



## Mutation analyses and prenatal diagnosis in families of X-linked severe combined immunodeficiency caused by *IL2R $\gamma$* gene novel mutation

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**ABSTRACT.** We investigated the feasibility of interleukin-2 receptor gamma (*IL2R $\gamma$* ) gene based on gene mutation analysis and prenatal diagnosis of X-linked severe combined immunodeficiency (X-SCID). Blood samples of patients and their parents of X-SCID (family 1) and X-SCID (family 2) were collected. *IL2R $\gamma$*  gene sequences of the 2 families were analyzed using bi-directional direct sequencing by polymerase chain reaction. DNA sequence changes in the *IL2R $\gamma$*  gene exon region and shear zone were also analyzed. We also sequenced the *IL2R $\gamma$*  gene in 100 healthy individuals. Prenatal genetic diagnoses for a high-risk fetus in family 1 were performed by chorionic villus sampling after determining each family's genotypes. The suspect female in family 1 underwent carrier detection. Two novel mutations of *IL2R $\gamma$*  gene were identified, including c.361-363delGAG (p.E121del) in the patient and his mother in family 1, and c.510-511insGAACT (p.W173X) heterozygous mutation in the proband's mother in family 2. These mutations were absent in the 100 controls. Prenatal diagnosis

of early pregnancy in the female fetus of family 1 was performed; the fetus was heterozygous, which was confirmed at postnatal follow-up. The suspect female in family 1 showed no mutation in carrier detection. The novel p.E121del and p.W173X mutations in IL2R $\gamma$  may have been the primary causes of disease in 2 families with X-SCID. In couples with an X-SCID reproductive history, prenatal gene mutation analysis of IL2R $\gamma$  can effectively prevent the birth of children with X-SCID and carrier detection for suspected females.

**Key words:** Gene mutation; Interleukin-2 receptor gamma gene; Prenatal diagnosis; X-linked combined immunodeficiency diseases

## INTRODUCTION

Severe combined immunodeficiency diseases (SCIDs) are a group of primary immunodeficiency diseases characterized by a severe lack of T cells (or T cell dysfunction) caused by various gene abnormalities and accompanied by B cell dysfunction (WHO, 1992; Buckley et al., 1997). The incidence rates in infants were 1/75,000-1/10,000 (WHO, 1992), but no morbidity statistics are available in China. The 2 genetic modes of SCID include X-linked recessive and autosomal recessive genetic inheritance. X-linked severe combined immunodeficiency (X-SCID) is the most common form, accounting for 50-60% of SCID cases (Noguchi et al., 1993). Immune system abnormalities in patients with X-SCID include T-B+NK<sup>-</sup>, in which T cells (CD3<sup>+</sup>) and natural killer (NK) cells (CD16<sup>+</sup>/CD56<sup>+</sup>) are absent or significantly reduced, and the number of B cells (CD19<sup>+</sup>) is normal or increased, causing reduced immunoglobulin production and class switching disorder (Buckley, 2004; Fischer et al., 2005). The IL-2R $\gamma$  gene mutation has been confirmed to be a major cause of X-SCID (Noguchi et al., 1993). In recent years, great progress has been made in understanding the pathogenesis of primary immunodeficiency disease and its application in clinical treatment, particularly regarding the development of critical care medicine and immune reconstruction technology. With timely control of infection and early bone marrow or stem cell transplantation, X-SCID patients can be treated, prolonging survival time. Therefore, early diagnosis of X-SCID is very important for patient treatment. Gene diagnosis has become a better early diagnosis or differential diagnosis method. In addition, familial X-SCID brings a great psychological burden to the relatives of patients. Ordinary chromosome analysis and immunological evaluation cannot be used for female carrier identification and fetal diagnosis, and gene diagnosis is the most effective method of carrier detection and prenatal diagnosis. In this study, we detected mutations in 2 families with X-SCID and identified 2 novel mutations, confirming the X-SCID pedigrees. Prenatal diagnosis was performed for the pregnant fetus in the mother of one of the probands based on gene diagnosis. Female individuals in this family were subjected to carrier detection.

## MATERIAL AND METHODS

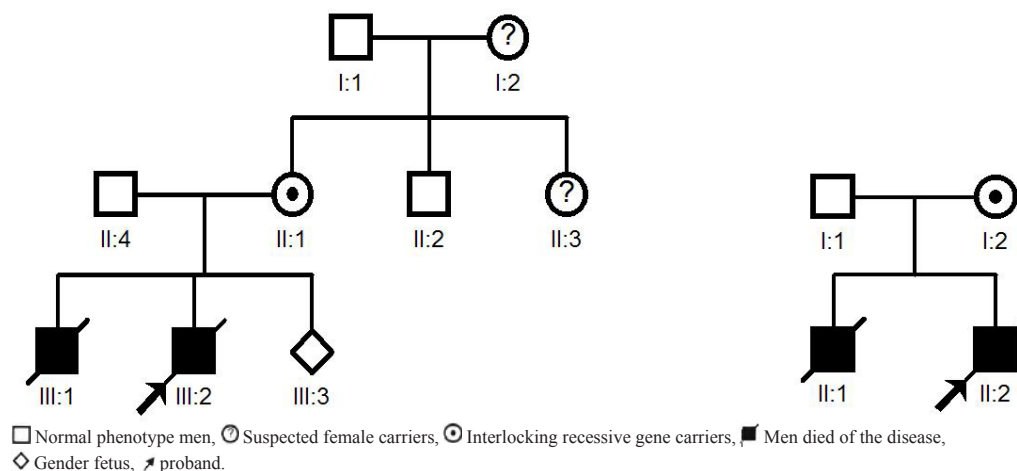
### Pedigrees

Both X-SCID families were recruited from the Department of Pediatrics in our hospital. According to the chief complaint of the family members, the child (Figure 1, III1) in fami-

ly 1 was hospitalized with pneumonia and blood abnormalities at 3 months. Lymphocyte count was decreased ( $0.3 \times 10^9/L$ ) and accompanied by disunion of the Bacillus Calmette-Guerin (BCG) vaccination area. The initial diagnosis was hemophagocytic syndrome and T-B+NK-SCID was suspected after further immunological analysis, which showed that T and NK cell counts were very low. The child died at 7 months. The child (Figure 1, III2) was normal at birth and had not received the GCG vaccine. The child was hospitalized with pneumonia because of poor appetite and recurrent fever at 3 months. Chest computed tomography scanning showed no thymus, and immunological analysis showed severe immunodeficiency disease. X-SCID was diagnosed. From the patient that died at 7 months, 2 mL anticoagulant blood samples were collected. The parents wished to have additional children and thus requested prenatal diagnosis in order to avoid the disease in future children. The child (Figure 1, II2) in family 2 was normal at birth and had recurrent pulmonary infection at 3 months; ulceration and fester was observed in the area of BCG injection. Immunological analysis showed T-B+NK-SCID and X-SCID was diagnosed. This child died at 8 months and no blood samples were collected. The family members stated that the first boy (Figure 1, III1) had similar symptoms and they wished to undergo genetic counseling in order to avoid these issues in the future. The study was approved by the medical Ethics Committee of the First Affiliated Hospital of Zhengzhou University, all gene diagnosis of patients and their families were agreed to. All subjects signed informed consent.

## Family 1

## Family 2



**Figure 1.** Pedigree chart of X-SCID family; arrow represents the proband

## Methods

### DNA extraction

First, 2 mL peripheral venous blood samples of the proband (Figure 1, III2) was collected and preserved from the mother (Figure 1, III1), aunt (Figure 1, II3), and grandmother (Figure 1, I2) in family 1 and mixed with the anticoagulant ethylenediaminetetraacetic acid

K2. In addition, 2 mL peripheral blood was extracted from the proband's parents (Figure 1, I1 and Figure 1, I2) in family 2. Prenatal diagnosis was performed in family 1 at 11 weeks of pregnancy with ultrasound-guided transabdominal fetal chorionic villus samples. We selected 100 healthy individuals and collected DNA for use as normal control specimens. The DNA from all subjects was extracted using the Beijing Tiangen DNA kit according to manufacturer instructions.

### ***Polymerase chain reaction (PCR) and product sequencing detection***

We extracted the relevant sequences of the IL2R $\gamma$  gene from the UCSC Genome Bioinformatics database (<http://www.genome.ucsc.edu>) and synthesized the IL2R $\gamma$  gene 1-8 exons and intron using 4 primer pairs (Table 1). We selected patients and the mothers in family 1 and the proband's mother's DNA in family 2, and the IL2R $\gamma$  gene was amplified by PCR in a total reaction volume of 25  $\mu$ L. The renaturation temperature used in the conventional PCR is shown in Table 1. The PCR product was confirmed by 2% agarose gel electrophoresis. After purification of PCR products, the products were bi-directionally sequenced using the BigDye v3.1 Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in an ABI 3130xl gene sequencing instrument (Applied Biosystems). The sequencing results were compared with the normal sequence using the Chromas software to identify gene mutations. Detection of novel mutation sites in 100 healthy control specimens was used to exclude the polymorphic loci of newly discovered loci.

**Table 1.** Primer sequences of IL2R $\gamma$  gene mutation and annealing temperature.

Primer name	Amplified region	Primer sequences	Annealing temperature (°C)
IL2R $\gamma$ -A	Exon 1	F: CATGGCATAGAACGGTGATGT R: CCACATGATTGTAATGGCCAGT	65
IL2R $\gamma$ -B	Exon 2-4	F: GTGCTTGGCCTCCTCTTCT R: GGAGCTTGATATTAGGTCCTTC	68
IL2R $\gamma$ -C	Exon 5	F: TGGCTTGAGTAGTCAAGAGATG R: GGAGAGATGGGGACCAAGT	65
IL2R $\gamma$ -D	Exon 6-8	F: CACAGGAGCTGTTGTGAGGAT R: CATCGTTTCAGGAACAATCGGA	61

### ***Mutation nomenclature and verification***

To identify initial sequence variation, the Human Gene Mutation Database (<https://portal.biobase-international.com/hgmd/pro/start.php>) and X-SCID gene database (IL2R $\gamma$ base) (<http://research.nhgri.nih.gov/scid/>) were searched. To exclude polymorphisms, we sequenced the corresponding exons of 100 healthy unrelated individuals. The new mutant was named based on the international gene mutation naming system (<http://www.hgvs.org/mutnomen>).

### ***Prenatal diagnosis***

After confirming the X-SCID gene mutation in the proband's mother, fetal chorionic villus samples were collected in the high-risk pregnant women of family 1 at 11 weeks' gestation for prenatal diagnosis. Extraction of fetal DNA and mutation detection of genomic DNA was conducted using DNA sequencing.

### ***Identification of fetus gender and elimination of fetal maternal contamination***

Using the Promega PowerPlex 16 HS system (Promega, Madison, WI, USA) to identify the gender of AME loci, the genes were amplified by PCR according to kit instructions, then the products were detected using the ABI3130XL sequencing instrument and analyzed using the Gene-Mapper ID v3.2 software. The AME (Amelogenin) site of male individuals produced 102- and 106-bp fragments, while female individuals produced 102-bp fragments. Maternal pollution exclusion criteria: the fluorescence peaks of all fetal alleles were from each parent; all fetal alleles were not from the second fluorescence peaks of their mothers.

### ***Carrier detection***

After detecting the X-SCID gene mutation in the patient and mother in family 1, the presence of the corresponding mutation was detected in the proband's grandmother (I2) and aunt (II3) in family 1. This was conducted to determine whether the grandmother and aunt were pathogenic mutation heterozygous carriers.

### ***Follow-up***

After delivery of the fetus, the umbilical cord blood was collected to verify the results of prenatal gene diagnosis and conduct follow-up of the baby after 1 year.

## **RESULTS**

### ***IL2R $\gamma$ gene mutation test***

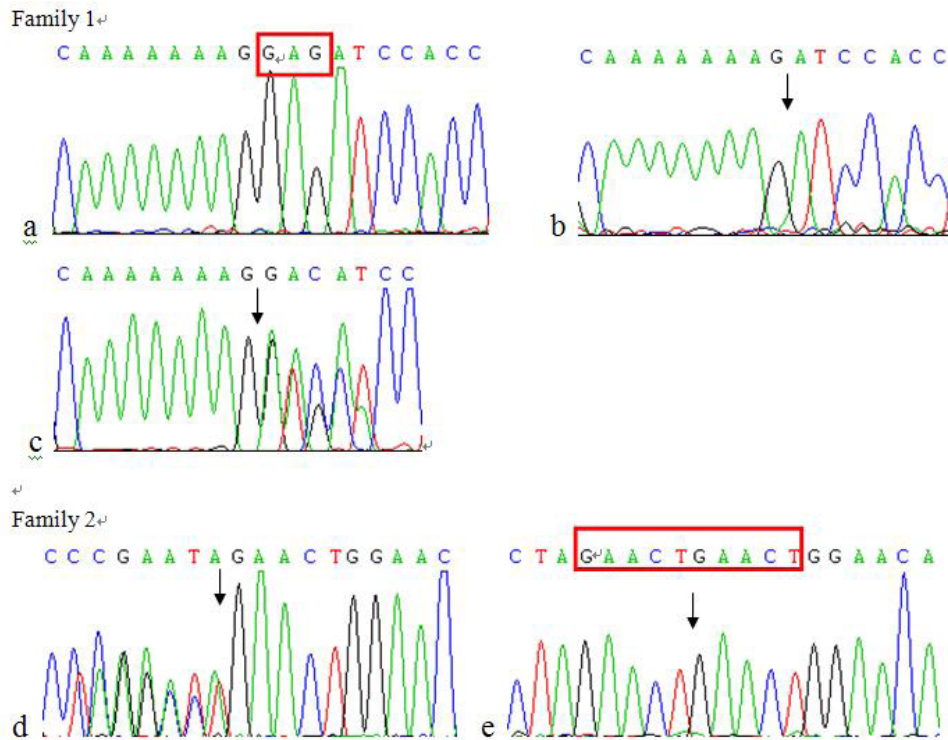
Direct sequencing of 1-8 exons and the flanking region of the *IL2R $\gamma$*  gene by PCR in family 1 showed that the 3rd exon of the proband contained the c.361-363delGAG heterozygous deletion mutation, which led to deletion of the 121st amino acid glutamate (p.E121del) in its coding product. There were no sequence variations in other coding regions or in the shear zone. The proband's mother carried the same heterozygous mutation, while his father did not carry the mutation site (Figure 2a, b, c). This mutation was not observed in any cases of the control group, and this family was identified as an X-SCID family. The c.510-511insGAACT insertion heterozygous mutation was present in the 4th exon of the proband's mother in family 2. This mutation was a 5-base repeat of GAACT, resulting in a change in amino acid 173 from tryptophan into a stop codon (p.W173X). While there were no sequence variations in other coding regions or in the shear zone, the patient's father did not carry the mutation (see Figure 2d, e). We did not find this mutation in the healthy control group. We presumed that the 4th exon of the deceased child in family 2 contained the c.510-511insGAACT insertion mutation, leading to X-SCID symptoms, and thus we speculated that this family was an X-SCID pedigree.

### ***Prenatal diagnosis***

We verified the chorionic villus status of the fetus in family 1 using the PowerPlex 16 HS System kit. The results of prenatal diagnosis showed that the fetal tissue contained no

maternal contamination and that this fetus was female.

The results of prenatal diagnosis showed that there was no c.361-363delGAG (p.E121del) heterozygous mutation in the female fetus of family 1.



**Figure 2.** Sequencing graph of *IL2R $\gamma$*  gene in 2 pedigrees with X-chain severe combined immunodeficiency. **a-c.** Family 1. **a.** Normal control (rectangle indicates 3 edentulous bases of this patient). **b.** Proband carrying the c.361-363delGAG (p.E121del) mutation (arrow indicates deletion of fragment connection sites). **c.** The proband's mother contained a c.361-363delGAG (p.E121del) heterozygous mutation (arrow). **d-e.** Family 2. **d.** The proband's mother carried the c.510-511insGAACT (p.W173X) heterozygous mutation (arrow indicates that the reverse sequencing graph was positive). **e.** Normal control (rectangular box indicates 2 normal copies of GAACT (the mutation fragment was 3 copies).

### Carrier detection results

For the c.361-363delGAG (p.E121del) site, the gene analysis results of the female individual in family 1 showed that I2 (proband's grandmother) was a heterozygous carrier and that II3 (proband's aunt) was a non-carrier and had no mutations.

### Follow-up

The fetus of family 1 did not carry the mutation from the mother, and the possibility of becoming the patient or female carriers in the future was lower. The mother in family 1 chose to continue the pregnancy after genetic counseling. Analysis of the umbilical cord blood of

the fetus after birth showed results that were consistent with prenatal diagnosis. The general development of infants was normal after 1-year follow-up by telephone.

## DISCUSSION

IL-2 can combine with the IL-2 receptor (IL-2R) of the immune cell membrane. IL-2R is composed of 3 subunits, including the IL-2R $\alpha$  chain (CD25), IL-2R $\beta$  chain (CD122), and IL-2R $\gamma$  chain (CD132). IL-2R $\gamma$  functional units in common with IL-4, IL-7, IL-9, IL-15, IL-21, and other cytokine receptors, and these regions are referred to as the total chain (Li et al., 2000). The IL-2R $\gamma$  chain can maintain the integrity of the IL-2R complex and is required for the internalization of the IL-2/IL-2R complex; it is also the link that contacts the cell membrane surface factor region and downstream cell signal transduction molecules. Therefore, the integrity of the IL-2R $\gamma$  chain is vital for the immune function of an organism (Malka et al., 2008; Shi et al., 2009).

Mutations in the *IL2R $\gamma$*  gene, which encodes IL-2R $\gamma$ , were identified to be a major cause of X-SCID in 1993 (Noguchi et al., 1993). The *IL2R $\gamma$*  gene is located on chromosome X q21.3-22, is 37.5 kb length, and contains 8 exons, which encode 369 IL-2R $\gamma$  amino acids. The IL2R $\gamma$  chain exhibits varying structural regions, such as the signal peptide [amino acids (AA) 1-22], extracellular domain (AA 23-262), transmembrane region (AA 263-283), and intracellular region (AA 284-369). The WSXWS motif is located in the extracellular region (AA 237-241), while Box 1 is located in the intracellular region (AA 286-294).

By the end of 2013, the Human Gene Mutation Database contained a total of 200 mutations in the *IL2R $\gamma$*  gene (HGMD Professional 2013.4). The most common mutation types in the *IL2R $\gamma$*  gene were the missense or nonsense mutations, which result from single base changes. A total of 100 missense or nonsense mutations have been identified, followed by insertion or deletion mutations in a total of 50 species. The 3rd most common type of mutations includes shear mutations in approximately 30 species. Eight exons contained mutations, and mutations in 3rd or 4th exons were the highest, accounting for a total mutation rate of 43% (86/200). According to the X-SCID gene database (IL2RGbase) (<http://research.nhgri.nih.gov/scid/>), the gene mutations in *IL2R $\gamma$*  mainly occurred in the extracellular region of the IL2R $\gamma$  chain (Fugmann et al., 1998). Zhang et al. (2013) reported that the *IL2R $\gamma$*  gene mutations in 10 patients with X-SCID in China were located in the extracellular region. Two mutations reported in our study were also located in the extracellular region. The mutation of *IL2R $\gamma$*  gene in family 1 was a codon mutation in the 3rd exon, resulting in a 3-base deletion. The c.361-363delGAG (p.E121del) mutation was located in the extracellular area of the IL-2R $\gamma$  subunit, and we inferred that the 121 glutamate deletion caused by the mutation would lead to changes in the structure of the peptide chain, affecting signal transmission and resulting in serious symptoms. The mutation of family 2 was a GAACT repeat of *IL2R $\gamma$*  gene; this repeat of 5 bases resulted in 173 codon changes from tryptophan into a stop codon. Generation of the peptide chain with the mutation lacked 196 amino acids compared to the normal chain, including the intracellular, transmembrane, and some extracellular regions, directly affecting the structure and function of receptors and causing disease. No studies have been reported regarding these 2 mutations. We combined with the mutation characteristics and clinical manifestations and diagnosed family 1 as X-SCID pedigrees. Although the patient in family 2 was deceased, it can be speculated that the 2 deceased patients in family 2 were X-SCID pedigrees caused by c.510-511insGAACT (W173X).

The genetic diagnosis of *IL2R $\gamma$*  is not only an early diagnosis or differential diagnosis

method for patients with X-SCID but also the best means of carrier detection and prenatal diagnosis (Wu et al., 2012). In this study, the proband's sibling in family 1 was suspected of having X-SCID, but did not undergo gene diagnosis, and symptoms were observed in the proband after birth. Although we failed to save this child, we combined immunological evaluation and gene diagnosis to confirm that this pedigree was X-SCID genetic disease. The proband's mother was pregnant again after genetic counseling, and the results of gene diagnosis showed that the female individual was not carrying virulence genes and was healthy after 1-year follow-up. Prenatal diagnosis can accurately identify fetal situations and be used to avoid birth defects, which can also ease the anxiety of the pregnant mother. Gene diagnosis for pedigrees of patients based on DNA samples has advanced recently, particularly with the application of high-throughput sequencing technology (Alsina et al., 2013). We can now perform gene analysis for varied clinical infectious diseases for differential diagnosis. However, the effectiveness of prenatal diagnosis for pedigrees in which the proband is dead remains unclear. Because the gene mutations in the proband is unknown in these cases, the patient's situation was only inferred by his mother's genotypes. However, we considered that for the deceased, if we can define the mother was a pathogenic gene carrier, even if the proband is not X-SCID, the woman also has a risk of having X-SCID children and this pedigree may be X-linked recessive inheritance. Prenatal diagnosis may provide a choice for preventing the birth of patients in these families in the premise of informed consent.

Gene diagnosis of IL2R $\gamma$  can also be used for carrier detection of suspected females in the family. In the present study, we performed carrier detection of the patient's grandmother and aunt in family 1 and determined that the patient's pathogenic mutations were from his grandmother. His aunt did not inherit the pathogenic gene, and thus she was a non-carrier and her fertility will not be affected.

In this study, we used direct sequencing of PCR products and identified IL2R $\gamma$  gene mutations in 2 pedigrees with X-SCID. We found 2 unreported mutations in the IL2R $\gamma$  gene, and prenatal diagnosis and carrier detection were conducted in 1 X-SCID family. Because the incidence rate of X-SCID is extremely low, it is difficult to promote the widespread use and application of genetic diagnosis. However, this study may provide some implications for the diagnosis of infants with immunodeficiency, and gene diagnosis techniques such as conventional or high-throughput sequencing should be used as soon as possible during pregnancy, which can be used to guide treatment. This method can also provide reliable prenatal diagnosis and carrier detection service for these families.

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