Case Reports

Rare gross deletion in T-cell immune regulator-1 gene in Iranian family with infantile malignant osteopetrosis

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ABSTRACT

تصخر العظم الطفولى الخبيث arOP من الاضطرابات الصبغية الجسدية المتنحية. الطفرات فى موروثة TCIRG1 وجدت أن تكون علة arOP. وجدنا أول مريضة إيرانية مع حذف جسيم في هذه الموروثة، تبلغ من العمر 5 أعوام، تعاني من كبر الرأس، سوء تشكل في الوجه، عمى، تخلف عقلي، توسيع كبدي – طحالي، نقص العناصر الدموية الشامل، وتصلب العظام فى الجمجمة والأطراف. تم التقييم الجزيئي عبر استخدام RT–PCR للـ10–19 exons من موروثة TCIRG1، ومن بعدها تعيين تسلسل الموروثة بأكملها. ظهر فى التحاليل قطعة متكاثرة غير متوقعة تبلغ من الحجم 275bp، كما وقد كشف تعيين التسلسل عن حذف جسيم فى الـ10–15 exons فى المنطقة المترجمة من TCIRG1 والتى تؤثر على الكودونات 389-518. هنالك تقارير تشهد على انواع مختلفة من الطفرات في موروثة TCIRG1 في الـarOP، لكن نادراً ما يوجد تقرير عن حذف جسيم. يعتبر هذا الحذف الجسيم أول طفرة يتم التبليغ عنها في هذه الموروثة لدى المرضى الإيرانيين، كما أنها أكبر حذف في موروثة TCIRG1 قد تم التبليغ عنها حتى الآن.

Infantile malignant osteopetrosis (arOP) is an autosomal recessive disorder. Mutations in the T-cell immune regulator 1 (*TCIRG1*) gene were found as the cause of arOP. We found the first Iranian patient with a rare gross deletion in this gene. The patient was a 5-year-old girl with macrocephaly, facial dysmorphism, blindness, mental retardation, hepatosplenomegaly, pancytopenia, and osteosclerotic changes in the skull and limb. Molecular analysis was performed using reverse transcriptase-polymerase chain reaction for exons 10-19 of the *TCIRG1* gene followed by whole gene sequencing. She showed a 275bp unexpected amplified segment. Sequencing revealed a gross deletion in exons 10-15 transcript region of *TCIRG1* that affected codon 389 to 518. Various types of mutations in the *TCIRG1* gene in arOP have been reported, however, gross deletions are reported rarely. This gross deletion is the first mutation reported among Iranian patients in this gene. This deletion is also the largest deletion of *TCIRG1* gene reported to date.

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Infantile malignant osteopetrosis (MIM:259700) is an autosomal recessive disorder, manifested by severe osteosclerosis within the first decade of life. It comprises increased bone density, intracerebral calcification, mental retardation, growth failure, and facial dysmorphism. It is caused by failure of osteoclasts function to resorb bone resulting in osteosclerosis. Common features are pathologic fractures, macrocephaly, hepatosplenomegaly, and pancytopenia. It is often associated with visual impairment and hearing loss due to cranial nerve compression, however, there is also evidence for a primary retinal degeneration.[1] Death occurs in early childhood due to severe anemia and infections. Without treatment, survival rarely exceeds 20 years. Mutations in the T-cell immune regulator 1 (*TCIRG1*) gene encoding the osteoclast-specific 116-kD subunit of V-type H^+-ATPase named as a 3 subunit leads to infantile malignant osteopetrosis type (arOP).[2]

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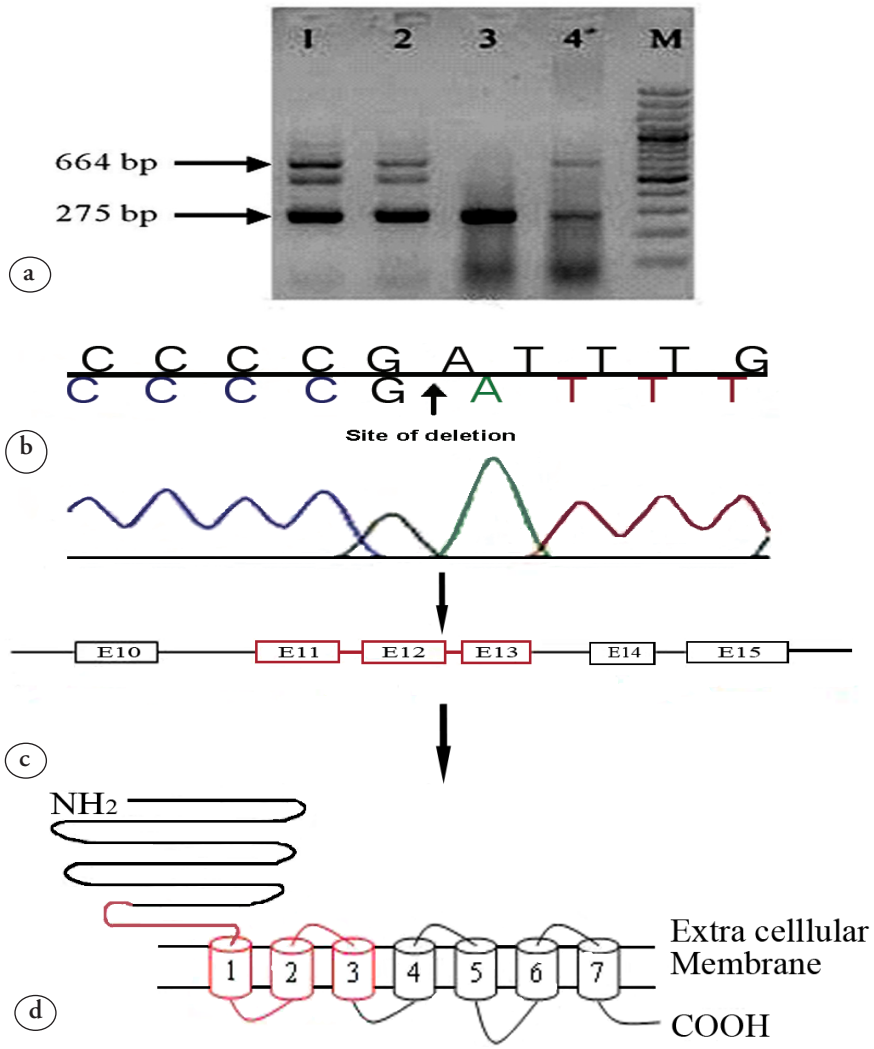

Figure 1 - a) The result of reverse transcriptase-polymerase chain reaction amplification of 664 bp target in patient and her family. 1 - mother, 2 - father, 3 - patient, 4 - brother. The 275 bp band resulted from delation mutation in *TCIRG1* allele. b) Sequencing chromatogram of polymerase chain reaction product demonstrated large deletion in patients including exon 11-13 *TCIRG1* gene. c) Schematic view of mutation site in the exon/intron boundaries. d) Schematic view of *TCRIG1* protein (Swissprot: Q13488). The site of mutation is indicated.

This gene contains 20 exons, mapped to 11q13.4-5 and specifically expressed in osteoclasts in the ruffled borders.[3] Recent research found that mutations in the *TCIRG1* account for approximately 50% of malignant osteopetrosis patients.[4] Various types of mutations in the *TCIRG1* gene in infantile malignant osteopetrosis have been reported in different populations including missense/nonsense, splicing, and small deletions, whereas, gross deletions are reported rarely.[5,6] This study reports the first Iranian patient with a rare gross deletion identified in this gene. This case was presented to report the first mutation of *TCIRG1* gene detected among Iranian osteopetrosis patients, which can help in development of suitable molecular genetic diagnosis of the disease in this region.

Case Report. The patient was a 5-year-old girl born from consanguineous parents. She also had a healthy 3-year-old brother. She was referred to the hematology department of Dr. Sheikh Pediatric Hospital, Mashhad, Iran, for management of weakness. On admission she was pale and ill. Macrocephaly and moderate exophthalmia were significant. She was diagnosed blind since 3 month of age. A history of several seizures was reported, and she was mentally retarded. Abdominal examination was significant for hepatosplenomegaly. Laboratory investigations revealed pancytopenia. Radiological images showed osteosclerotic changes in the skull and limb. With these findings, she was referred for molecular analysis of osteopetrosis disease. Appropriate informed consent was obtained from all family members

prior to blood collection and molecular analysis. After total ribonucleic acid (RNA) extraction and first-strand complimentary deoxyribonucleic acid (cDNA) synthesis, reverse transcriptase-polymerase chain reaction (RT-PCR) was performed on exons 10-19 of the *TCIRG1* in 25 ml final volume with 1U Taq polymerase, 1.5 mM magnesium chloride, 200 mM deoxynucleotide triphosphate, 10 pmol of each oligonucleotide primer as previously described.[5] The thermal condition was 30 cycles of denaturation at 94°C for 30 seconds, annealing at 64°C for 30 seconds, and 72°C for 30 seconds. The expected PCR products were 706 bp for exons 15-19 and 664 bp for exons 10-15. When resolved on 1.7% agarose gel, the normal 706 bp PCR product was observed in our patient, her parents and brother. However, this patient displayed a 275 bp band instead of the 664 bp band. Her parents and her brother showed both 644 bp and the 275 bp band (**Figure 1**). Direct sequencing was performed to find the reason for the presence of the 275 bp band using an ABI 3730 capillary system sequencer. The sequencing result of the 275 bp PCR product revealed a gross deletion in the exons 10-15 transcript region of *TCIRG1* when compared to genomic sequence (accession number for cDNA sequence of *TCIRG1*: U45285.1). The sequencing showed that the 275 bp product resulted from a 389 bp deletion in transcript region from exons 10-15 of the *TCIRG1* gene. This deletion affected codon 389-518 including entire exons 11-13 of the gene (**Figure 1**).

Discussion. Several mutations in the *TCIRG1* gene in infantile malignant osteopetrosis have been reported in many populations based on the sequencing of the coding exons as well as the exon/intron junctions.[4,5,7] In these reports, mutations of the *TCIRG1* was detected in approximately 50% of cases.[4] Splicing site mutations composed the major cause of severe abnormalities in the protein and represent a large portion (40%) of the *TCIRG1* variations.[5] In our study, sequencing of the PCR product of the patient confirmed a gross deletion spanning 3 exons in the *TCIRG1* mRNA. This deletion of exons 11-12-13 was reported for the first time by Susani et al[6] at the genomic DNA level (the human gene mutation database, accession number: CG044459). We report this mutation in a patient with arOP detected at the mRNA level. It is the first mutation reported among Iranian patients, and also it is the largest mutation of *TCIRG1* reported to date.

The *TCIRG1* codes V-type ATPase 116 kD subunit a3, which consists of 830 amino acids. The 3D structure of this protein has yet to be determined in humans. However, according to available data on this protein (VPP3, SwissProt: Q13488), the protein consists of 7 transmembrane regions (21AA length each), an extracellular domain (containing 397AA) and 3 glycosylation sites. Therefore, we made a schematic representation of *TCIRG1* protein and determined the site of this large mutation on the protein (**Figure 1**). As shown in **Figure 1**, the mutation starts at the end of the extracellular domain and spans 3 transmembrane regions. This mutation also affects 2 glycosylation sites. The first Iranian osteopetrosis patient with a Gross deletion in TCIRG1 gene has been reported. Further studies are recommended to determine common mutations among Iranian osteopetrosis patients to develop suitable molecular genetic diagnosis of disease in this region. This would also help in prenatal diagnosis of disease.

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References

1. Thompson DA, Kriss A, Taylor D, Russell-Eggitt I, Hodgkins P, Morgan G, et al. Early VEP and ERG evidence of visual dysfunction in autosomal recessive osteopetrosis. ***Neuropediatrics*** 1998; 29: 137-144.
2. Kornak U, Schulz A, Friedrich W, Uhlhaas S, Kremens B, Voit T, et al. Mutations in the a3 subunit of the vacuolar H+-ATPase cause infantile malignant osteopetrosis. ***Hum Mol Genet*** 2000; 9: 2059-2063.
3. Heaney C, Shalev H, Elbedour K, Carmi R, Staack JB, Sheffield VC, et al. Human autosomal recessive osteopetrosis maps to 11q13, a position predicted by comparative mapping of the murine osteosclerosis (oc) mutation. ***Hum Mol Genet*** 1998; 7: 1407-1410.
4. Sobacchi C, Frattini A, Orchard P, Porras O, Tezcan I, Andolina M, et al. The mutational spectrum of the human malignant autosomal recessive osteopetrosis. ***Hum Mol Genet*** 2001; 10: 1767-1773.
5. Scimeca JC, Quincey D, Parrinello H, Romatet D, Grosgeorge J, Gaudray P, et al. Novel mutations in the TCIRG1 gene encoding the a3 subunit of the vacuolar pump in patients affected by infantile malignant osteopetrosis. ***Hum Mutat*** 2003; 21: 151-157.
6. Susani L, Pangrazio A, Sobacchi C, Taranta A, Mortier G, Savarirayan R, et al. TCIRG1-dependent recessive osteopetrosis: mutation analysis, functional identification of the splicing defects and in vitro rescue by U1 snRNA. ***Hum Mutat*** 2004; 24: 225-235.
7. Del Fattore A, Peruzzi B, Rucci N, Recchia I, Cappariello A, Longo M, et al. Clinical, genetic, and cellular analysis of 49 osteopetrotic patients: implications for diagnosis and treatment. ***J Med Genet*** 2006; 43: 315-325.