *ADAR1* mutations causative of DSH have been truncation mutations, except for the substitution mutation p.Arg26Lys.

We evaluated the RNA editing ability of several mutant ADAR1 p150 isoforms including homozygous and heterozygous p.Pro193Ala, as previously reported.[4] The A-I editing efficiencies are summarized in Supplementary Figure 1b. The A-I editing efficiencies of the ADAR1 p150 isoform with homozygous and heterozygous p.Pro193Ala were 95.7% and 98.8% of that of the wild-type plasmid transfectants, respectively. The average editing efficiency of two transfectants with BSN-causative compound heterozygous mutations—one with [p.Pro193Ala + p.Cys1036Ser] (the

present patient's genotype) and the other with [p.Pro193Ala + p.Leu1186Glu]—was 69.1%. The average editing efficiency of two transfectants with DSH-causative heterozygous mutations—one with [wild type + p.Lys1003Arg] and the other with [wild type + p.Cys1036] (the genotype of the present patient mother)—was 71.8% and that of two transfectants with AGS6-causative genotypes—one with [p.Asp1113His + p.Asp1113His] and the other with [wild type + p.G1007R]—was 34.8%. We found that the p150 isoform with p.Pro193Ala largely retained its RNA editing ability. Judging from the assay result, the phenotype associated with p.Pro193Ala may be due to the remaining editing ability of p150 isoform with p.Pro193Ala. Also, we were able to speculate that homozygous p.Pro193Ala shows no manifestations of DSH/BSN/AGS6. Although other unknown factors are thought to affect the phenotypes, we speculate that phenotypes of these three entities might be associated with the levels of RNA editing ability.

In conclusion, we reported the first BSN patient with DSH and clarified that most common mutation p.Pro193Ala is semi-pathogenic mutation, which cannot impair the phenotype by itself.

Conflicts of interests

None of the authors has any conflicts of interests relevant to this letter.

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Figure legends

Fig. 1. The proband's pedigree and clinical features, an MRI image, and the causative *ADAR1* mutations detected in her family. (a) The pedigree of the proband's family. Her father and elder brother had no skin manifestations. According to the proband's mother, her deceased sister was suspected of DSH. There was no history of similar neurological symptoms in her family including the deceased mother's sister. (b-e) Clinical features of the proband and her mother. (b) The proband can stand by herself, but shows dystonic posture of the limbs. (c-d) She has a mixture of 5-mm hyper- and hypo-pigmented macules on the dorsa of the hands and the posterior of the ankle. (e) Faintly pigmented macules (arrows) are seen on the dorsal foot of the proband's mother. (f) MRI (fluid-attenuated inversion recovery (FLAIR) image) of the brain showing bilateral symmetric linear hyperintensity in the posterior putamen (arrows). (g) Two previously reported heterozygous substitution mutations, c.577C>G (p.Pro193Ala) in exon 2 (paternal) and c.3107G>C (p.Cys1036Ser) in exon 12 (maternal), were detected in *ADAR1*.