RESEARCH PAPER

Hunter Syndrome in Northern India: Clinical features and Mutation Spectrum

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Objective: To study the clinical profile and mutation spectrum of Hunter syndrome.

Methods: Evaluation of 18 cases of Hunter syndrome from 17 families was done.

Mutation analysis of Iduronate sulfatase (IDS) gene was done in 9 families, and mothers of four affected children with no family history.

Results: Joint contracture, hepatomegaly and radiological changes were present in all children. 6 (33%) children had normal cognitive function at presentation. Point mutations were identified in all the 9 families for whom mutation analysis was done. Among 4 mothers tested from families without any family history, 2 (50%) were found to be carriers.

Conclusion: Accurate etiological diagnosis by mutation analysis of *IDS* gene is important in Hunter syndrome.

Keywords: *Diagnosis, Iduronate sulfatase gene, Lysosomal storage disorder, Presentation*.

Hunter syndrome (Mucopolysaccharidosis Type II) is an X linked multisystem disorder caused due to deficiency of iduronatesulphatase. Mutation in *IDS* gene is responsible for this disease. No definite phenotypic-genotypic correlation has been established for point mutations in *IDS* gene [1], except for the pathogenic variant c.1122 C>T which is associated with a slowly progressive form of disease [2]. Mutation analysis is essential for diagnosis, carrier testing in females, and in providing prenatal diagnosis. We herein, present data on clinical features of 18 patients with Hunter syndrome from 17 families.

METHODS

Clinical data from suspected Hunter syndrome patients were collected since 2007. The diagnosis was confirmed by enzyme assay of iduronate sulfatase. Informed consent was obtained and blood samples were collected from 9 families in EDTA tubes. DNA was isolated by using QIAamp DNA Mini Kit (Qiagen), which uses silica-membrane-based DNA extraction technology. Primers were designed for all 9 exons of *IDS* gene. In 10 patients from 9 families, PCR amplification of all 9 coding exons was done. Sanger sequencing was done using ABI 310. To look for carrier status in non-familial cases, mutation status was checked in mothers of four affected children, who had no family history. A correlation of the phenotype and genotype was attempted.

RESULTS

Four of the 17 families (24%) had multiple affected members. In a family with two affected siblings, the 12-year-old was more severely affected and had lower IQ than the 8- year-old sibling.

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In majority of patients (78%), the age of presentation was 5 years and above. Hepatomegaly and joint contractures were present in all the patients. Fifteen children (83%) had splenomegaly at presentation and 11 (61%) had umbilical hernia. Intelligence quotient assessment revealed that 6 (33.3%) patients had normal IQ at presentation. Short stature, respiratory symptoms and macrocephaly were present in 11, 8 and 9 patients, respectively. All patients had radiological changes of dysostosis multiplex. Hearing impairment was detected in 3 out 15 patients in whom it could be assessed. Valvular heart disease was observed in two children out of 13 (15%) for whom echocardiography was done. The mean iduronate sulphatase enzyme level was 0.11 nmol/4 hr/mL.

Hemizygous mutations in *IDS* gene were identified in all the 10 patients with Hunter syndrome, including a pair

WHAT THIS STUDY ADDS?

- Four novel mutations in *IDS* gene are reported among 9 families of Hunter syndrome tested by Sanger sequencing of *IDS* gene.

of siblings (***Table* I**). All of them were point mutations. Five out of nine mutations were previously reported as disease causing mutations. The four novel mutations were predicted to be pathogenic by in-silico analysis, by predicting softwares like MutationTaster, SIFT and Polyphen. Mothers of two out of four (50%) children were found to be carriers even without any family history.

A similar mutation c.1454 T>A was identified in two unrelated children. Of the two siblings with the same mutation, c.1327 C>T (R443*) in exon 9, the younger sibling has been started on enzyme replacement therapy (Idursulfase) since last one year. He is getting weekly infusion and does not have any serious reactions till date. His liver has regressed in size by clinical examination, after a year of ERT. His age and gender matched 6-minutes-walk test before ERT was 422 meters (between 10^{th} and 25^{th} centile). After 6 months of ERT it has improved to 578 meters (between 25^{th} and 50^{th} centile for age and sex).

DISCUSSION

Mucopolysaccharidosis Type II or Hunter syndrome is a common lysosomal storage disorder, which is easy to suspect clinically in any male with clinical and radiological features of mucopolysaccharidosis and a clear cornea.

Confirmation of diagnosis by enzyme assay and preferably by mutation analysis is essential not only for genetic counseling, but also for starting Enzyme Replacement Therapy (ERT) which has shown success, especially in those without neurological involvement. One third of the cases in this series had normal cognition which is comparable to Hunter Outcome survey [3]. Our only patient on ERT has shown improved physical activity and a reduction in hepatosplenomegaly, which is similar to what is reported in the literature [9].

Being an X-linked disorder, it is essential to offer carrier screening to female members in extended family on maternal side. For carrier screening and prenatal diagnosis, mutation analysis in *IDS* gene in the proband is essential. More than 300 pathogenic variants have been described in *IDS* gene, the majority of which are point mutations [6]. A great degree of phenotypic variability is observed in Hunter syndrome, but no genotype-phenotype correlation has been identified. Intra-familial variability is rare [8].

High cost of ERT and lack of its efficacy on neurological manifestation stresses the need for genetic counseling and prevention by prenatal diagnosis. Enzyme assay of chorionic villi is used for prenatal diagnosis in the absence of facilities for molecular diagnosis. However, the use of enzyme assay for prenatal diagnosis has limitations and mutation based prenatal diagnosis is more accurate. For carrier detection, enzyme assay cannot be used and mutation analysis is a must.

TABLE I MUTATION SPECTRUM IN *IDS* GENE

Age at diagnosis	*Family history*	*Mutation analysis*	*Type of mutation*	*Novel / reported*	*Carrier status of mothers*
3 yr	Yes	c.1454 T>A	Missense	Known	Not done (obligate carrier)
3 yr	No	c.418+4 C>G	Splice site	Novel	Sample not available at present
3.5 yr	No	c.1047 C>A	Missense	Novel	Carrier
5 yr	No	c.162 T>A	Non sense	Known	Carrier
5 yr & 12 yr sibling	Yes	c.1327 C>T	Non sense	Known	Not done (obligate carrier)
5 yr	No	c.964 C>T	Non sense	Novel	Not carrier
5 yr	No	c.957 C>A	Missense	Novel	Sample not available at present
6 yr	No	c.1019 G>A	Missense	Known	Not carrier
12 yr	Yes	c.1454 T>A	Missense	Known	Sample not available at present

In the era of availability of enzyme-replacement therapy, accurate differentiation of Hunter syndrome from other mucopolysaccharidoses with overlapping clinical features is important. Mutation analysis of *IDS* gene helps in confirmation of the diagnosis and prevention by carrier testing and prenatal diagnosis.

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