Molecular Analyses in Families with Hunter Syndrome Indicate Unequal Male and Female Mutation Rates in the Iduronate-2-Sulfatase Gene

Peining Li*

Department of Genetics, Yale University School of Medicine, New Haven, CT, 06520, USA

*Corresponding author: Peining Li, Department of Genetics, Yale School of Medicine, New Haven, CT, USA, Tel: (203) 758-6317; Fax: (203) 785-7342; E-mail: peining.li@yale.edu

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Abstract

Molecular diagnosis for patients with mucopolysaccharidosis type II (MPS II, Hunter syndrome) has detected a spectrum of mutations including large deletions, intragenic rearrangements, recurrent nucleotide substitutions at CpG sites, and high proportion of novel point mutations in the X-linked iduronate-2-sulfatase (IDS) gene. The mutational heterogeneity and the X-linked inheritance make the IDS gene a good disease model for studying human germine mutations and underlying mechanisms. Pooling data from 135 MPS II families observed that 8% were sporadic cases from de novo maternal germine mutations and 92% were transmitted cases from carrier females. Haplotyping analysis on transmitted cases showed preference of germine mutations from maternal grandfather. Estimation of male-to-female ratio of mutation rates indicated a 3.76-to-10.5-fold higher mutation rate in males for mutations in the IDS gene. However, little is known on gender-specific mutation rate for different type of mutations in the IDS gene. A large-scale population based collaborative study to construct a database of detailed mutational composition and family pedigrees is needed to further characterize the mutation origin and male/female mutation rates in the IDS gene. This information will be helpful in genetic counseling and disease prevention and also gain insight into mutagenesis mechanisms for various types of mutations.

Keywords: Hunter syndrome; Mucopolysaccharidosis Type II; Iduronate 2-Sulfatase; Carrier detection; Haplotype analysis; Mutation origin; Mutation rate

Introduction

Hunter syndrome (mucopolysaccharidoses type II, MPS II, OMIM 309900) is an X-linked lysosomal storage disorder caused by the deficiency of iduronate 2-sulfatase (IDS, EC 3.1.6.13) [1]. The detection of IDS enzyme deficiency has been routinely used for the diagnosis of MPS II [2,3]. Because of the X-linked recessive inheritance, female relatives of MPS II patients are at risk of being carriers of the mutant alleles. In accordance with the Lyon hypothesis, the heterozygous females are observed to have two populations of cells, those enzyme-deficient cells with the active X-chromosome carrying the mutant allele and those with the active X-chromosome expressing the normal allele. The enzyme-deficient cells could achieve metabolic correction by uptake of enzyme from the neighboring normal cells. This “cross-correction” has made it difficult to distinguish the heterozygous cell populations from the normal homozgyotes in carrier detection using enzyme activity analysis. Several biochemical methods have been proposed for MPS II carrier detection. These methods include the cloning analysis of fibroblasts and the measurement of enzyme activity in serum and lymphocyte or fibroblast extracts [4-6]. However, the practical application of these methods has been limited due to factors such as reliability, technical difficulty, time consuming and expense. According to the studies done by Zlotogora and Bach [7] and Schorder et al. [8], about 10% false positives or false negatives can be assumed for carrier detection performed with enzymatic methods.

The characterization of the IDS cDNA sequence [9] and its genomic structure [10] has made possible the exploration of molecular defects in the IDS gene [11]. To date, the disease-causing genetic defects detected in the IDS gene comprise large deletions encompassing neighboring genes, intragenic partial deletions and rearrangements, recurrent nucleotide substitutions at CpG hotspots, and a high proportion of novel point mutations private to the affected probands and families [12-27]. This mutational heterogeneity has made the identification of the “private mutation” in a proband a prerequisite for carrier detection using molecular methods. Once the mutation in a particular family is known, carrier detection in female relatives is accurate, relatively simple, and inexpensive. Several molecular approaches have been used in carrier detection for the IDS gene. These approaches include the Southern blot hybridization analysis using the IDS cDNA probe [18,28], a modified primer extension method [29], and restriction enzyme digestion assays [15,22,30]. Because of the high proportion of novel mutations and a spectrum of various types of mutations involved, MPS II families represent a good disease model for analyzing the male-to-female ratio of mutation rates and mutagenesis mechanisms underlying various types of germine mutations. This report outlines Haldane’s initial approach to estimate mutation rate and a modified formula to estimate male-to-female ratio of mutation rates in X-linked genes, reviews MPS II case series to update the mutational profile for the IDS gene, and reveals unequal male and female mutation rate from carrier genotyping and haplotyping of MPS II families. The identification of mutations and the detection of carriers not only provide valuable information for genetic counseling and clinical management, but also gain significant insight for mutagenesis mechanisms and mutation rates in the IDS gene during male and female gametogenesis.
Haldane's method to estimate human mutation rate

Haldane JBS proposed an indirect method to estimate mutation rate on an X-linked gene based on the assumed equilibrium balance between arising new mutations and reproductive loss of affected cases [31]. The pedigrees of X-linked recessive condition like Hunter syndrome, in which affected males do not reproduce, present sporadic cases resulted from the maternal de novo germline mutations (Type S, Figure 1) and transmitted cases with heterozygous mother inherited the mutation from heterozygous grandmother or from de novo germline mutation of a grandparent (Type T, Figure 1) [32]. The proportion of sporadic cases and transmitted cases is determined by mutation rates μ and ν for female and male germ cells, respectively.

Figure 1: The sporadic cases (type S) and transmitted cases (type T) for MPS II families. The proportion of type S and type T and the probability of maternal grandfather germline mutation are a function of ν/μ ratio. (ν, male mutation rate, μ, female mutation rate, adapted from Refs [31,32]).

The proportion of observed sporadic cases is: \( s = \frac{\mu}{2\mu + \nu} = 1/(2 + \nu/\mu) \). The proportion of transmitted cases is: \( t = (1-s) = \frac{(\mu+\nu)}{2(\mu+\nu)} = \frac{1}{2 + \nu/\mu} \). While within the transmitted cases, the probability of cases with mutation originated from maternal grandfather (MGF) is: \( P_{mgf} = \frac{\nu}{4\mu + 2\nu} \).

Molecular Analyses on MPS II Case Series

Spectrum of mutations in the IDS gene

To update the spectrum of mutations in the IDS gene, a literature review of molecular analyses of MPS II case series (n>10) found 16 reports with patients from North America, Europe, Asian and Latin America populations [12-27]. Pooling together, there were a total of 519 MPS II cases with defined mutations in the IDS genes. Even though the relative frequencies of various types of mutations showed regional variations, a similar pattern of mutational heterogeneity was observed in different populations. On average, approximately 3% (ranging 3%-8%) of the MPS II patients were caused by gross IDS gene deletions. Approximately 13% (3%-37%, with Chinese and Japanese data showing 27% and 37%, respectively) were caused by partial deletions and IDS-2 induced rearrangements. The remaining 84% (63-95%) were caused by small nucleotide substitutions, deletions, insertions and mutations affecting mRNA splicing (Table 1).

The mutational heterogeneity of the IDS gene indicated that different mutagenesis mechanisms involve in different type of mutations with different mutation rates in the IDS gene. For example, ‘hotspot’ mutations at codons R88, S333L, R443, R468 and G374 (c. 1122C>T, splicing out 20 amino acids) all occur at a CpG site as a transitional event and were found in different populations [12-27,35]. It was estimated that 35% of point mutations in the IDS gene are found at CpG sites; methylation profiling noted CpG hypomethylation at the R88 codon but hypermethylation at the S333L, R443, R468 and G374 codons [36]. Many other small lesions are reported as novel or ‘private’ mutations and likely caused by recent germline mutations. Because of the presence of the IDS2 pseudogene 20 Kb distal to the IDS gene, recombination events between the homologous sequences can result in intragenic deletion and inversions [13,37]. Large deletions including the entire IDS gene, the proximal FMR2 gene and distal genes are usually associated with severe phenotypes [38]. The presence of unique IDS-IDS2 genomic structure and a spectrum of heterogeneous mutations in the IDS gene made it an ideal model to study mutation origin and male-to-female ratio of mutation rates for different types of mutations.
cases were from non-carrier mothers, thus suggesting a small deficit of de novo mutations. Among 12 Jewish families with Hunter patients in Israel, 10 families were transmitted cases and two may be from de novo mutation [7]. Results of carrier detection by biochemical tests and genmic RFLP analysis in 10 families with German origin found that seven families were informative as transmitted cases while three families were not informative [8]. Although the biochemical analysis and indirect molecular testing may be biased due to a relatively high false positive and false negative rate, the above testing results suggested approximately 20% cases in MPS II families were sporadic cases. BIOCHEMICAL ASSAY AND INDIRECT MOLECULAR TESTING IN FAMILIES OF GERM-CELL ORIGIN

Table 1: Mutational spectrum in the IDS gene and carrier detection in the MPS II families

**Estimate of male-to-female mutation ratio by carrier detection**

For an X-linked recessive disorder, in which affected males do not reproduce and the mutation rates in egg and sperm are assumed to be equal, it could be expected that 1/3 of cases were the result of de novo mutations and 2/3 were transmitted mutations inherited from a carrier mother. Skewed ratio in family studies is indicative of unequal male and female mutation rates. Different mutation rates in males and females have been shown in several X-linked genes. For example, a study on X-linked factor VIII gene observed approximately 18% of hemophilia patients caused by de novo maternal mutations. Point mutations showed a 5-to-10-fold higher and inversions a >10-fold higher mutation rate in male germ cells, whereas the deletions showed a >5-fold-higher mutation rate in female germ cells [34].

Carrier detection conducted by Chase et al. [5] using hair root enzyme assay in 31 MPS II families found that 23% (7 out of 31) of the cases were from non-carrier mothers, thus suggesting a small deficit of de novo mutations. Among 12 Jewish families with Hunter patients in
could be affected by factors such as sample size, ascertainment bias for sporadic and transmitted cases, population differences, various mutation types, and type-specific mutation rate. It is also worth to point out that carrier detection information were found in only 26% (135/519, Table 1) of cases reported from the case series. Some reports provided partial information [22] and some did not include the familial data [20-23,25,26]. It is strongly recommended that familial data should be included as a confirmitory for mutations detected in the probands and an inference for mutation origin and segregation.

Tracking grand-paternal origin of mutations by haplotyping and genotyping

Sporadic cases of MPS II provide direct estimation of female germline mutations in the IDS gene, while transmitted cases originated from the MGF represent male germline mutations. Haplotyping methods have been introduced to track the grandparental origin of IDS gene mutations in the female carriers [15,22,35]. To construct haplotypes flanking the IDS locus, four dinucleotide repeat markers, DXS1193, DXS1123, DXS1113 and DXS8011, two trinucleotide repeat markers, FRAXA and FRAXE, and a tetranucleotide repeat marker HPRRTB were selected. According to the public human genome browser (www.genome.ucsc.edu) and genome database (GDB, www.gdb.org), the polymorphic markers HPRRTB (Xq26.2), FRAXA (Xq27.3), FRAXE (Xq28), DXS1193 and DXS1123 are located approximately 14.9 megabase (Mb), 1.6 Mb, 487 kilobase (kb), 188kb and 183 kb proximal to the IDS gene, respectively. Markers DXS1113 and DXS8011 are located 11 kb and 1.2 Mb distal to the IDS gene, respectively. Figure 2A shows a schematic map of the genes HPRRTB, FMRI, FMR2, IDS, and the IDS-2 locus, as well as the selected seven polymorphic markers. All seven markers were readily studied by PCR using flanking oligonucleotide primers [39,40]. Haplotypes can be constructed using a combination of four to seven of the selected polymorphic markers and presented in the order of HPRRTB-FRAXA-FRAXE-DXS1193-DXS1123-(IDS)-DXS1113-(IDS2)-DXS8011.

Haplotyping analysis was performed on 12 families (including 12 probands, carrier mothers, 10 MGMs, two MGFs, and other 26 female and four male relatives) with previously detected IDS enzyme deficiency and gene mutations [13]. In family #122 with a large deletion, the haplotyping result showed loss of heterozygosity in carrier females and MGF germline mutation for the deletion (Figure 2B). The pedigrees of other 11 families with mutations in the IDS gene detected as de novo mutations from spermatogenesis of the presumed normal MGFs. Of the 12 families, nine mutations were inferred as de novo mutations from spermatogenesis of MGFs. The high proportion of MGF germline mutations in the transmitted cases strongly argues against equal male and female mutation rate and infers a higher mutation rate in the IDS gene during male spermatogenesis than female oogenesis. Among the tested nine sibs of the affected probands from the 12 families, one normal male, three normal females, and five carrier females were noted, suggesting the possibility of a deficit of male offspring from carrier mothers. Froissart et al. found that mutations occurred preferentially in the grandfather’s gamete in 9 out of 10 families [15]. MGF origin of IDS mutation was reported in one family in the Taiwan case series [22]. Rathmann et al. studied 21 families with maternal grandparents and found 10 maternal grandmothers were carriers, 10 mutations originated from MGF and one uninformative case; the lower limit of k compatible with the observed data would be 3.76 [35]. The probability of cases with mutation originated from MGF (Fmgf) should not excel 0.5 for the estimation of male-to-female ratio (Figure 1). Case ascertainment bias and the small sample size are the likely explanations for skewed MGF cases. The calculation from the collected 135 familial cases and Rathmann’s estimation indicated a 3.76-to-10.5-fold higher mutation rate in males for mutations in the IDS gene. The exclusion of mutant haplotype from the maternal grandmother’s haplotypes and a shared haplotype with different IDS genotypes indicated that the mutations occurred during spermatogenesis of the presumed normal MGFs. Of the 12 families, nine mutations were inferred as de novo mutations from spermatogenesis of MGFs. The high proportion of MGF germline mutations in the transmitted cases strongly argues against equal male and female mutation rate and infers a higher mutation rate in the IDS gene during male spermatogenesis than female oogenesis. Among the tested nine sibs of the affected probands from the 12 families, one normal male, three normal females, and five carrier females were noted, suggesting the possibility of a deficit of male offspring from carrier mothers. Froissart et al. found that mutations occurred preferentially in the grandfather’s gamete in 9 out of 10 families [15]. MGF origin of IDS mutation was reported in one family in the Taiwan case series [22]. Rathmann et al. studied 21 families with maternal grandparents and found 10 maternal grandmothers were carriers, 10 mutations originated from MGF and one uninformative case; the lower limit of k compatible with the observed data would be 3.76 [35]. The probability of cases with mutation originated from MGF (Fmgf) should not excel 0.5 for the estimation of male-to-female ratio (Figure 1). Case ascertainment bias and the small sample size are the likely explanations for skewed MGF cases. The calculation from the collected 135 familial cases and Rathmann’s estimation indicated a 3.76-to-10.5-fold higher mutation rate in males for mutations in the IDS gene.
transmission in other populations. With the accumulation of more pedigrees with defined molecular defects, statistical analysis in larger numbers of MPS II pedigrees could help to answer this question. Further characterization of the mutation rates and transmission preference on various types of IDS mutations could gain important insights into mutagenesis mechanisms of various germline mutations and provide useful information for genetic counseling and disease management. Large-scale population-based collaborative effort to construct confidential database of mutation composition and family pedigrees for estimation of type-specific mutation rates in males and females is needed for MPS II and other X-linked disorders.

Acknowledgements

This manuscript is dedicated to the memory of Jerry N Thompson.

References


Summary and Future Direction

Coupling genotyping with haplotyping analyses are extremely useful for transmitted cases involving three generations but it can also be applied to two-generation family study with the inclusion of maternal uncles and aunts. With the clinical application of exome sequencing, haplotyping could be constructed using SNP alleles [41,42]. The coupling analyses have several advantages. It is obvious that haplotyping analysis can track mutation segregation and infer origin of mutation. Furthermore, haplotyping can define large IDS gene deletions as shown in this report and also analyze female cases with somatic and germline mosaicism [43] and affected female caused by skewed X inactivation [44]. Lastly, haplotyping could be considered as a quality control measure to rule out DNA sample contamination and nonpaternity cases in general molecular diagnostic practice.

Molecular analyses on families with X-linked IDS gene mutation provide not only molecular diagnosis for affected males and carrier females but also insight for mutagenesis mechanisms and gender-specific mutation rates. Collected data from carrier detection indicated a higher mutation rate in male than female in the IDS gene. However, gender-specific mutation rates for large deletions, IDS2-induced intragenic rearrangements, 'hotspot' mutations and novel mutations could be different due to different mutagenesis mechanisms. Another related question is the pattern of mutant allele transmission within the MPS II families. It has been reported that prenatal selection favoring the transmission of IDS mutant alleles among Jewish MPS II patients [45]. Little is known for the skewing pattern on IDS mutant allele transmission in other populations. With the accumulation of more pedigrees with defined molecular defects, statistical analysis in larger numbers of MPS II pedigrees could help to answer this question. Further characterization of the mutation rates and transmission preference on various types of IDS mutations could gain important insights into mutagenesis mechanisms of various germline mutations and provide useful information for genetic counseling and disease management. Large-scale population-based collaborative effort to construct confidential database of mutation composition and family pedigrees for estimation of type-specific mutation rates in males and females is needed for MPS II and other X-linked disorders.