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Research Article

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Mutational Analysis in Gaucher Disease: Implications in Genetic Counseling and Management

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Abstract

Gaucher disease (GD) is the most common LSD worldwide. The disease is caused due to mutations in β-glucocerobrosidase (*GBA)* gene located on chromosome 1. The mutations results in the deficient activity of acid β-glucosidase (glucocerebrosidase) enzyme. It is inherited in an autosomal recessive fashion and both men and women are affected equally. We report here two families wherein the mutation analysis for the disease was performed as the clinical features of the children were suggestive of GD. In the first family the enzyme analysis reports of the children were normal but GD was confirmed upon mutation analysis. In another family who had come for prenatal diagnosis, the parents were confirmed to be heterozygote of normal mutation whereas the foetus was found to be of carrier status. The family had already lost two children who had clinical features suggestive of Gaucher. We conclude that in some cases the enzyme analysis report may not be conclusive and mutation analysis has to be carried out to confirm the disorder. Prenatal diagnosis for lysosomal storage disorders like GD is also recommended among high risk couples.

Keywords

Lysosomal Storage Disorders (LSD); Gaucher Disease (GD); Acid β-glucosidase glucocerebrosidase enzyme

Introduction

Lysosomal storage disorders (LSDs) are a group of more than 50 disorders that arise because of genetic mutations resulting in non-functioning or dysfunctional lysosomal enzymes. Therefore, the enzyme's target molecule remains undigested and starts getting gets accumulated inside the lysosomes leading to cell degeneration. This in turn leads to accumulation of substrates in various tissues and organs affecting the function of these organs, resulting in progressive deterioration in physical as well as mental state and ultimately death. The condition is manifested with broad spectrum depending on the severity of the mutation and specific target molecule [1]. Mode of inheritance in all LSDs is autosomal recessive, except for Fabry disease, Mucopolysaccharidosis II and Danon disease which are inherited as X-linked disorders [2].

The worldwide epidemiological data on LSDs is not available as very few studies have been carried out and the reports are based on selected sub-populations. However, a thirty years long study has reported the total LSD incidence of 16 per 100 000 births with the incidence of an individual disorder ranging from to 0.05 per 100 000 for mucolipidosis II and Danon disease to 2.55 per 100,000 Krabbe disease [3].

In India the actual incidence rate of LSDs is not known. However, many isolated studies have been reported from different places and the largest study has been carried out on 1558 patients with clinical suspicion of various LSDs [4]. About 30% of the cases were positive, with sphingolipidoses as the most common subgroup, followed by mucopolysaccharidoses. The incidence of LSDs reported from India is lower in comparison to other parts of the world which might be on account of under diagnosis due to late suspicion or delay in diagnosis [5].

Gaucher disease (GD) is the most common LSD worldwide [6] as well as in India [4,7-9]. The disease is caused due to mutations in β-glucocerobrosidase (*GBA)* gene bearing 11 exons and located on chromosome 1q21. The mutations reflect in the deficient activity of acid β-glucosidase (glucocerebrosidase) enzyme resulting in accumulation of glucosylceramide in different organs [10]. The disease is inherited in an autosomal recessive fashion. The disorder affects men and women in equal numbers and occur in all ethnic groups and with an estimated worldwide incidence of 1/20,000 to 1/2000,000 in general population. However, the disease is more prevalent in Ashkenazi Jews, with a frequency of 1: 450 live births [11].

The clinical phenotype of GD follows a spectrum ranging from severe early-onset to milder late-onset disease [12]. The clinical features include skeletal disorders, enlarged spleen and liver and anemia. Based on the presence/absence of neurological involvement, GD is defined as non-neuronopathic type I (OMIM # 230800), whereas acute neuronopathic features define type II (OMIM # 230900) and subacute neuronopathic features define type III (OMIM # 231000). Type II is usually fatal by 2 years of age whereas Type III may have onset before age of two years with slow progression. Visceral disease in Type III is often more severe than in Type I. Type I is the most common [13] whereas Type III is seen in 5% of patients overall especially in Northern Europe, Egypt and East Asia [14]. A high incidence of type III is found in the Swedish region of Norrbotten and is therefore also referred to as the Norrbottnian type of GB [15].

Glucocerebrosidase acts on the glucocerebroside component of the cell membrane. In normal lysosome, the protein saposin C present's glucocerebroside to GBA which activates the enzyme. Deficiency of the enzyme leads to accumulation of glucosylceramide and other glycolipids in the lysosomes of macrophages, primarily in the spleen, liver, osteoclasts, bone marrow, brain and less often the lungs, skin, kidneys, conjunctivae and heart [16].

We report here two families with children affected with GD in whom mutation analysis was performed. In addition a carrier fetus determined by prenatal mutation analysis and carried to term has also been reported.

Cases

The families under report were selected from the patients attending the Genetic Clinic and Genetic ward of Advanced Paediatric centre, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

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Case 1

A 10 year old male child with his younger sibling, a 3 year old girl child presenting with hepatosplenomegaly and anemia, from Nalanda, Bihar state, were evaluated in the Genetic Ward of the Institute by a qualified pediatrician and clinical characteristics were noted down.

Case 2

The family 2 constituted a consanguineous couple from Ladakh, Jammu and Kashmir who had lost two children at the age of 2 ½ years and 1 ½ years with splenomegaly and anemia and had come for prenatal diagnosis.

Complete hemogram, reticulocyte count and Hb electrophoresis was also performed using the blood samples from the two siblings in family 1, to rule out hemolytic anemia especially thalassemia. Radiographs of the hands and spine were done to look for dysostosis and ultrasonography of the abdomen was performed to see for any renal anomalies. Informed consent was taken from the parents for enzyme and DNA analysis.

Enzyme Analysis

Enzyme analysis was carried out in blood of the affected siblings and parents for GBA. For fetus, the enzyme analysis was carried out in the amniotic fluid for three commoner LSDs namely Gaucher, Niemann Pick or GM1 gangliosidosis. The enzyme analysis was carried out from leucocytes using 4-MU specific synthetic substrate for β-glucosidase using standard protocol [17]. The β-galactosidase was used as the reference enzyme.

Mutation Analysis for Family 1

Targeted mutation analysis

Sample preparation: Genomic DNA was extracted from blood of the 10 years male child and was quantified using a Qubit and 50 ng of DNA was taken for library preparation using a transposon based shearing of the genomic DNA.

Genes evaluated: Nineteen genes namely *AGL, ATP7B, FUCA1, G6PC, G6PC3, GAA, GBA, GBE1, GNPTAB, LYST, MAN2B1, MCOLN1, NEW1, NPC1, PSAP, PYGM, SLC17A5, SLC37A4, SLC37A4, SMPD1* were analysed for mutation detection. These genes have been implicated in the pathogenesis of GD and other related storage disorders.

Target enrichment: The tagged and amplified sample libraries were checked for quality and quantified. Two simultaneous enrichment steps were performed to optimize the pull down of the regions of interest using target specific probes. Target libraries were amplified using limited PCR steps and 6-10 pM were loaded for sequencing.

Sequencing details: Sequencing was performed using Illumina Trusight inherited disease panel, on Illumina NGS platform (Miseq) with the expected data output of 4.0-5 GB. Variations were then analysed and interpreted with available bioinformatics platforms including Strandomics. The variants were filtered using standard methods.

Mutation Analysis for Family 2

Sample preparation

Genomic DNA was extracted from the husband and wife from EDTA blood. Genomic DNA was also extracted from chorionic villus sample (CVS) for prenatal diagnosis in the fetus.

Targeted Mutation analysis

The mutation analysis was performed for four common Gaucher mutations namely N 370S, L444P, 84GG and IVS 2+1 in the *GBA* gene using PCR and RFLP techniques as described previously [18,19].

Results

More than 60 cases of lysosomal storage disorders were identified in the Genetic Clinic and Genetic ward of Advanced Paediatric centre, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India over a period of 8 years (Oct 2007-Sep 2015). Out of these 12 cases were confirmed to be affected by GD through bone marrow and enzyme analysis with or without DNA analysis. The mutation analysis could be carried out in two cases only.

Both the siblings from Family 1, one boy and another girl, had anemia, growth retardation, and hepatosplenomegaly. No evidence of dysmorphism, coarse facies or cherry red spots in fundus were observed. Both of them scored average grades in the school. There was no history of consanguinity. The X-rays did not show any dysostosis multiplex and the complete hemogram with reticulocyte count and red cell indices was not suggestive of hemolytic anemia. The Hb electrophoresis was normal for the family 1 whereas the enzyme analysis report indicated a borderline value of beta-glucosidase enzyme. The girl's enzyme analysis showed a value of 22.7 units which is almost normal (Normal values-22-86 nmol/hr/mg protein). Nineteen genes namely *AGL, ATP7B, FUCA1, G6PC, G6PC3, GAA, GBA, GBE1, GNPTAB, LYST, MAN2B1, MCOLN1, NEW1, NPC1, PSAP, PYGM, SLC17A5, SLC37A4, SLC37A4, SMPD1* were analyzed for mutation analysis. A known homozygous pathogenic variant, 1448T>C or p.Leu483Pro in the *GBA* gene was detected in the boy on mutational analysis.

In the family 2, the two deceased siblings had abdominal distension and enlarged spleen and history of blood transfusion. No detailed investigation reports were available. Both husband and wife were tested by mutation analysis, and showed heterozygosity for L444P mutation in the GBA gene. DNA diagnosis in the fetus also revealed heterozogysity for the same mutation indicating a carrier status. Examination of the child on follow up was normal.

Discussion

GD an autosomal recessive disorder is the commonest LSD in India and worldwide. More than 300 mutations have been reported in GBA gene [20]. These include missense and nonsense mutations, small insertions or deletions that lead to frameshifts or in-frame alterations, splice junction mutations, and complex alleles carrying two or more mutations in cis [10]. Majority of these mutations are single nucleotide substitutions. The mutations resulting from N370S and L444P substitutions account for approximately 70% of mutations in non-Ashkenazi European patients [21]. Two cases of type 1 non-neuropathic and type 3 juvenile subacute neuropathic variant of adult Gaucher disease have been reported in two of three siblings in a family from India [22]. A study had identified two novel missense mutations G289A (c.866G>C) and I466S (c.1397T>G) in exons 7 and 10, respectively in 6.06% GD destabilize the protein structure [23]. L444P (c.1448T>C) was found to be the most common mutation in non-neuronopathic and sub-acute neuronopathic form. Other nine rare mutations: R463C (c.1504C>T), R395C (c.1300C>T), R359Q (c.1193G>A), G355D (c.1181G>A), V352M (c.1171G>A) and S356F (c.1184C>T) were also observed. Compound heterozygous mutation L444P (c.1448T>C)/R496C (c.1603C>T) in exon 10/11 and L444P

(c.1448T>C)/R329C (c.1102C>T) were observed in exon 10/8 in one each patient (6.06%). The study concluded that L444P is the most common mutant allele with exons 8 and 10 as the hot spot region of GBA gene observed in Indian GD patients [23]. Another study identified twenty two targeted mutations F251L,C342G, W312C, P415R, R463C, D127V, A309V, G46E, G202E,P391L, Y363C, Y205C, W378C, I402T, S366R, F397S, Y418C, P401L,G195E, W184R, R48W, and T43R using seven different algorithms [24].

DNA analysis is used in combination with the enzyme assay test to diagnose GB and is helpful in defining the subtype. Genetic mutation analysis highlights the most common and least common, genetic mutations usually associated with GD and aids in classifying which type of Gaucher a person has. Neither disease type nor severity of disease is defined by enzyme assay.

Individuals affected with GD have been reported with low β-glucosidase activity in leukocytes and/or fibroblasts. The value of enzyme beta-glucosidase has been found to vary from 0.65 nmol/hr/mg to 2.5 nmol/hr/mg in these patients [25]. In family 1 in the present report, the enzyme analysis did not reveal enzyme deficiency but the clinical features were consistent with GD in both siblings. There was no dysostosis on Xrays, no cherry red spots on fundus evaluation and no evidence suggesting hemolytic anemia.

To the best of our knowledge this is the first report of GD where the enzyme activity level has been found to be almost normal. Previous reports have shown that small pharmacological chaperone isofagomine (IFG) binds and stabilizes L444P GCase resulting in increased Lysosomal trafficking and cellular activity. The oral administration of IFG resulted in significant increase in the GCase activity in disease relevant tissue including brain [26]. There might be some natural chaperons available which might be responsible for normal levels of the enzyme. However, information on the natural chaperons in this regard is missing.

In addition to this, presence of near value of enzyme in GD also might be on account of modifier genes. Based on the expression studies, certain variants of the GBA gene like c.1093G>A (p.E326K) and c.680A>G (p.N188S) should be considered as "modifier variants" rather than a neutral polymorphism and mild mutation as reported previously [27]. The authors suggested that the modifier variants may be non-pathogenic, on their own, but have an additive effect, making a pathogenic double mutant. Recently in a summary on the current knowledge regarding the incidence, cause and mechanisms interplaying in GD, conclusion has been made that although several mutations have been identified to be responsible for GD, the mechanisms by which the defects in glucocerebrosidase enzyme lead to GD remain poorly understood [11].

Since, in this family the enzyme levels were found to be nearly normal. We went ahead with mutation analysis which revealed a known pathogenic 1448 T>C mutation. The homozygous missense variant, p.Leu483Pro, detected in family 1 in this report alters a conserved amino acid is predicted to be damaging by four out of five in silico predictors (Mutation Taster, SIFT, Polyphen 2 HDIV and Polyphen 2 HVAR). The variant p.Leu483Pro is one of common variants of GBA to be associated with type I, II and III GD. The homozygous variant is reported to be more often associated with neuronopathic form of GD.

In family 2, who had lost two children on account of suspected GD, both husband and wife were found to be heterozygous for L444P mutation. DNA analysis of the fetus revealed heterogygosity of the same mutation. The child was found to be normal on follow up examination. The L444P mutation found in this family is a variant which is associated more commonly with type 3 GD. Therefore, prenatal diagnosis and counselling helped the parents to have a normal child.

Gene therapy for the treatment of LSDs is emerging as a promising tool for the treatment in near future [28]. Although, Enzyme Replacement Therapy (EPR) is approved worldwide for 6 LSDs namely GD, Fabry disease, Mucopolysaccharidosis types I, II, and VI, and Pompe disease, the therapy with infused protein is life-long and disease progression is still observed in treated patients [29]. An earlier report of Type 3 GD showed R463C/Rec Nci I mutation [30]. Type 3 GD is more severe and requires high doses of enzyme replacement therapy (ERT).

Since there is higher incidence of consanguinity/inbreeding in certain communities in India, some mutations especially with Type 3 Gaucher are detected. Therefore, the focus must be on early diagnosis and early treatment for the beneficial clinical outcomes. This would require a high index of suspicion. Few mutations are more common in Southern part of the subcontinent like D309H mutation, associated with cardiac involvement. In case the enzyme analysis is not conclusive, mutation analysis should be performed if the phenotype is clinically consistent with GD.

Since the LSDs including GD have no treatment available or the cost of treatment is prohibitive for most of the patients thereby making prenatal diagnosis an important tool to prevent its occurrence. Prenatal diagnosis of LSDs is possible using variety of samples like cultured chorioinic villi, uncultured chorionic villi and amniotic fluid [31]. Uncultured chorionic villi have been recommended for reliable prenatal biochemical diagnosis of nineteen LSDs [32]. A greater awareness of LSDs may help to reduce misdiagnosis and promote the early detection of LSDs [33]. Early diagnosis may be important for timely initiation of treatment to prevent disease complications.

Conclusion

In conclusion the enzyme analysis which is considered as the gold standard for diagnosing the GD may not give the correct diagnosis in few cases. Therefore, mutation analysis must be carried out to confirm the disease. The identification of different genetic modifiers by which they effect the phenotypic variability in GD might help us in understanding the mechanisms by which they effect the phenotypic variability. Identification of mutant allele is helpful in facilitating genetic counseling in high risk couples. Further, prenatal diagnosis for lysosomal storage disorders like GD is also recommended in selected families.

Conflict of Interest

The authors Inusha Panigrahi, Jaswinder Kalra, Prasoon Goyad, Preeti Khetarpal and Anjana Munshi declare no conflict of interest. Informed consent was obtained from all individual participants included in the study.

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