Complex Heterozygous Mutation in the T-cell Immune Regulator 1 Gene Associated with Severe Ocular Characteristics of Osteopetrosis in an Infant

Wen-Hong Cao[1,2], Wen-Bin Wei[2], Gang Yu[1], Li Li[1], Qian Wu[1]

[1]Department of Ophthalmology, Beijing Children's Hospital, National Center for Children's Health, National Key Discipline of Pediatrics, Capital Medical University, Beijing 100045, China

[2]Beijing Tongren Eye Center, Beijing Key Laboratory of Ophthalmology and Visual Science, Beijing Key Laboratory of Intraocular Tumor Diagnosis and Treatment, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China

Infantile malignant osteopetrosis (IMO) is a rare congenital disease that is characterized by an impaired function or differentiation of osteoclasts. IMO is the most severe type of osteopetrosis. Patients usually present various fatal manifestations soon after birth and die in infancy or childhood. Clinical features include bone marrow failure resulting in pancytopenia, hepatosplenomegaly, blindness secondary to optic nerve compression, hydrocephalus, and other neurological complications.[1] T-cell immune regulator 1 (TCIRG1, Gene ID: 10312) is one of the main genes that are responsible for the majority of IMO cases. Mutations in TCIRG1 associated with general clinical features have been reported in some studies.[2] In this study, we reported the ocular manifestations and genetic findings of an IMO patient.

A male infant, whose eyes were unable to follow the light at birth, had a tilted mouth at 4 months of the age. His parents were no consanguineous couple. At the age of 5 months, he was referred to the Department of Hematology, Beijing Children's Hospital, and was diagnosed IMO with general examination and gene mutation detection. Mutation analysis of the TCIRG1 gene was performed using direct DNA sequencing of polymerase chain reaction-amplified exons. Prediction of the protein domains was based on the potential topological domains reported in UniProt database (Q13488; http//www.uniprot.org). After 6 months, he received successful hematopoietic stem cell transplantation (HSCT). We performed detailed eye examination before and after HSCT, including orbital three-dimensional computer tomography (CT) scan to measure the optic canal diameters, RetCam III fundus examination, flash visual evoked potential (FVEP), and flash electroretinogram (FERG) using the standard FVEP protocols recommended by the International Standard EEG 10–20 System (ISCEV) (Roland Inc., German).

The principles outlined in the *Declaration of Helsinki* were followed. The Ethics Committees for Human Studies of Beijing Children's Hospital had approved this study. Informed consent was obtained from his parents.

We identified a possible novel frameshift mutation c.1007_1013del (p.L336pfs*8) [Figure 1a] and a reported missense mutation c.1213G>A (p.G405R) [Figure 1b] in the patient. MutationTaster predicted the effect of the c.1007_1013del mutation as "disease causing," which caused by deletions of a number of 1007_1013 nucleotides in a DNA sequence that is not divisible by there. The c.1007_1013del mutation was predicted to disrupt the ninth exon, change the reading frame (the grouping of the codons), resulting in a completely different translation from the original. This mutation was not reported in HGMD professional database and ExAC database. c.1213G>A was a missense mutation, a previously reported osteopetrosis-causing mutation (Pangrazio, *et al*. Osteoporos Int., 23, 2713, 2012), which was deemed to "disease causing" by MutationTaster, causing guanine to become adenine in the coding area of 1213. The mutation was predicted to disrupt

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Address for correspondence: Prof. Wen-Bin Wei, Beijing Tongren Eye Center, Beijing Key Laboratory of Ophthalmology and Visual Science, Beijing Key Laboratory of Intraocular Tumor Diagnosis and Treatment, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China
E-Mail: weiwenbintr@163.com

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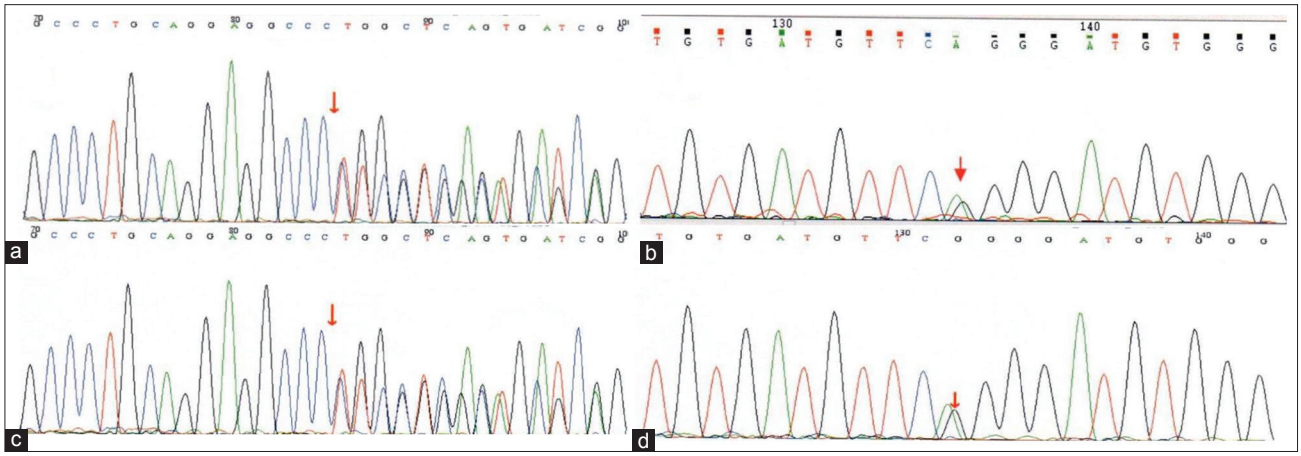

Figure 1: Partial nucleotide sequences of the T-cell immune regulator 1 gene. Arrows point to a possible novel frameshift mutation c.1007_1013del (p.L336pfs*8), and a reported missense mutation c.1213G>A (p.G405R). The patient (a) and his mother (c) carry the c.1007_1013del (p.L336pfs*8) mutation. The patient (b) and his father (d) have the c.1213G>A (p.G405R) mutation.

the eleventh exon, leading to the change of amino acids (glycine to arginine). This mutation was reported in HGMD professional database.[2]

Analysis of the parental DNA showed that the patient's mother carries the c.1007_1013del mutation [Figure 1c], and his father has the c.1213 G>A mutation [Figure 1d]. Thus, it followed that this patient's pathogenic genetic mutation was inherited from both of his parents, and the mutation was complex heterozygous mutation in the TCIRG1 gene.

Before HSCT, the patient was in poor growth, with square skull, forehead protruding, and facial palsy. After transplantation, all reduced peripheral blood cells gradually turned to be stable, the bone mineral density decreased, and the hepatosplenomegaly was getting better.

The eyes were dull to light, with nystagmus showing pendulum-like movement; for 1 month after transplantation, the eyes have been responded to light, nystagmus seemed mitigated. The preoperative fundus examination indicated the pale papilla of optic nerve, gloomy center of the macula, and thin retinal arteries, some of which were occluded like white line [Figure 2a]; postoperative photograph showed no change yet. Orbital CT scan showed that the diameter of bilateral narrow visual neural tubes was 1.5–1.7 mm [Figure 2b]; after 3 months of HSCT, they seemed slightly wider (1.7–1.9 mm). Before HSCT, the waveform of FVEP P2 was not elicited; Photopic 3.0 ERG displayed blanking type in the light adaptive stimulation; Scotopic 3.0 ERG in the dark adaptation state showed that latency period of the right/left eye in a/b wave was 27.0/24.4 ms and 76.9/78.4 ms and the amplitude was 0.83/1.62 μV and 67.5/102 μV, respectively. These outcomes indicated that the function of the binocular rods and cones was badly damaged. After HSCT, FVEP P2 latency period was 187.1/174.3 ms and the amplitude increased (5.23/2.89 μV); the corresponding values of Scotopic 3.0 ERG were 17.0/18.5 ms and 41.4/40.8 ms and 77.9/45.7 μV and 229/174 μV.

Osteopetrosis is a sort of very heterogeneous disease. The heterogeneity depends on the causal genes, modes of inheritance, and disease severity/phenotypes. To date, more than 10 osteopetrosis-causing genes have been identified.[3] The inheritance patterns contain autosomal dominant, autosomal intermediate, and autosomal recessive. The disease severity also varies from malignant, intermediate, to mild manifestations. This patient's disease is TCIRG1-related disease, which occurs due to the loss of function of both alleles on the homologous chromosomes. It is generally recessive and malignant. It is proved that the pathogenesis of osteopetrosis is due to the insufficient acidification of the microenvironment of osteoclasts, which requires an acidic environment for reabsorption and remodeling of bones. During acidification, H+ is transported into secondary lysosomes by vacuolar type H+ adenosine triphosphatase (V-ATPase), and TCIRG1 encodes its a3 subunit.[1]

In this patient, the inheritance pattern is obviously recessive, as the parents are both healthy heterozygous mutation carriers. The disease severity in our patient is also consistent with the recessive pattern. The paternally inherited known missense mutation, c.1213G>A, combined with possible unknown mutated genes, c.1007_1013del, maternally inherited on the other chromosome, resulted in the severe phenotypes in our patient. Frameshift mutation and missense mutation have been regarded as "loss of function mutations." The guidelines of the American College of Medical Genetics and Genomics have also been classified these types of mutation as "pathogenic mutations."[4]

Approximately 10% of children with IMO in the TCIRG1 gene have extensive and severe neurological damage, including optic atrophy, VEP loss, nystagmus, and blind. These children with severe visual impairment within 1 year of age are up to 75%. At present, most scholars hold that the neurological symptoms are due to the thickening of the bone plate at the bottom of the skull and stenosis or occlusion of channels in which cranial nerves, spinal cord, and major

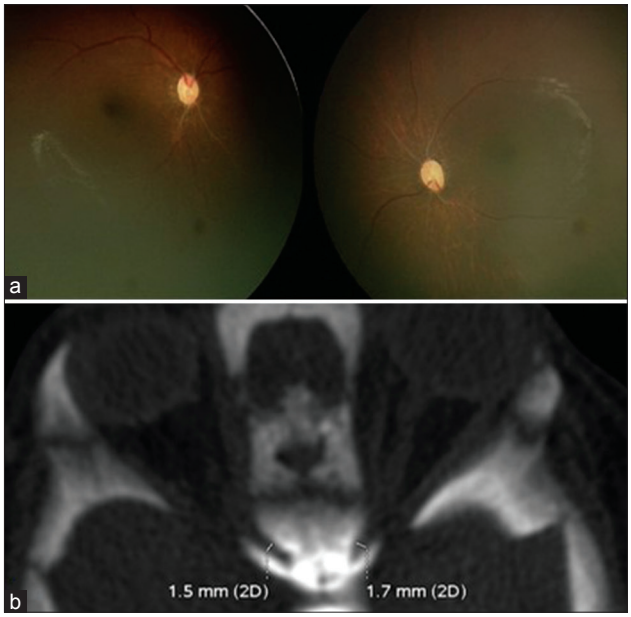

Figure 2: (a) Fundus examination indicated the pale papilla of optic nerve, thin retinal artery – even some occlusion like white line – and gloomy center of the macula. (b) Orbital computed tomography scan showed the bone density of the orbital bone increased thickening, the density of the medullary cavity increased, the bilateral orbital volume was small, the bilateral visual neural tubes were very thin, and the narrowest diameter was 1.5–1.7 mm.

blood vessels run, including the optic nerve compression caused by the optic canal stenosis.[5] The mechanism of optic nerve damage is still not fully understood. The FVEP and FERG test indicated that this infant's vision had been severely impaired. Although we fully informed the parents of the risk of transplantation, they persisted with HSCT for their baby. The infant has been still in the early stage of recovery; however, we saw an exciting and unexpected result that FVEP and FERG outcomes indicated a probably improved visual function. Due to the insignificantly widen optic nerve tube, we look forward to the patient's long-term observations.

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Conflicts of interest

There are no conflicts of interest.

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