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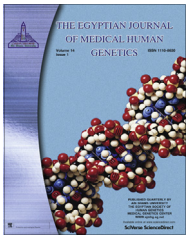

ORIGINAL ARTICLE

Prenatal genotyping of Gaucher disease in Egypt

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KEYWORDS

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Abstract *Objective:* To use chorionic villi sampling (CVS) and amniocentesis to determine the genotyping of Gaucher Disease (GD) of fetuses of pregnant mothers who had a previous child affected by GD.

Methods: The study was conducted between January 2009 and December 2012. It included 42 pregnant women that gave informed written consent. Thirty mothers presented early so they underwent CVS at 10–12 weeks of pregnancy while 12 mothers presented later and underwent amniocentesis at 14–16 weeks. Strip assay for the identification of Glucocerebrosidase (GBA) gene mutations in the samples of chrorionic villi and amniotic fluid was based on polymerase chain reaction (PCR) and reverse hybridization.

Results: The age of the studied pregnant women ranged from 19 to 26 years. Consanguinity was present in 38 cases. Eighteen women were pregnant in affected fetuses. The results of genotyping revealed 15 cases were homozygous L444P/L444P and one case homozygous (N370s/N370s) while two cases were heterogeneous (L444P/D409H). Twenty-four pregnant women had carrier fetuses which were all heterozygous L444P.

Conclusion: This study highlights the findings of an extended gene mutation examination for prenatal diagnosis of Guacher Disease. The study found out that the most common mutation was L444P/L444P.

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1. Introduction

Gaucher disease (GD) is a rare disease but is the commonest enzyme deficiency disorder among the group of inherited lysosomal storage diseases [1]. It is caused by a deficiency in glucocerebrosidase, a lysosomal hydrolase involved in the stepwise degradation of complex glycosphingolipids, or in rare cases,

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by a deficiency in the activator protein saposin C [2]. GD is classified based on clinical characteristics into three types: Type 1 is a chronic non neuropathic form involving viscera and blood forming tissues especially in Jewish population. Type II is the infantile neuropathic form which always appears by 6 months of age; it is rarer than type I and does not have predominance in Jewish population, most patients die before 2 years of life. Type III is the chronic neuropathic form which includes a heterogeneous group of patients with signs of slowly progressive neurological disease that begins during childhood or adolescence [3].

The diagnosis of GD is based on the assay of glucocerebrosidase activity in peripheral blood leucocytes or cultured fibroblast that is significantly lower than normal (< 30%) [4]. More than 300 mutant alleles have been identified. The presence of at least one N370s allele is associated with Type 1 disease and usually excludes neuropathic involvement [5] while individuals who were homozygous for the L444P mutation tend to have severe disease, with neurological complications. This mutation results in an unstable enzyme with little or no residual activity. In a study of 31 individuals with neurological complications, L444P accounted for 25 alleles (40%) [6]. Mutation analysis is the method of choice for the identification of Gaucher disease carriers. Carrier detection via enzymatic assay is unreliable because approximately 20% of obligate carriers demonstrate normal Glucocerebrosidase activity (false negative) i.e. an individual with normal enzyme activity but has one copy of any Gaucher disease mutation is an unaffected carrier.

Prenatal diagnosis for GD is now available at many centers. This allows parents to consider termination or prepare for enzyme replacement therapy for the coming baby hoping to change the disease nature course. The identification of carriers can be done by enzyme assay and molecular studies [5]. Carrier and prenatal testing for people with family history of GD should be offered in conjunction with genetic counseling [7].

The aim of this work was to undertake prenatal genotyping diagnosis of Gaucher Disease for Egyptian pregnant mothers who have a child suffering from GD.

2. Patients and methods

The registry of affected families was accessed through the Hematology outpatient clinic of the New Children Hospital (Cairo University) and Neuro-Pediatric Unit at Fayoum University. A GD Registry is now maintained by several University Hospitals in Egypt as part of an informal network of GD researchers. The development of this registry aims to offer to treat affected cases using imiglucerase enzyme replacement in line with the guidelines of the International Gaucher Disease registry. The enzyme replacement therapy is sponsored by the Gaucher Initiative U.S. Expert Medical Committee (US-EMC) in collaboration with Project HOPE (http://www.projecthope.org/) in Egypt. Currently there are around 130 children treated under the project and every year 12–15 new cases are added to the registry. The Fayoum University Research Ethics Committee which is a member of Egyptian Network Research Ethics Committee (ENREC) was informed of this study.

The present study included 42 pregnant women with previous children of GD who were diagnosed by enzyme assay of Beta glucosidase activity in peripheral leucocytes and genotype by molecular diagnosis (PCR). Those women had previously given birth to a child with GD and were currently pregnant with a child at risk of having GD. Mothers had hopes of having unaffected children, or their future children could escape the disease as carriers. After detailed counseling, informed written consent was received from all the studied couples in Arabic. A detailed description of the procedure (amniocentesis or chorionic villus sampling (CVS)) according to the gestational age at presentation, the time interval between the procedure and the availability of the report, and risks (such as fetal or maternal trauma, infection, vaginal bleeding, feto-maternal hemorrhage, or abortion) were explained adequately for couples at risk. All pregnant women were subjected to complete physical and systemic examination. Comprehensive family history including consanguinity, affected siblings and a similar condition in the family was taken.

According to age at presentation, 30 mothers underwent CVS at 10–12 weeks of pregnancy and 12 mothers went for amniocentesis at 14–16 weeks. The participating obstetrician performed detailed abdominal ultrasound of the uterus and pregnancy. None of the cases examined showed uterine or fetal abnormalities detected by ultrasound. The abdomen was cleaned using chlorhexidine solution. No local anesthesia and the freehand technique were used for both procedures. An obstetrician and an assistant controlling the scanning transducer performed the procedure.

For the CVS a 22 gauge single needle was used and passed through the placenta in order to aspirate chorionic tissue. The aspirated chorionic tissue was examined under microscopy to exclude maternal contamination. The aspirated tissue was then transported in Ham F-10 media to the laboratory. For the amniocentesis, a 22 gauge single needle was passed under ultrasound to a large pocket of amniotic fluid away from the fetus. A syringe was used to aspirate first 1 cc and after confirming there was no maternal blood contamination 15–20 cc of amniotic fluid was aspirated and sent for analysis. Samples collected were clearly labeled with the women's information and sent for analysis. All women were shown the fetus and fetal heart rate before and after the procedure. All women were asked to report immediately pain, bleeding or any concern. Women returned back after 5–7 days to perform another ultrasound scan follow up. Eight Rh negative women received the Anti-D antibodies after the procedure.

Strip assay for the identification of Glucocerebrosidase (GBA) gene mutations in the samples of chrorionic villi, was based on polymerase chain reaction (PCR) and reverse hybridization. The procedure included three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification products to a test strip containing allele – specific oligonucleotide probes immobilized sequences are detected using streptavidin–alkaline phosphatase and color substrates. The assay covers 8 common GBA mutations: 84GG[452 + G], IVS2 + 1[484G > A], N370S[1226A > G], V394L[1297G > T], D409H[1342G > C], L444P[1448T > C], R463C[1504C > T], R496H[1604G.A], as well as 2 recombinant alleles derived from crossover between the GBA functional gene and pseudogene. (The Vienna Lab Gaucher Disease Strip Assay @4–250 (20 tests/kit) 2007). The same technique for the identification of Glucocerebrosidase (GBA) gene mutations was used for the amniotic fluid sample in amniocentesis which was performed when mothers arrived for prenatal diagnosis at 14–16 weeks which was later than the time for CVS.

Table 1 Frequency of cases and carriers among study group.

Cases/carriers	Frequency/ percentage	Homozygous/ heterozygous
Cases (L444P/L444P)	15	Homozygous
Cases (L444P/D409H)	2	Heterozygous
Cases (N370s/N370s)	1	Homozygous
Carriers (L444P)	24	Heterozygous

Table 2 Gene mutations among the 18 affected fetuses.

	Frequency	Percentage (%)
N370s /N370s	1	5.6
L444P/D409H	2	11
L444P/L444P	15	83.4

Table 3 Allele frequency among the studied fetuses.

Allele	Frequency	Percentage (%)
L444P	41	97.6
N370s	1	2.4
D409H	2	4.8

3. Results

The mean age of the studied pregnant women was 23.6 years and ranged from 19 to 26 years. Positive consanguinity was present in 38 (90.5%) of the studied population, first cousin consanguinity in 30 (71.4%) of them and remote degree consanguinity in 8 (19.1%). Negative consanguinity was present in only 4 (9.5%) of the studied group. The median gestational age when carrying out the procedure was 13.8 weeks (range 10–16 weeks). There were no fetal losses related to the prenatal diagnostic procedure. However, mild bleeding was encountered in two patients during CVS for which they needed rest and observation for few hours and the bleeding stopped spontaneously.

Twenty-four fetuses (57.1%) proved to be carrier (trait) while 18 (42.9%) proved to be affected. Five women out of 18 who were pregnant in affected fetuses decided to terminate their pregnancy, while the remaining women decided to continue the pregnancy according to their religious believes. Babies to mothers that underwent CVS or amniocentesis were born without evidence of complications due to the procedure. Genotype analysis of the studied individuals is shown in Tables 1–3 as well as Figs. 1–3.

4. Discussion

Gaucher disease is the most prevalent lysosomal storage diseases which results from inherited deficiency in the glucocerebrosidase enzyme.

Previous molecular studies of the disease mutations revealed that although more than 50 mutations were identified in the glucocerebrosidase gene, only four of them frequently occurred. These four common mutations are L444P,D409H, N370s and IVS2 + 1, representing 90–95% of the mutations GD in the Ashkenazi Jewish population, and 50–75% of mutations in general population [8,9]. Recently, more than 200 mutations have been found in patients with GD, and certain mutations have a higher frequency in certain populations. El morsy and co-workers (2011) found that type II and III GD patients had Rec alleles/L444P genotypes (100%) with a poor phenotype/genotype correlation in Egyptian population [10].

In the current study the IVS2 + 1 mutation was not involved in our molecular diagnosis, which is in agreement with Koprivica and co-workers who reported that IVS2 + 1 mutation accounted for 1% only in non Ashkenazi Jewish [6]. Tsujii in 1987 [11] reported that L444P mutation occurs more frequently in non Jewish population, moreover, El-Beshlawy with her colleagues in 2006 [12] found that 14 of 22 patients (63.6%) were accounting for L444p mutation as homozygous or heterozygous with D409H mutations. In the study by Khalifa and co-workers in 2011, molecular analysis was performed over 23 patients, and the commonest genotype was homozygous L444P which was present in 13 patients (56.5%) in their study followed by homozygous N370S; found in three patients (13.04%) [13]. The percentage of occurrence of

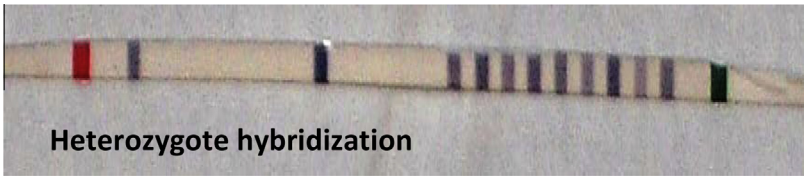

Figure 1 Strip showing heterozygote hybridization.

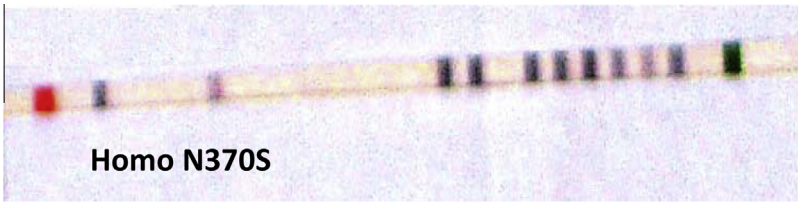

Figure 2 Strip showing N370/N370 mutation.

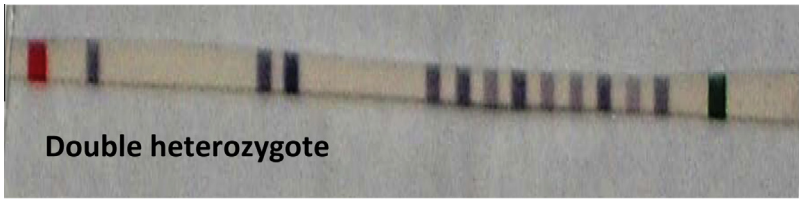

Figure 3 Strip showing a double heterozygote L444P/D409H mutation.

L444p mutation in this current study was exceeding 90% either homozygous or compound heterozygous with D409H mutations and N370s /N370s (homozygous) was found in only one patient (5.6%).

Since most of Egyptian children with Gaucher disease have type III disease and L444P/L444P genotype, a minimum dose of recombinant enzyme imiglucerase (cerezyme) 60 U/kg/2 weeks should be maintained until adulthood and higher doses started at an early age may delay the progression of neurological symptoms [13]. These previous results drove our attention to the importance of what we found as the most common mutations encountered in the current study which were L444p/L444p (homozygous) in 15 cases out of 18 affected fetuses (83.4%), followed by L444P/D409H(double heterozygous) in two cases (11%), whereas N370s /N370s (homozygous) was found in only one (5.6%). This highlights the importance of early enzyme replacement therapy for the coming baby to improve the disease nature course and delay the progression.

All cases with the genotypes L444p/L444p and N370S/N370S were from consanguineous parents i.e. 15 cases of 15 and one case of 1 respectively. Although 24 cases were carrier for GD with heterozygous L444p mutation, only four cases had negative consanguinity. So this study confirmed that about 100% of our affected cases were from consanguineous pedigree while 16.6% of carrier state was from non consanguineous marriage. These results exceeded what was reported by El-Beshlawy with her colleagues in 2006 who found that two thirds of their patients were from consanguineous marriage [12], while El-Gawhary in 1999 revealed that 80% of cases were from consanguineous parents and 30% with affected sibilings [14]. In another study, of El-Gawhary and co-authors stressed the significance of premarital as well as pre-natal counseling of the disease for families with affected children [15]. New developments which will allow for preimplantation genetic diagnosis for those women that can afford to undergo intra cytoplasmic sperm injection (ICSI) will allow couples to avoid having children with GD and the need to undergo prenatal testing.

5. Conclusions

The high frequency of positive consanguinity and the mode of inheritance drive the attention to the importance of genetic counseling and pre-natal diagnosis of the GD among high risk cases in Egypt. The most common mutation found in our study was L444p/L444p in the affected fetuses that may need a higher dose of enzyme replacement starting at an early age of life to delay the progression of possible neurological symptoms. The low acceptance rate to termination of pregnancy highlighted the need for one of the earlier preventive approaches such as preimplantation genetic diagnosis for those women that can afford to undergo intra cytoplasmic sperm injection (ICSI).

Conflicts of interest

The first author has been involved with the Gaucher Disease Initiative implemented by the Project HOPE and supported by Genzyme Corporation (a Sanofi Company) for around 12 years. The second two authors are Principal GD Researchers at their Universities which is affiliated to the informal network of GD researchers supported by Project HOPE. All authors have not received any funding to conduct this study and declare no conflict of interest with regards to this research.

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