Ilkogretim Online - Elementary Education Online, 2021; Vol 20 (Issue 6): pp. 501-508
http://ilkogretim-online.org
doi: 10.17051/ilkonline.2021.06.053

Molecular and Genetic Analysis of Selected Exons of GBA gene in Pakistani Families affected with Gaucher's Disease

Faiza Sattar, Department of Biosciences, University Of Wah.
Tayyaba Shan, Department of Biosciences, University Of Wah, Department Of Zoology, Quaid e Azam University.
Razia Virk, Department of Biosciences, University Of Wah.
Syed Waqas Hassan, Department Of Biosciences, University Of Wah. Waqas.Hassan@uow.edu.pk
Bushra Gul, Department of Biosciences, University Of Wah, Department Of Zoology, Quaid e Azam University.
bushra.gul@uow.edu.pk

Abstract- Gaucher disease, the most prevalent lysosomal storage disease and transmitted as an autosomal recessive trait results from an inherited deficiency in the enzyme glucocerebrosidase. The enzyme substrate glucocerebroside accumulate in the patient's spleen, liver and bone marrow. The resultant accumulation in several tissues and organs leads to numerous manifestations like anemia, hepatosplenomegaly, thrombocytopenia, growth retardation and skeletal disease. Three clinical forms of Gaucher disease have been described: Type 1 non-neuronopathic, Type 2 acute neuronopathic, and Type 3 subacute neuronopathic. The gene responsible for glucocerebrosidase is GBA, located on chromosome lq21. There are 11 exon and 10 introns are present in the gene. The exon 9 and 10 is the hot spot exon of said disease in Asia and also reported in worldwide. The current study was about on exon 10 because its most common exon. In current study, 7 families were involved for sequencing and molecular analysis. For sequencing, genomic DNA extraction was done by the phenol- chloroform method and PCR reaction were done. Sequencing of 7 patients revealed 12 different variants including 5 polymorphisms (Homo/hetero). 3 variants out of these 5 were non-synonymous. The other 7 variants were predicted to be disease causing mutation by mutation taster. 4 out of 7 mutations were non-synonymous, while 3 were synonymous. 6 novel mutations were found, 02 of them were homozygous and 04 are heterozygous mutations, and one already reported mutation c.1152G>A with rs12747811. While we found 4 novel polymorphisms, 02 heterozygous and 02 homozygous polymorphisms and one of them was already reported polymorphism that is chr1:155205170A>G having rs143255568. This shows comprehensive picture of molecular properties of GBA gene and related phenotypic variations in the patients. We need further evaluation of remaining exons in order to determine the carrier ratio of Gaucher disease in our population or whether we should screen our neonates for such lysosomal disorders.

Keywords: lysosomal storage disease, Gaucher disease glucocerebrosidase, polymorphism, mutation

I. INTRODUCTION

Gaucher disease (GD) is a rare genetic and autosomal recessive disorder. It is caused by deficiency of the lysosomal hydrolase glucocerebrosidase (GBA) (EC 3.2.1.45; Brady et al., 1965), required for the degradation of glycosphingolipids (Thomas et al., 2014).
Gaucher disease is the most prevalent disease among lysosomal storage disease. The prevalence rate is roughly 1 out of 100,000. The occurrence in everybody is around 1 out 60,000 individuals each year, yet it can reach up to 1 out of 1,000 people (Martin-Banderas et al., 2016).
Gaucher disease was first portrayed in 1882 by Phillipe Gaucher. As a clinical understudy he analyzed a 32-year-old female and described of an enlarged spleen and resulting tiny examination uncovered engorged cells, at first idea to be dangerous (Burrow et al., 2011).
The sign and symptoms of disease are hepatosplenomegaly, anemia, thrombocytopenia, growth retardation, low level of enzyme (Gcas) activity, consanguinity, bone crisis and sometimes neurological issues.
In Gaucher disease, storage fundamentally happens all through the reticulo-endothelial framework. Lysosomes inside macrophages become stimulated with the put away glycolipid, offering ascend to ''Gaucher cells,'' macrophages with an indicatively trademark appearance. By light microscopy, they are PAS positive, have dislodged cores and a cytoplasm that has been portrayed as taking after ''wrinkled tissue paper.'' Electron microscopy uncovers wound, prolonged, rounded constructions that absolutely damage the lysosome (Sidransky 2004).

Relationship of Gaucher disease with other organs:
The Gaucher disease is a heterogenetic, rare autosomal recessive disorder. The fatty molecules are accumulated in different organ of body because of less activity of GCas activity. The glucocerebroside is unable to break down into glucose and ceramide.

Splenomegaly:
Splenomegaly is a hallmark in the clinical presentation of this disease. During physical examination splenomegaly is present in all affected patients while normal size spleens can be present in asymptomatic patients. The degree of splenic enlargement is highly variable. The size of spleen become 5- 25 times larger as compare to the normal one depends on the weight of body. (Nussbaum et al., 2004).
Hepatomegaly:
Hepatomegaly is by and large surrendered to be characterized as liver mass is 1.25 occasions higher than assessed ordinary volume which is 2.5% of all out-body weight and has been portrayed as a typical finding in patients with GD. By the by, hepatomegaly is generally less enormous than GD splenomegaly (Adar et al., 2018). The pace of gallstones in GD1 is 35%, that is multiple times higher than in everyone (Stirnemann et al., 2017).
Bone manifestation:
Bone signs in GD, which absolutely show up in pre-adulthood, are changed and can incorporate osteonecrosis, bone agony emergencies, lytic sores, osteoporosis (across the life expectancy), obsessive breaks. Diminishes in bone thickness during this season of bone development prompted a decline in generally speaking pinnacle bone mass. Indeed, even in asymptomatic patients with typical bone marrow thickness, there might be hidden interruption of the bone's trabecular engineering, causing a lessening in bone soundness (Marcucci et al., 2014).
Growth retardation (GR):
GR is most common in the pediatric non-neuronopathic GD population. (Kaplan et al., 2006).GR usually is contrary with clinical, x-ray or MRI evidence of bony disease. Such growth retardation may represent a 'bioassay' or 'biomarker' of the extent of involvement. (Grabowski et al., 2005). Growth retardation most likely reflects overall metabolic derangement of Gaucher disease and the total disease burden. Delayed puberty is a less well documented disruption of development. Major abnormalities in growth hormone, other growth promoting hormones or the pituitary are rare (Pastores et al., 2004).
Thrombocytopenia:
Thrombocytopenia is a condition in which platelet number becomes less and prevent the clotting of blood. It is noticeable anomaly in GD1 and is often the first or sole hematological indication of the problem. The platelet tally has demonstrated to be a touchy marker of generally speaking treatment responsiveness (Hollak et al., 2012).
Anemia:
Low level of blood in a body termed as anemia condition. It might result from hypersplenism, assumption condition, iron inadequacy or B12 insufficiency and in cutting edge infection, diminished erythropoiesis because of bone marrow disappointment from Gaucher cell invasion or renal issues (Pastores & Hughes 2018).
Nervous system:
Non-neuronopathic GD is the most prevailing structure (94%) and is recognized from the intense neuronopathic (1%) and ongoing neuronopathic (5%) structures by the nonappearance or presence of focal sensory system CNS inclusion. Type 1 GD is nonneuropath while type2 and type3 is neurological structure (Adar et al., 2018).
Neurological appearances are a sign of GD3 and can introduce in youth, puberty, or even adulthood. The most widely recognized appearance is flat supranuclear look paralysis, and in certain people, this is the lone neurological indication. Other neurological effect includes strasbismus, incoordination, mental deterioration, and myoclonic seizures. Sever neuro effect leads toward the Parkinson disease, dementia and other genetic disorder (Gary et al., 2018).
Types
Usually, Gaucher disease has been comprehensively characterized into three principal structures dependent on the absence (type I) or presence and seriousness of neurological manifestations (type II and type III).
The most widely known type of the disease, Gaucher type I, doesn't include the sensory system and shows with enlarged liver and spleen and irregular bone marrow, prompting weakness, thrombocytopenia and skeletal changes. GD type I can have adolescence or grown-up beginning and get by as long as 80 years (Bongarzone et al., 2012).
GD type II is portrayed as the intense neuronopathic type of Gaucher infection. The beginning of the disease is in the neonatal period and death happens by age two to four years. In any case, a few instances of perinatal-deadly Gaucher disease related with hepatosplenomegaly, skin anomalies and intrauterine death have been accounted for (Weiss et al., 2015). Gaucher disease type II presents a similar instinctive indication of type I, with huge hepatosplenomegaly and aspiratory contribution (pulmonary effect). Patients create

ichthyosis, going from mellow skin stripping to the "colloid infant" aggregate (Roshan et al., 2017). The most punctual neurological side effects are strabismus and flat look paralysis, hypertonic acting and retroflexion of the head (Pastores & Hughes 2015).

Type 3 (subacute and persistent neuronopathic) is described by a later beginning of neurological side effects, which incorporate strabismus, incoordination, mental disintegration, and myoclonic seizures. There is a variable level of hepatosplenomegaly and skeletal inclusion. Demise happens in youth. Although this sort of the infection is uncommon and panethnic (Davies et al., 2007).

Genetics of Gaucher Disease:

GD is a rare, autosomal, recessive genetic disease achieved by mutation in the GBA gene, located on chromosome 1 (1q21). This prompts an especially decline action of the lysosomal enzyme, glucocerebrosidase (GCase, in like manner called glucosylceramidase or acid β-glucosidase, EC: 4.2.1.25), which hydrolyses glucosylceramide (GlcCer) into ceramide and glucose.

Structure of GBA Gene:

The GBA gene (OMIM#606463, Human Reference Genome: GRCh37/hg19 Chromosome 1: 155,204,239 to 155,214,653), encoding the lysosomal storage enzyme b-glucosidase (GBA; E.C number 3.2.1.45), have 11 exons and 10 introns spreading over 7.6 kb. It is organized on chromosome 1q21 inside a flighty locus comprising seven gene and two pseudogenes, likely beginning from a repetition occasion of this chromosomal locale (Zampieri et al., 2017). Without a doubt, a particularly homologous 5.7-kb pseudogene (GBAP1; OMIM# 606463; Human Reference Genome: GRCh37/hg19 Chromosome 1: 155,183,616 to 155,197,325) is originate about 16 kb downstream of the respected GBA gene (Martinez-Arias et al., 2001). The exonic district of the GBAP1 gives 96% progression homology to the coding territory of the GBA gene, however the plan homology shows up at 98% in the area between intron 8 and the 30 untranslated regions (Lesage et al., 2011). The GBA gene arrangement is profoundly saved among various species, specifically among human and various types of vertebrates. Unexpectedly, the GBAP pseuodogene is just present in primates (Chenna et al., 2003)

GBA Gene Mutation:

G More than 250 pathogenic changes have been accounted for the GBA gene including missense, garbage, inclusion/cancellations, graft intersection variations and complex alleles conveying at least two transformations coming about because of quality recombination or transformation with the pseudogene. Various aggregates of GD can prompt various alterations. The N370S alteration is connected uniquely with Gaucher's infection type 1 and it appear as though to be defensive for the improvement of the neurological submersion normal for GD type 2 and 3. Without a doubt, patients who present the N370S alteration on at any rate one allele of the GBA gene will show just GD type 1. (Lepe-Balsalobre et al., 2020). Stimulatingly, subjects who are homozygous for the N370S variation can likewise stay asymptomatic for the infection. Then again, L444P mutation is normally connected with GD type 2 or 3, in any event, while introducing in a compound heterozygous state (Dandana et al., 2016). Homozygous L444P transformation with no recombinant alleles can be related with extremely serious yet in addition milder aggregates. The D409H variation is liable for GD type 3 which gives trademark cardiovascular valve calcifications. N188S, G377S, and V394L are bound to be related with myoclonic epilepsy (Graham et al., 2020).

Diagnosis:

Finding of Gaucher disease is made based on clinical history, actual assessment, lab test and affirmed by a blood test indicating inadequate glucocerebrosidase enzyme and hereditary change examines when the conclusion is suspicious. History of association and family background of suspected or demonstrated GD will uphold the analysis.

Laboratory test:

Thrombocytopenia, sickliness and leucopenia are seen on the blood tallies. Liver catalysts might be raised and coagulation factor insufficiencies causing irregular thickening have been portrayed notwithstanding platelet work issues. Platelet grip imperfection and not conglomeration deserts add to the thrombocytopathy in GD patients and thusly incline to an expanded inclination to dying (Rosenbloom et al, 2011).

Scanning of organs:

Ultrasonography CT examine or preferably MRI sweep of the midsection are utilized to decide liver and spleen volumes. Central collections of Gaucher cells might be found in the liver or spleen. In asset helpless setting like our own MRIs are once in a while performed. Likewise, need for sedation in more youthful kids makes it all the more testing. CT filters have the burden of rehashed radiation and are hence not regularly suggested (Di Rocco et al., 2014).

Prenatal diagnosis:

Pre-birth determination of GD can be performed by hereditary examination, utilizing either amniotic liquid cells or chorionic villus testing, however just if the record case genotype has been recently distinguished. It should likewise be possible by estimating glucocerebrosidase movement on new chorionic villi or refined amniotic cells. Preimplantation hereditary determination is additionally conceivable (Hughes et al., 2007).

Treatment

There are two direct kinds of treatment for GD: enzyme replacement treatment (ERT) and substrate replacement treatment (SRT). The goal is to treat patients prior to the start of setups, the sequelae of which are crippling or not improved by extra treatment, including enormous strong splenomegaly, AVN, assistant osteoarthritis, vertebral pressing factor and various breaks, hepatic fibrosis and lung fibrosis (Martinez-Arias et al., 2001).

Ideally, bone marrow transplantation could fix patients with GD, anyway this treatment isn't, now offered given its low benefit/risk extent and the current availability of convincing, all around suffered medicines (Stirnemann et al., 2017).

Table 1. list of pervious reported exonic mutations of GBA gene

Exon number	Mutation	Reference
1	-	-
2	IVS2+1	(Rosenbloom et a.l, 2011)
3	-	-
4	del55	(Alfonso et al., 1999)
5	R120W	(Rosenbloom et a.l, 2011)
6	N188S G202R F213I	(Serra-Vinardell et al., 2014)
7	R257Q	(Serra-Vinardell et al., 2014)
8	G325R R359Q V352M	(Olga Amaral et al.,2000)
9	N370S G377S D409	(Rosenbloom et al., 2011)
10	L444P R463C	(Migdalska-Richards et al., 2017)
11	R496H	(Brautbar et al, 2003)

II. METHODOLOGY

There are the two major steps involve in this research work that are molecular and genetic context of GBA gene of Gaucher disease. 07 Pakistani families with least one affected individual were involved in this study. Blood samples of affected and normal individuals of family were collected using 5-10 cc disinfected needles. Formerly further processing, blood was shifted to EDTA containing vacutainer tubes and placed at 4°C temperature in the research laboratory. There were Phenol- chloroform DNA isolation method used for genomic DNA extraction. After isolation of genomic DNA, amplification of PCR for exon 10 of GBA gene the

following primer sequence was used. After getting pure product of PCR, sanger sequencing was carried. Analysis of sequenced data was analyzed by sequencer and for finding pathogenic variants mutation taster, provean, polyphen 2 and mutation accessors used.

Table 2. Description of Primers used in PCR amplification

Primer name	Sequence (5'-3')	Primer length	Tm	Product size
GBAEX10-F	GCAGAAAAGCAGGGTCAGTG	20	61	575
GBAEX10-R	TGCTGTGCCCTCTTTAGTCA	20	59.6	575

III. RESULTS

Detailed mutation analysis of affected member of 7 enrolled families having one and more than one variant discussed below with chromatograms shown in the form of figures.

Mutational analysis of affected person of family 1 revealed that two variation one is polymorphism that is synonymous homozygous with RS_ID rs143255568 (g.9484T>C) with no amino acid change (fig.1) and other is synonymous homozygous pathogenic mutation (g.9551G>A) without any amino acid change (fig 2). Another family 2 proband shows novel non-synonymous homozygous mutation (c.1135A>T) with change in amino acid I379F (fig 3.)and novel Non-synonymous heterozygous polymorphism (c.1169C>A) is also found with amino acid change A390D in same affected member (fig 4.). The two novel non- synonymous heterozygous mutations are found with amino acid change E381D and R385K respectively (fig 5 & fig 6) in affected person of another family. Proband of another family revealed that non- synonymous heterozygous mutation (c.1170C>A) with no amino acid change (fig 7.) and a non- synonymous homozygous mutation (c.1183G>C) with amino acid change(D395H) found in same person (shown in fig 8.). Affected member of another family shows known synonymous heterozygous mutation (c.1152G>A) with no amino acid change having Reference ID is rs12747811(fig 9.) but non- synonymous heterozygous mutation (c.1198G>C) with amino acid change(A400P) found in same member (fig 10.). An affected person of another family revealed that a novel non- synonymous heterozygous polymorphism with change in amino acid N394K shown in fig 11. A synonymous heterozygous polymorphism (g.9725C>A) with no amino acid change was found in proband of another family (fig 12.)

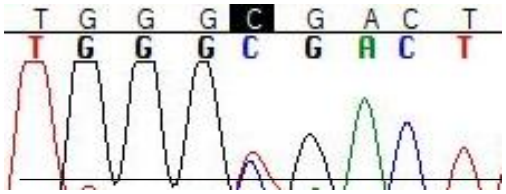

Fig 1. g.9484T>C(polymorphism)

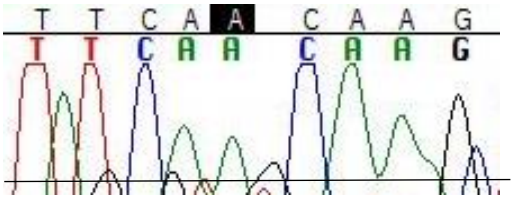

Fig 2. g.9551G>A (mutation)

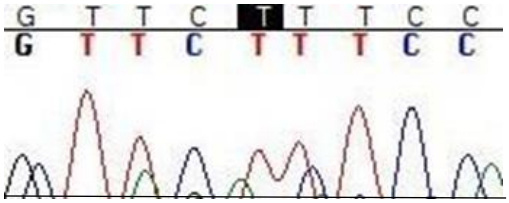

Fig 3. c.1135A>T (mutation, I379F)

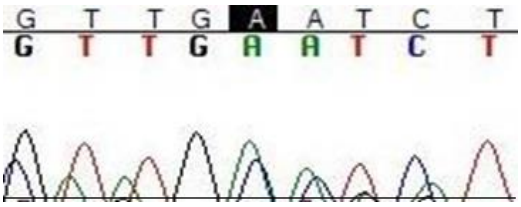

Fig 4. c.1169C>A (polymorphism, A390D)

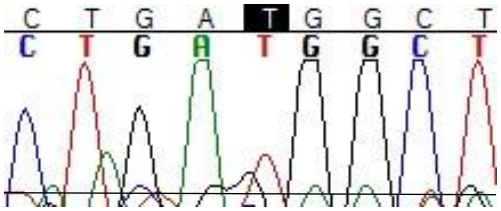

Fig 5. c.1143G>T (mutation, E381D)

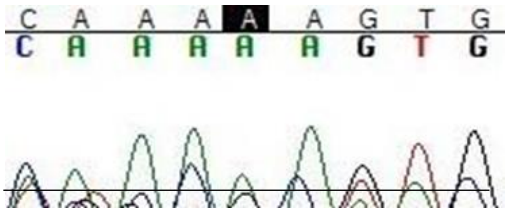

Fig 6. c.1154G>A (mutation, R385K)

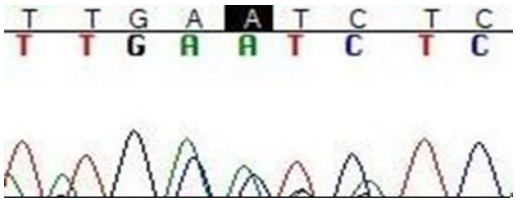

Fig 7. c.1170C>A (mutation)

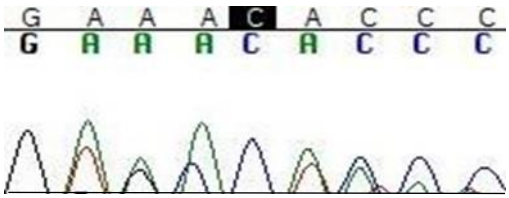

Fig 8. c.1183G>C (mutation, D395H)

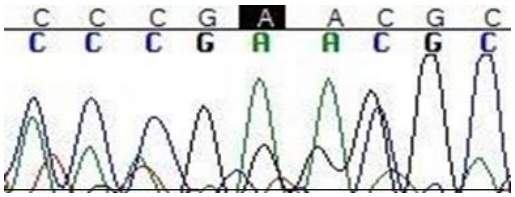

Fig 9. c.1152G>A(mutation)

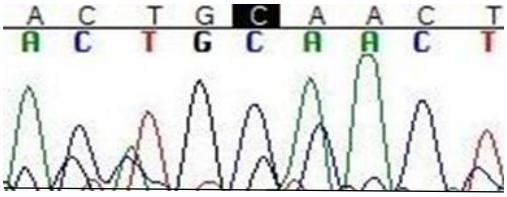

Fig 10. c.1198G>C (mutation, A400P)

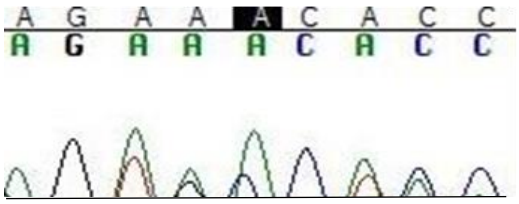

Fig 11. c.1182C>A (polymorphism, N394K)

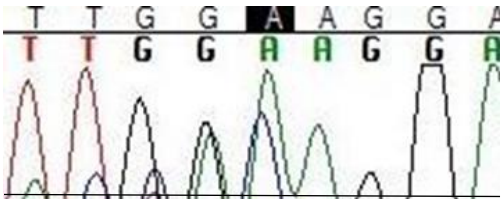

Fig 12. g.9725C>A(polymorphism)

Table 3: Mutations found in GD patients in exon 10

Patient ID	Hg19 coordinate	HGVSc	HGVSp	RS_ID	ExAC	Mutation taster
001	chr1:155205170A>G	Intron	No AA change	rs143255568	11	P
001	chr1:155205103C>T	Intron	No AA change	Not found	N/A	DC
002	chr1:155205095T>A	c.1135A>T	I379F	Not found	N/A	DC
003	chr1:155205087C>A	c.1143G>T	E381D	Not found	N/A	DC
003	chr1:155205076C>T	c.1154G>A	R385K	Not found	N/A	DC
002	chr1:155205061G>T	c.1169C>A	A390D	Not found	N/A	P
004	chr1:155205060G>T	c.1170C>A	No AA changes	Not found	N/A	DC
006	chr1:155205048G>T	c.1182C>A	N394K	Not found	N/A	P
004	chr1:155205047C>G	c.1183G>C	D395H	Not found	N/A	P
005	chr1:155205078C>T	c.1152G>A	no AA changes	rs12747811	N/A	DC
005	chr1:155205032C>G	c.1198G>C	A400P	Not found	N/A	DC
007	chr1:155204929G>T	g.9725C>A	no AA changes	Not found	N/A	P

*AA: amino acid *DC: disease causing *P: Polymorphism

IV. DISCUSSION AND CONCLUSION

Gaucher disease is the most prevalent lysosomal storage disease. It is transfer as an autosomal recessive way. The enzyme substrate glucocerebroside aggregate in the patient's spleen, liver and bone marrow. Osteoarticular manifestations are often contribute the injury and disability related to Gaucher Disease. The

resultant accumulation in several tissues and organs leads to numerous manifestations like anemia, hepatosplenomegaly, thrombocytopenia, growth retardation and skeletal disease. In light of the clinical seriousness, GD was isolated into three clinical subtypes: type 1, non-neuronopathic; type 2, acute neuronopathic; type 3, subacute neuronopathic. Glucocerebrosidase is a lysosomal layer bound enzyme that hydrolyses the (3-glucosidic linkage of glucocerebroside. In the event that the enzyme movement of glucocerebrosidase is significantly insufficient, the substrate won't be debased to glucose and ceramide appropriately. As a result, it amasses in the lysosomes of cells, and causes the disease known as Gaucher disease.

The gene for glucocerebrosidase is GBA located on chromosome lq21. There are 11 exon and 10 introns are present in the gene. The exon 9 and 10 is the hot spot exon of said disease in Asia and also reported in worldwide. The current study is about on exon 10 because its most common exon. The literature reports p. Leu483pro, p. Asn409ser, c.84dupg (84GG) and IVS2 + 1G>A as the most well-known mutations in GD. (Zampieri et al., 2017). The mutations can be more addressed specifically ethnic gatherings just as specifically aggregates. The N370S or N409S change is the most broadly remembered one among Ashkenazi Jew (AJ) patients, followed by the 84GG modification, which is more surprising. The IVS2 + 1, R463C and R496H are normally found with GD type 1. Interestingly, the N370S change is occasionally found among Chinese and Japanese patients. Among Asian ethnic gatherings, the L444P, or L483P and F252I generally connected with GD type 2 and 3, are more prevailing. L444P is moreover the most redundant mutation between Caucasians with a non-Ashkenazi Jew legacy. (Hruska et al., 2008). The given study shows the change range of GD in Pakistani patients. It uncovers 20 cases of GD (clinical range), with molecular investigation of 7 cases.

Variation in these observations can be likely due to different ethnic group involved in these studies. The majority of the patients, in the given study, are from children hospital Lahore, Punjab that provides spectrum of GD mutations spectrum in local population. From those 7 patients, 12 different variants were found 05 were polymorphism both heterozygous and homozygous, 03 of them were non- synonymous polymorphism causing amino acid change and 07 were disease causing predicted by mutation taster, two homozygous and others heterozygous mutations, 04 of them causing amino acid change and others shows splice sites changes.

We found 6 novel mutations, two of them were homozygous and four are heterozygous mutations, and one already reported mutation c.1152G>A with rs12747811. While we found 4 novel polymorphisms, two heterozygous and two homozygous polymorphisms and one of them was already reported polymorphism that is chr1:155205170A>G having rs143255568.

This shows comprehensive picture of molecular properties of GBA gene and related phenotypic variations in the patients. We need further evaluation of remaining exons in order to determine the carrier ratio of Gaucher disease in our population or whether we should screen our neonates for such lysosomal disorders.

REFERENCES

1. Adar, T., Ilan, Y., Elstein, D., & Zimran, A. (2018). Liver involvement in Gaucher disease–Review and clinical approach. Blood Cells, Molecules, and Diseases, 68, 66-73.
2. Bongarzone, E. R., Givogri, M. I., Darryl, C., & DiMauro, S. (2012). Inborn Metabolic Defects of Lysosomes, Peroxisomes, Carbohydrates, Fatty Acids and Mitochondria. In Basic Neurochemistry (pp. 755-782). Academic Press.
3. Burrow, T. A., Barnes, S., & Grabowski, G. A. (2011). Prevalence and management of Gaucher disease. Pediatric Health, Medicine and Therapeutics, 2, 59-73.
4. Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G., & Thompson, J. D. (2003). Multiple sequence alignment with the Clustal series of programs. Nucleic acids research, 31(13), 3497-3500.
5. Dandana, A., Khelifa, S. B., Chahed, H., Miled, A., & Ferchichi, S. (2016). Gaucher disease: clinical, biological and therapeutic aspects. Pathobiology, 83(1), 13-23.
6. Davies, E. H., Erikson, A., Collin-Histed, T., Mengel, E., Tylki-Szymanska, A., & Vellodi, A. (2007). Outcome of type III Gaucher disease on enzyme replacement therapy: review of 55 cases. Journal of inherited metabolic disease, 30(6), 935-942.
7. Di Rocco, M., Andria, G., Deodato, F., Giona, F., Micalizzi, C., & Pession, A. (2014). Early diagnosis of Gaucher disease in pediatric patients: proposal for a diagnostic algorithm. Pediatric blood & cancer, 61(11), 1905-1909.
8. Gary, S. E., Ryan, E., Steward, A. M., & Sidransky, E. (2018). Recent advances in the diagnosis and management of Gaucher disease. Expert review of endocrinology & metabolism, 13(2), 107-118.
9. Grabowski, G. A. (2005). Recent clinical progress in Gaucher disease. Current opinion in pediatrics,

17(4), 519-524.
10. Graham, O. E., Pitcher, T. L., Liau, Y., Miller, A. L., Dalrymple-Alford, J. C., Anderson, T. J., & Kennedy, M. A. (2020). Nanopore sequencing of the glucocerebrosidase (GBA) gene in a New Zealand Parkinson's disease cohort. Parkinsonism & Related Disorders, 70, 36-41.
11. Hollak, C. E., Belmatoug, N., Cole, J. A., vom Dahl, S., Deegan, P. B., Goldblatt, J., ... & Cappellini, M. D. (2012). Characteristics of type I Gaucher disease associated with persistent thrombocytopenia after treatment with imiglucerase for 4–5 years. British journal of haematology, 158(4), 528-538.
12. Kaplan, P., Andersson, H. C., Kacena, K. A., & Yee, J. D. (2006). The clinical and demographic characteristics of nonneuronopathic Gaucher disease in 887 children at diagnosis. Archives of pediatrics & adolescent medicine, 160(6), 603-608.
13. Lesage, S., Anheim, M., Condroyer, C., Pollak, P., Durif, F., Dupuits, C., ... & Vidailhet, M. (2011). Large-scale screening of the Gaucher's disease-related glucocerebrosidase gene in Europeans with Parkinson's disease. Human molecular genetics, 20(1), 202-210.
14. Lepe-Balsalobre, E., Santotoribio, J. D., Nuñez-Vazquez, R., García-Morillo, S., Jiménez-Arriscado, P., Hernández-Arévalo, P., ... & Macher, H. C. (2020). Genotype/phenotype relationship in Gaucher disease patients. Novel mutation in glucocerebrosidase gene. Clinical Chemistry and Laboratory Medicine (CCLM), 1(ahead-of-print).
15. Martínez-Arias, R., Calafell, F., Mateu, E., Comas, D., Andrés, A., & Bertranpetit, J. (2001). Sequence variability of a human pseudogene. Genome research, 11(6), 1071-1085.
16. Martin-Banderas, L., Holgado, M. A., Duran-Lobato, M., Infante, J. J., & FernándezArévalo, M. (2016). Role of nanotechnology for enzyme replacement therapy in lysosomal diseases. A focus on Gaucher's disease. Current Medicinal Chemistry, 23(9), 929-952.
17. Marcucci, G., Zimran, A., Bembi, B., Kanis, J., Reginster, J. Y., Rizzoli, R., ... & Brandi, M. L. (2014). Gaucher disease and bone manifestations. Calcified tissue international, 95(6), 477-494.
18. Nussbaum, R. L., McInerney-Leo, A., & Sidransky, E. (2004). Parkinsonism among Gaucher disease carriers. Journal of medical genetics, 41(12), 937-940.
19. Pastores, G. M., Weinreb, N. J., Aerts, H., Andria, G., Cox, T. M., Giralt, M., ... & Tylki-Szymańska, A. (2004, October). Therapeutic goals in the treatment of Gaucher disease. In Seminars in hematology (Vol. 41, pp. 4-14). WB Saunders.
20. Pastores, G. M., & Hughes, D. A. (2015). Non-neuronopathic lysosomal storage disorders: disease spectrum and treatments. Best Practice & Research Clinical Endocrinology & Metabolism, 29(2), 173-182.
21. Pastores, G. M., & Hughes, D. A. (2018). Gaucher disease. In GeneReviews®[Internet]. University of Washington, Seattle.
22. Rosenbloom, B., Balwani, M., Bronstein, J. M., Kolodny, E., Sathe, S., Gwosdow, A. R., ... & Weinreb, N. J. (2011). The incidence of Parkinsonism in patients with type 1 Gaucher disease: data from the ICGG Gaucher Registry. Blood Cells, Molecules, and Diseases, 46(1), 95-102.
23. Roshan Lal, T., & Sidransky, E. (2017). The spectrum of neurological manifestations rassociated with Gaucher disease. Diseases, 5(1), 10.
24. Sidransky, E. (2004). Gaucher disease: complexity in a "simple" disorder. Molecular genetics and metabolism, 83(1-2), 6-15.
25. Stirnemann, J., Belmatoug, N., Camou, F., Serratrice, C., Froissart, R., Caillaud, C., ... & Berger, M. G. (2017). A review of Gaucher disease pathophysiology, clinical presentation and treatments. International journal of molecular sciences, 18(2), 441.
26. Thomas, A. S., Mehta, A., & Hughes, D. A. (2014). Gaucher disease: haematological presentations and complications. British journal of haematology, 165(4), 427-440.
27. Weiss, K., Gonzalez, A. N., Lopez, G., Pedoeim, L., Groden, C., & Sidransky, E. (2015). The clinical management of type 2 Gaucher disease. Molecular genetics and metabolism, 114(2), 110-122.
28. Zampieri, S., Cattarossi, S., Bembi, B., & Dardis, A. (2017). GBA analysis in next-generation era: Pitfalls, Challenges, and Possible Solutions. The Journal of Molecular Diagnostics, 19(5), 733-741.