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SHORT COMMUNICATION

Siblings with optic neuropathy and *RTN4IP1* mutation

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Inherited optic neuropathies (IONs) are neurodegenerative disorders affecting the optic nerve and the nervous system. Dominant and recessive IONs are known. Many of the dominant IONs are caused by mutations of *OPA1*. Autosomal-recessive IONs are rare. OPA10 is an autosomal-recessive ION due to mutations in *RTN4IP1*. Patients with *RTN4IP1* mutations show extraocular manifestations. We report brothers with optic neuropathy who had novel mutations in the *RTN4IP1* gene. This is the first report of Japanese patients with OPA10. They showed extraocular manifestations resembling mitochondrial encephalopathy.

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INTRODUCTION

Inherited optic neuropathies (IONs) affect the optic nerve and the nervous system. Both dominant and recessive IONs are known. Many of the dominant IONs are caused by mutations of *OPA1* (MIM: 605290).[1,2] Some patients have *OPA3* (MIM: 606580) mutations.[3] Both *OPA1* and *OPA3* encode inner mitochondrial proteins. Some patients with the *OPA1* gene mutations show extraocular features, including hearing disorder, progressive external ophthalmoplegia and various neurological manifestations.[4] Patients with the *OPA3* gene mutations show dominant IONs and cataracts.

The autosomal-recessive IONs with known molecular defects are OPA7 (MIM#612989), OPA9 (MIM#616289), OPA10 (MIM#616732) and OPA11 (MIM#617302). They are caused by mutations of *TMEM126A* (MIM*612988),[5] *ACO2* (MIM*100850),[6] *RTN4IP1* (MIM*610502)[7] and *YME1L1* (MIM*607472),[8] respectively. Angebault *et al.*[7] reported four families with OPA10. They showed early-onset optic neuropathy and some neurological manifestations.[7] *RTN4IP1* mutations are designated as 'optic atrophy 10 with or without ataxia, mental retardation and seizures.' *RTN4IP1* encodes a mitochondrial ubiquinol oxydo-reductase. Angebault *et al.*[7] revealed that fibroblasts from patients with a *RTN4IP1* mutation showed decreased activities of mitochondrial respiratory complex I and IV. The cells also showed susceptibility to ultraviolet light. Depletion of Rtn4ip1 by short hairpin RNA resulted in abnormal growth of mouse retinal ganglion cell dendrites.

We report brothers with optic neuropathy who had mutations of the *RTN4IP1* gene. They showed extraocular manifestations resembling mitochondrial encephalopathy.

CLINICAL REPORT

The 15-year-old male (II-1) was the first child of healthy and non-consanguineous Japanese parents (Figure 1). He had presented with poor vision since early childhood. He also showed color blindness and congenital nystagmus. His visual acuity was 0.02 in each eye. He presented with epileptic seizures and visual blurring. The electroencephalography showed spikes and high voltage slow waves from the left occipital region. Transient hyperlacticacidemia was noted on laboratory investigations (lactic acid 74.8 mg dl^{-1}, pyruvic acid 3.22 mg dl^{-1}). Leber's hereditary optic neuropathy was suspected.

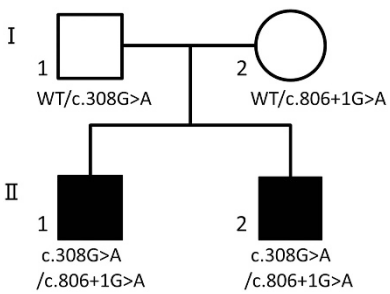

Figure 1 Pedigree of the family.

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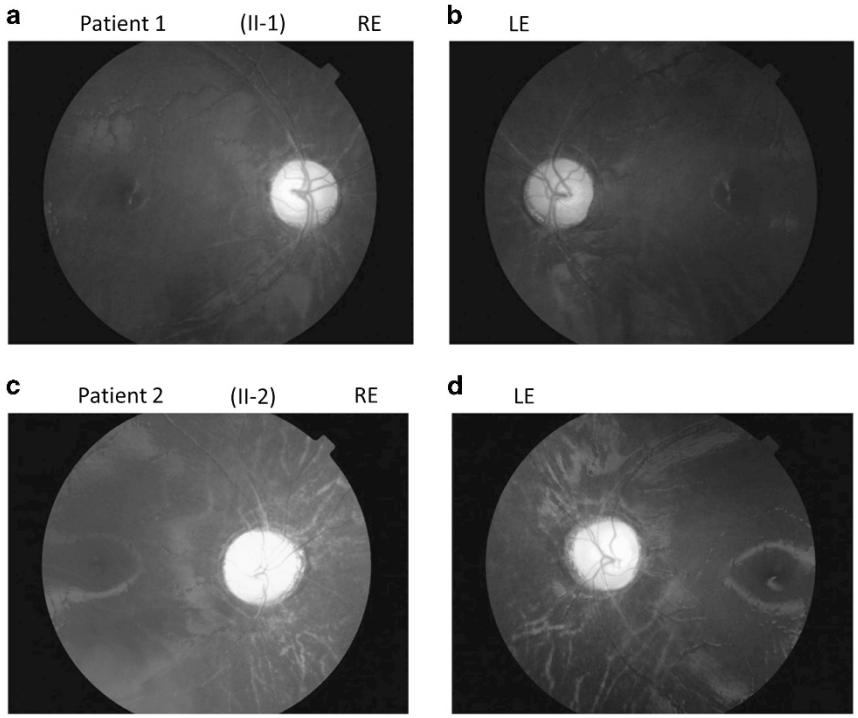

Figure 2 Fundus examinations revealed pallor of the optic discs and depigmented areas around the disc. (**a**,**b**) Patient 1 (II-1) (**c**,**d**) Patient 2 (II-2) Depigmentation was more prominent in Patient 2 (II-2). LE, left eye; RE, right eye. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

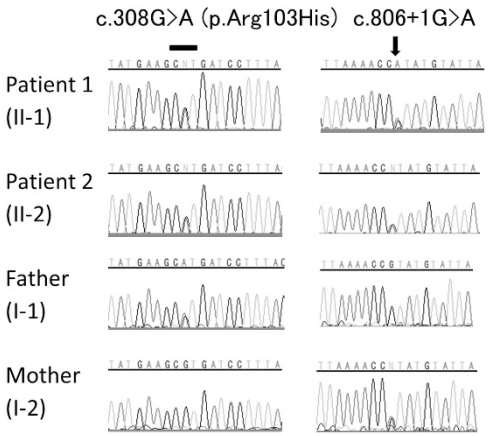

Figure 3 *RTN4IP1* mutations in the family of Patients 1 (II-1) and 2 (II-2) were compound heterozygous for the mutation. The parents were each heterozygous for one of the each mutated alleles. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

However, mitochondrial DNA mutation was not found by the extensive screening system.[9] A brain magnetic resonance imaging showed no significant abnormalities except for hypoplastic optic nerves. Magnetic resonance spectroscopy showed no lactate peak.

Physical growth was also within normal standards for a Japanese male. He had learning difficulty. Neurological examinations were normal expect for the ocular symptoms. He has been seizure-free for a number of years. Ataxia was not observed. Funduscopic examination revealed that bilateral optic disc was moderately pallor and there were depigmented areas around the disc (Figures 2a and b).

The 10-year-old male (II-2) was the younger brother of Patient 1. He presented with poor vision, color blindness and congenital nystagmus. His visual acuity was 0.03 in each eye. He did not show hyperlacticacidemia or epileptic seizures. Physical growth was normal. His bilateral optic disc was pallor and depigmented area was found around the disc (Figures 2c and d). Depigmentation was more prominent in Patient 2 (II-2).

Psychomotor development was mildly delayed. His development quotient was 72 based on the Kyoto Scale of Psychological Development 2001 at 5 years old. Macular function was disturbed in both patients. The visual fields were severely constricted in both patients. The brothers did not show sensitivity to ultraviolet in daily activities. Their parents (I-1, I-2) with the heterozygous variant did not show ocular symptoms.

MATERIALS AND METHODS

The study was approved by the ethics committees of the respective institutions. All biological samples were obtained after written informed consent from the individuals of the family. The samples were analyzed using whole-exome sequencing. Three μg of DNA was sheared to 150–200 bp using the Covaris DNA Shearing System (Woburn, MA, USA). To capture the exonic DNA, we used the SureSelect XT Human All Exon V4/V5 capture library (Agilent, Santa Clara, CA, USA). The sequence library was constructed with the SureSelect XT Target Enrichment System for Illumina Paired-End Sequencing Library kit (Agilent) according to the manufacturer's instructions. We performed DNA sequencing of 101 bp paired-end reads using the Illumina (San Diego, CA, USA) HiSeq 2000 sequencer. We analyzed exome data as described previously.[10] The computer software ANNOVAR (http://annovar.openbioinformatics.org/en/latest/) was used to annotate of sequence data.

RESULTS

We identified a c.308G>A NM_032730.5:exon2:c.G308A:p.R103H, substitution and a c.806+1G>A in the splice site substitution of *RTN4IP1* (Figure 3). Patients 1 (II-1) and 2 (II-2) had compound

heterozygous mutations. The parents were heterozygous for one of the each mutated alleles.

DISCUSSION

We identified a c.308G>A (p.Arg103His) substitution and a c.806+1G>A splice site substitution of *RTN4IP1*. The c.308G>A (p.Arg103His) substitution has been previously reported by Angebault *et al*.[7] The splice site variant is believed to have a deleterious effect. It is interesting that this mutation was repeatedly found in unrelated families. The splice site variant is a novel mutation. No other variants in optic atrophy-related genes including *OPA1*, *OPA3*, *TMEM126A*, *ACO2* and *YME1L1* were found.

Patient 1 (II-1) had been more severely affected by epileptic seizures from the age of 3 years. Transient hyperlacticacidemia was noted. These findings were suggestive of a mitochondrial encephalopathy. He is now free from seizures and there has been no further progression of disorder. Angebault *et al*.[7] reported on a patient that showed myoclonic seizures and two patients who exhibited mild intellectual disability. Magnetic resonance imaging of the brothers showed only hypoplasia of optic nerve. No significant abnormalities including stroke-like lesions were observed.

Angebault *et al*.[7] assessed the subcellular localization of RTN4IP1 with the outer membrane mitochondria. Rtn4ip1 plays important roles in controlling the Rtn4 function and disruption of Rtn4ip1 results in abnormal retinal ganglion cell neurite outgrowth.[7] Ocular size of the morpholino-injected zebrafish was small. Looping swimming behavior suggesting visual impairment was observed in the zebrafish. Our patients showed congenital nystagmus and low visual acuity. Their ocular size was normal.

Our results further support the suggestion that *RTN4IP1* abnormalities result in early-onset optic neuropathy and neurological features including mild intellectual disability and epilepsy. The clinical manifestations may resemble mitochondrial encephalopathy. We should consider *RTN4IP1* mutations in similar patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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