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A new mutation (1062 del 16) of iduronate-2-sulfatase gene from a Chinese patient with Hunter syndrome*

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Abstract: Objective: To identify the mutations of iduronate-2-sulfatase (*IDS*) gene, to reveal its mutation features, and to establish a basis for genetic counseling and prenatal gene diagnosis of Hunter syndrome. Methods: Urine glycosaminoglycans (GAGs) assay, PCR and DNA sequencing were performed to detect mutation of *IDS* gene of the patient and his parents. Results: The result showed that the patient was: DS(++), HS(++), KS(−), CS(−), and that both of his parents were negative. A frame-shift deletion mutation (1062 del 16) was identified in exon 7 of the patient's *IDS* gene. His parents' genotypes were normal. Conclusion: The patient's mutation was not inherited by his parents but a novel one. The mutation probably altered the primary structure and tertiary structure of IDS enzyme protein remarkably and lowered the activity of IDS enzyme greatly. Therefore it is supposed to be the direct cause of the disorder.

Key words: Hunter syndrome, Mucopolysaccharidosis type II (MPS II), Glycosaminoglycan (GAG), Iduronate-2-sulfatase (IDS), Mutation, Gene diagnosis, DNA sequencing

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INTRODUCTION

Hunter syndrome (MIM 309900), also called mucopolysaccharidosis type II (MPS II), is worldwide distributed and a severe disabling, fatal monogenic X-linked recessively inherited disease. The morbidity is 1/132000 males (Du and Liu, 1992). It is the basic cause of Hunter syndrome that iduronate-2-sulfatase (*IDS*) gene mutation lowers the activity of IDS enzyme (EC 3.1.6.13) and influences the degradation of heparan sulfate (HS) and dermatan sulfate (DS). Investigation of the *IDS* gene mutation is the key to diagnose the disorder and the necessary premise for genetic counseling, prenatal gene diagnosis and early prevention and cure. The new mutation rate of *IDS* gene is high (Hopwood *et al*., 1993) and *IDS* gene has high genetic heterogeneity. The mutation patterns of patients from different races, nations and areas are diversified, and mutation hot spots also are various. In order to investigate the mutations of Chinese patients with Hunter syndrome, search for mutation hot spots and identify new mutation, we have collected and further studied the *IDS* gene of a typical Hunter syndrome pedigree from Guangdong recently. We identified a novel mutation which had not been reported, and the following is the detail.

MATERIALS AND METHODS

Proband

He was 5 years old and suffered from short stature, mental retardation, a coarse face, stiff joints, swollen abdomen and navel hernia.

Physical examination

He was 103 cm tall and 18 kg, with a head cir-

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cumference of 54 cm. He suffered from a coarse face including protruding forehead, coarse eyebrows, low nose bridge, big tongue, wide distance between two eyes, full tips, sparse teeth and short neck. He also suffered from remarkable mental retardation, hearing decline, obscured pronounciation, clawing of hands, ulna and radius stiff, funnel chest, asthma, swollen abdomen, navel hernia and so on. But his eyesight was normal and his corneas were not turbid. His parents' phenotypes were normal and they were non-consanguineously married.

Laboratory examination

Urinary glycosaminoglycans (GAGs) collected from middle stage of urination of the patient and his parents were tested with methods of agarose gel electrophoresis and toluidine blue established by ourselves with standard dermatan sulfate (DS), heparan sulfate (HS), keratan sulfate (KS) and chondroitin sulfate (CS) as positive controls and with normal people as negative controls (Xu *et al*., 2005; Huang *et al*., 2007). Two to three millilitres EDTA anticoagulated blood of patient and his parents was extracted respectively to be used for preparing DNA samples. Thereafter, exons 1~9 of *IDS* gene were tested with methods of PCR and DNA bidirectional sequencing (Guo *et al*., 2005; 2006; Guo and Du, 2006a; 2006b).

1. PCR amplification

Primer pairs were designed according to *IDS* gene sequence mapped on Xq27.3~q28 (ACCESSION: NC_000023; REGION: complement 148371663~148394841; SPAN: 23179 bp). The PCR reactions were carried out in a reaction volume of 30 μl which contained 3 μl 10×Taq buffer, 3 μl dNTPs (2 mmol/L), 1 μl each primer (10 μmol/L), 1.5 μl (300 ng) genomic DNA, 2.5 U Taq DNA polymerase and 19 μl ddH_2O. PCR was performed using the Hema type 480 PCR cycler (Guo *et al*., 2005; 2006; Guo and Du, 2006a; 2006b).

2. DNA sequencing

Sequencing was performed using the ABI PRISM 377 or 3730 DNA sequencer by Shanghai Invitrogen Company.

RESULTS

1. The result of patient's urine GAGs assay was: DS(++), HS(++), KS(−), CS(−), and both of his parents' were: DS(−), HS(−), KS(−), CS(−).

2. In the encoding region of patient's exon 7, there is a frame-shift deletion mutation (1062 del 16, cDNA 1062~1077 bp, codon 313th~318th) shown in Figs.1d and 2d), while his parents were normal shown in Figs.1b, 1c, 2b and 2c). The sequence of exon 7 was mapped on X chromosome 148379697~148380006, spaning 310 bp.

DISCUSSION

Hunter syndrome is a lysosomal storage disease caused by the deficiency of IDS enzyme or lowered activity and incomplete degradation or lysosomal accumulation of HS and DS. The surplus GAGs can be drained from urethra, so urinary GAGs qualitative assay can be used for preliminary diagnosis. The types of MPS are various and MPS can be classified into three types (MPS I, II, VII) only by the difference of DS and HS upraising (Luo, 2000). Therefore, urinary GAGs assay cannot be used for classification, diagnosis, and prenatal diagnosis. Gene diagnosis is essential to diagnose the disease. According to the evidence that Hunter syndrome is X-linked recessively inherited and patients always were male whose corneas were not turbid, we firstly examined patient's *IDS* gene. Thereafter, a novel deletion mutation (1062 del 16) in the encoding region of exon 7 was identified. This mutation resulted in loss of 6 amino acids of RLLSAL in position 313th~318th of IDS enzyme and the base sequence after mutation spot occurred dislocation. It caused elongation of the amino acid chain to a terminal codon TAG at position 334, thus the peptide chain was shortened from 550 to 333 amino acids and lost 217 amino acids. By searching HGMD database (http://www.hgmd.org) and document research, we confirmed that this mutation was a novel one that was discovered firstly. The parents' genotypes were normal, which suggested that the mutation was not inherited by his parents but was changed at the first time. Early in 1991, some cases had been reported abroad (Machill *et al*., 1991). This mutation altered the primary structure and tertiary structure of IDS enzyme protein remarkably and lowered the activity of IDS greatly. Therefore it is supposed to be the direct cause of the patient with Hunter syndrome.

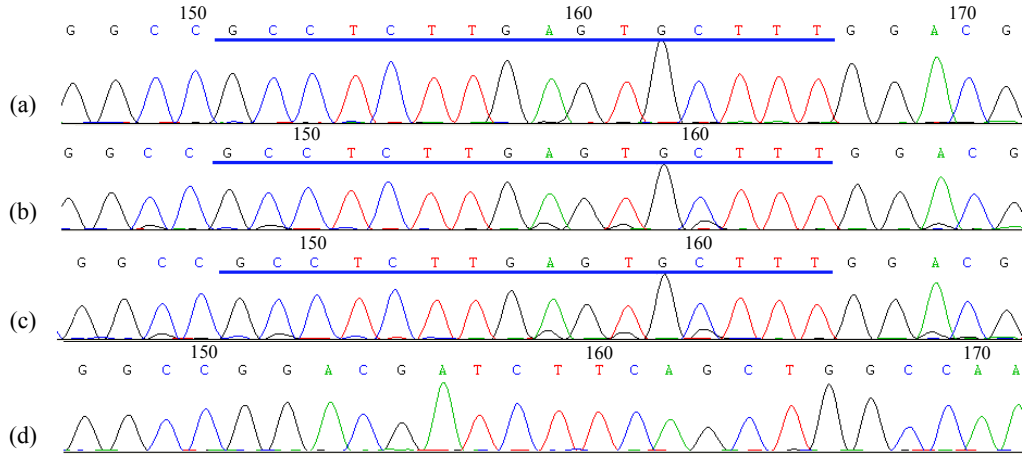

Fig.1 The comparison of forward sequences in exon 7 of *IDS* gene

(a) Normal control; (b) Father; (c) Mother; (d) Patient. The underline shows the normal sequence which contains 16 base pairs. The sequences of patient's father and mother are the same as the normal control's, while the patient's sequence deletes 16 bp of "GCCTCTTGAGTGCTTT" (cDNA 1062~1077 bp)

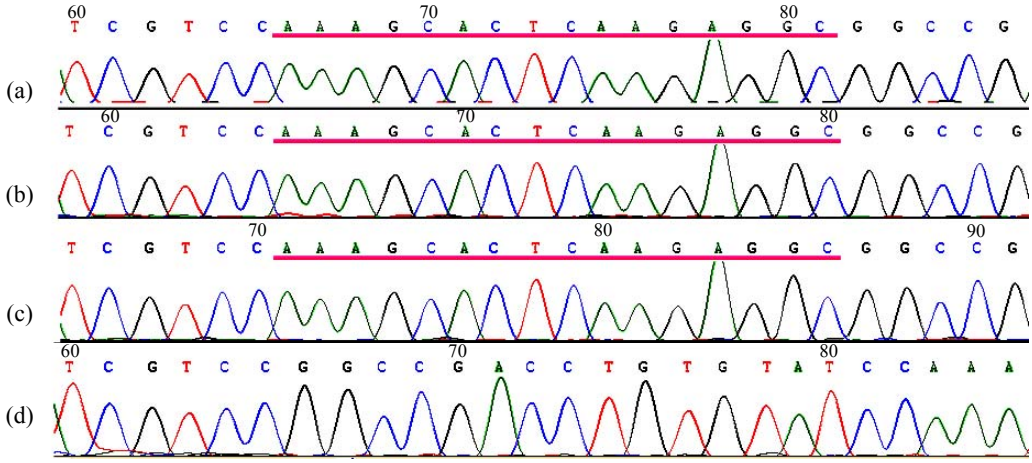

Fig.2 The comparison of reverse sequences in exon 7 of *IDS* gene

(a) Normal control; (b) Father; (c) Mother; (d) Patient. The underline shows the normal sequence which contains 16 base pairs. The sequences of patient's father and mother are the same as the normal control's, while the patient's sequence deletes 16 bp of "AAAGCACTCAAGAGGC" (cDNA 1077~1062 bp)

We have discovered that 1062 del 16 is the ninth kind of mutation from Chinese patients with Hunter syndrome in recent years. The detection of this mutation is helpful for demonstrating that *IDS* gene has a high new mutation rate and remarkable hot spots (Rathmann *et al*., 1996). Recently, Alves *et al*.(2006) confirmed that *IDS* gene has a high mutation rate of splicing site. The study of Lualdi *et al*.(2006) revealed that point mutation of *IDS* gene can activate multiple larvaceous splicing sites, leading to mis-splicing RNA. Their results verified again that *IDS* gene had a high new mutation rate and remarkable hot spots. According to the data of *IDS* gene mutations from Chinese patients with Hunter syndrome (Guo *et al*., 2005; 2006; Guo and Du, 2006a; 2006b; Liu *et al*., 2002; Zhang *et al*., 2004; Dou *et al*., 2007), the mutation frequency of exon 7 (which contains the splicing sites) is the highest one, next exon 9, against exons 8 and 4, which is different from the result of overseas. Of course, it is necessary to collect more cases and test more mutations for determining the final result.

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