A Homozygous Mutation in 5′ Untranslated Region of TNFRSF11A Leading to Molecular Diagnosis of Osteopetrosis Coinheritance With Wiskott-Aldrich Syndrome

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Summary: Wiskott-Aldrich syndrome (WAS) and osteopetrosis are 2 different, rare hereditary diseases. Here we report clinical and molecular genetics investigations on an infant patient with persistent thrombocytopenia and prolonged fever. He was clinically diagnosed as osteopetrosis according to clinical presentation, radiologic skeletal features, and bone biopsy results. Gene sequencing demonstrated a de novo homozygous mutation in 5′-untranslated region of TNFRSF11A, c.−45A > G, which is related to osteopetrosis. Meanwhile, a hemizygous transition mutation in WAS gene, c.400G > A diagnosed the infant with WAS. This is the first clinical report for the diagnosis of osteopetrosis coinheritance with WAS in a single patient.

Key Words: Wiskott–Aldrich syndrome, osteopetrosis, next-generation sequencing, TNFRSF11A, untranslabeled region

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BACKGROUND

Osteopetrosis is a genetic disorder characterized by an increased bone mass due to defects in osteoclast formation and function. It mainly includes 2 forms: autosomal recessive osteopetrosis (ARO), also known as infantile malignant osteopetrosis for its early onset and high mortality; and autosomal dominant osteopetrosis.1 The genetic basis of osteopetrosis has now been extensively studied: mutations in TCIRG1, CLCN7, OSTM1, SNX10, and PLEKHM1 induce osteoclast-rich ARO (in which osteoclasts are abundant but functionally impaired), whereas mutations in TNFSF11 and TNFRSF11A induce osteoclast-poor ARO.2 TNFSF11 encodes tumor necrosis factor ligand superfamily member 11 (better known as RANKL), produced mainly by osteoblasts and stromal. TNFRSF11A encodes tumor necrosis factor receptor superfamily member 11A (RANKL cognate receptor, RANK) mainly expressed by osteoclast precursors and mature osteoclasts. It is now well established that loss-of-function mutations in TNFRSF11A and TNFSF11 involved in the RANK/RANKL signaling pathway lead to ARO.3

Wiskott-Aldrich syndrome (WAS), a rare X-linked recessive disease, is caused by mutations of the WAS protein (WASP) gene and characterized by microthrombocytopenia, eczema, recurrent infections, autoimmune phenomena, and increased incidence of malignancy.4 As an important regulator of the actin cytoskeleton, WASP, expressed by all hematopoietic cell lineages and precursor cells, plays an important role in hematopoietic and immune cell functions including effective migration, phagocytosis, and immune synapse formation. Loss of WASP activity leads to immunodeficiency, autoimmunity, and microthrombocytopenia.5 To date, over 300 kinds of mutations involved with WAS gene have been described. Most nonsense mutations are located in exons 1 to 4, whereas splice-site mutations predominantly in introns 6 to 10. Typical WAS was diagnosed depending on the clinical presentations and gene mutation analysis.3,6

Although the rare genetic diseases osteopetrosis and WAS have been occasionally described, the coinheritance of both osteopetrosis and WAS in 1 patient, to our best knowledge, has never been reported before. In this report, a case was radiologically and clinically diagnosed as infant osteopetrosis correlated with a single nucleotide transition in the 5′ untranslated region (5′ UTR) of the TNFRSF11A gene. Furthermore, this infant was genetically diagnosed with WAS, a missense mutation in exon 4 of WAS gene detected by the next-generation sequencing (NGS) analysis. Our results indicate that autosomal recessive and X-linked recessive diseases can occur simultaneously. These clinical findings together with the results of exome sequencing throw some light on the diagnosis of rare diseases.

Case Presentation

Clinical Data

A 1.5-month-old male infant was admitted to our hospital due to persistent thrombocytopenia and prolonged fever for 10 days. The patient had been physically examined at birth and reported as grossly normal. His platelet counts...
and mean platelet volume (MPV) was normal at birth. Furthermore, his parents denied any positive family history for bleeding disorders or hematologic malignancies.

To evaluate the disease severity and to identify the causes, comprehensive physical examination and necessary laboratory tests were performed. The infant’s physical examination was unremarkable except for rales in the right middle lobe and the skull deformities of cephalus quadratus. Neither obvious hearing difficulty and visual disturbances nor eczematous skin lesions were detected. His routine blood test revealed leukocytosis, anemia, and thrombocytopenia with normal MPV (Table 1). Then the bone marrow aspirate was performed to exclude the possibility of leukemia (Fig. 1A). Skeletal radiography was carried out as the skull deformities of cephalus quadratus were usually associated with the dry tap of bone marrow aspiration. Radiographs showed a generalized increase in bone mass density, a typical marker of osteopetrosis (Figs. 1B, C). In addition, bone biopsy shows a significant decrease in osteoclasts and an increased number of cancellous substances in the iliac crest of this patient (Figs. 1D, E).

Serologic tests for immunoglobulins (Igs) revealed a decrease in IgM and an increase in IgG and IgA concentration (Table 1). Immunophenotyping of lymphocytes from peripheral blood showed a low level of both CD3\(^-\)CD16\(^+\)CD56\(^+\) NK and

| TABLE 1. Blood Examinations |
|----------------------------------|----------------------------------|----------------------------------|
| **Routine Blood Test** | **Immunoglobulin** | **Biochemical Examination** |
| Items | WBC (10\(^9\)/L) | Hb (g/L) | PLT (10\(^9\)/L) | MPV (fL) | IgM (g/L) | IgA (g/L) | IgG (g/L) | AST (U/L) | ALT (IU/L) | LDH (IU/L) | GGT (IU/L) | Ca\(^{2+}\) (mmol/L) | CRP (mg/L) |
| Value | 18.59 | 74 | 50 | 10.8 | 0.14 | 0.46 | 16.48 | 105 | 134 | 367 | 709 | 2.17 | 59.2 |
| Age-matched normal values | 8-12.5 | 120-170 | 100-300 | 7-11 | 0.23-0.91 | 0.13-0.35 | 0.23-0.91 | 0-60 | 0-60 | 80-285 | 0-50 | 2.2-2.6 | 0-8 |
| Date of test | October 14, 2016 | October 14, 2016 | October 14, 2016 |

ALT indicates alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; HB, hemoglobin; IG, immunoglobulin; LDH, lactate dehydrogenase; MPV, mean platelet volume; PLT, Platele; WBC, white blood cell.
CD3−CD19+ B lymphocytes by Flow Cytometry (FC500) (Table 2, Supplementary Fig. 1, Supplemental Digital Content 1, http://links.lww.com/JPHO/A360). Comprehensive analysis of metabolic disease ruled out common metabolic conditions. The further biochemic investigation suggested that hypocalcemia and subsequent liver defects were established (Table 2). Additional etiologic diagnosis of cytomegalovirus (CMV) infection was confirmed by the positive results of CMV-antibodies and CMV-DNA (3.22 × 10⁴ copies).

On the basis of the clinical presentation and ancillary findings, this patient was primarily diagnosed with osteopetrosis, thrombocytopenia, bronchopneumonia, CMV infection, and common variable immunodeficiency. Meanwhile, genetic analysis for the detection of inherited platelet disorders and osteopetrosis was investigated immediately using NGS. Because of the patient’s persistent thrombocytopenia and no response to conventional therapy, 71 candidate genes related to inherited platelet disorders were analyzed.8 His parent’s blood samples were also sent to confirm the sources of mutation.

NGS and DNA sequence analysis was used in this case. Briefly, the sheared genomic DNA, sheared by sonication, was then hybridized with a NimbleGen probe capture array. The array covered about 400 genes including all the thrombocytopenia and osteopetrosis related genes from the OMIM database (www.omim.org) (Joy Orient, China). The libraries were first tested for enrichment by quantitative polymerase chain reaction (PCR) and for size distribution and concentration using the Agilent Bioanalyzer 2100. The samples were then sequenced on an Illumina Hiseq. 2500. Raw image files were processed by the BclToFastq (Illumina) for base calling and the raw data generating. Sanger sequencing was used to confirm the mutation in WAS and TNFRSF11A gene of the proband (Supplementary Table 1, Supplemental Digital Content 2, http://links.lww.com/JPHO/A361, list The PCR primers and length of PCR product).

In the first place, NGS analysis showed a pathogenic variant in accordance with guidelines,9 consisting of a hemizygous transition mutation in WAS gene, c.400G>A, causing substitution of alanine for threonine at amino acid position 134 (p.A134T, NM_000377). His mother was confirmed to be a carrier of this WAS mutation by Sanger sequencing (Fig. 2A). This nucleotide transition, located in Exon 4 of the WAS gene, had been identified as the mutation responsible for the X-chromosome-linked recessive WAS.10 Judging from the genetic result and his clinical

| Cell Type | CD (Specify Markers) | Percentage | Age-matched Normal Percentage Values | Absolute Values (×10⁹/L) |
|-----------|----------------------|------------|-------------------------------------|--------------------------|
| B-lineage | CD3−CD19+/CD19+      | 2.4        | 9.02-14.1                           | 0.17                     |
| Lymphocyte (total) | CD3+         | 81.7       | 61.7-77                             | 6.01                     |
| Helper/inducer | CD3+CD4+      | 33.7       | 25.8-41.6                           | 2.48                     |
| Cytotoxic/suppressor | CD3+CD4+/CD3−CD8+ | 1.5        | 0.9-1.9                             | 0.11                     |
| Natural killer cells | CD3−CD16+CD56+ | 6.7        | 10.4-19.78                          | 0.49                     |

NGS as previously recommended.2 Because of the patient’s persistent thrombocytopenia and no response to conventional therapy, 71 candidate genes related to inherited platelet disorders were analyzed.8 His parent’s blood samples were also sent to confirm the sources of mutation.

Investigations
Considering that this infant presented with typical radiologic skeletal features of osteopetrosis, 7 identified pathogenicity genes of osteopetrosis were analyzed by the

![FIGURE 2. Molecular genetic analysis. A, Validation by Sanger sequencing of the c.400G>A mutation in Wiskott-Aldrich syndrome (WAS). The arrow indicates the position of the mutated base. The propositus was hemizygous and the mother heterozygous for the mutation. B, Sequencing of TNFRSF11A revealed a homozygous single nucleotide transition (c.–45A>G). C, This mutation is in the upstream of exon 1, at the 5′ untranslated region (5′ UTR) of TNFRSF11A gene. 3′ UTR indicates untranslated region.](c266 | www.jpho-online.com)
presentation, the infant was diagnosed with WAS with a clinical score 3.11

To further study the genetic evidence underlying the clinical finding of osteopetrosis, the molecular analysis of genes known to be responsible for the different types of osteopetrosis (TCIRG1, CLCN7, PLEKHMI, RANKL, RANK, and SNX10) was performed by NGS of exons and intron-exon boundaries as previously described.12 As a result, a homozygous single nucleotide transitions, c.-45A>G (Fig. 2B), in the 5′ UTR of TNFRSF11A gene had been uncovered (Fig. 2C). The infant’s father was found to be heterozygous for this mutation. This variant has not been recorded in any of the publicly available single nucleotide polymorphism database (1000Genomes, ExAC, ESP, HGMD, Clinvar) and inhouse database in Chinese Han people. To date, no literature or other genetic database records have reported that this 5′ UTR of TNFRSF11A gene leads directly to the pathophysiology of osteopetrosis. On the basis of the clinical presentation and genetic analysis, we identified a likely pathogenic variant according to the guidelines.9

**DISCUSSION AND CONCLUSION**

In the present case, WAS was not initially considered as the priority diagnosis due to normal MPV and the absence of eczema. In fact, a number of cases diagnosed with WAS have been described with normal or even increased MPV.13,14 Thus, the refractory thrombocytopenia to first-line therapy and immune deficiency may be the common features despite the distinctive atypical clinical presentation in this inherited thrombocytopenia. It’s concluded that both normal platelet size and the absence of eczema should not exclude the diagnosis of WAS, and the high-throughput sequencing will help to disclose the genetic basis.

In the past few years, exome sequencing has gradually promoted the discovery of the genetic defects of osteopetrosis underlying Mendelian disorders.15,16 Mutations in the coding region of the TNFRSF11A gene may lead to a defect in osteoclast formation and result in clinical manifestations.17 However, current sets of probes for exome capture target not only coding regions but also 5′ UTR and 3′ untranslated regions and stretches of intronic regions. Recently, several cases have demonstrated that the mutations in the noncoding region even in the intronic region can contribute to inherited diseases including ARO.12,16-18

In the present case, with a clinical diagnosis of osteopetrosis based on radiologic findings and hematologic defects, a single nucleotide mutation in the 5′ UTR of TNFRSF11A gene were identified. These findings provide a de novo likely pathogenic mutation involved in osteoclast-poor formatted recessive osteopetrosis complex. Although mutations in 5′ UTR usually have significant effects on transcription at the level of mRNA, it is difficult to decide whether 5′ UTR mutation is also significant in this case as the infant died.

In conclusion, the present case reported a de novo single nucleotide transitions in the 5′ UTR of TNFRSF11A as the basis of molecular diagnosis of osteopetrosis. The findings reported here are the first to identify the coinheritance of 2 rare inherent diseases, ARO, and X-linked recessive WAS. Further studies are needed to elucidate the molecular pathophysiology of this mutation in 5′ UTR and additional families are needed for the full description of the phenotypic manifestations.

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