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Original scientific article

NEUROLOGICAL MANIFESTATIONS IN PAKISTANI LYSOSOMAL STORAGE DISORDERS PATIENTS AND MOLECULAR CHARACTERIZATION OF GAUCHER DISEASE

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Lysosomal storage disorders (LSDs) are a large group of inborn errors of metabolism each caused by genetic mutations of a particular lysosomal protein encoding gene. These inherited conditions are characterized by lysosomal dysfunction with wide variety of organ impact sometimes organ failure with growing age. Neurological complications in LSD cases range from severe neurodegenerations in 70% cases to mild symptoms or absence of neuropathy in others. Each LSD is monogenic but heterogeneous from a molecular standpoint with a large number of mutations described in the respective gene. Some mutations are particular to specific populations, reflecting consequences of founder effect. Present study aimed to access the demographic and clinical profiles of forty-five LSD affected families enrolled during January 2018 to December 2019 at local hospitals to find out neurological symptoms in Pakistani LSD cases. Furthermore, molecular genetic analysis of Gaucher's disease affected families was performed to unveil underlying disease causing mutation/s. Neurological manifestations were present in twenty-eight families including eleven Mucopolysaccharidosis-1 (MPS-I), four Gaucher's disease (GD) and all MPS-II, MPS-III, Niemann-Pick, Griscelli and Chediak-Higashi cases. Neurological involvement was not found in eight MPS-I, one GD, all MPS-IV and Pycnodysostosis affected families. Screening of *GBA* gene in GD families revealed a

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reported missense mutation p.L483P in all analyzed families. Clinical heterogeneity of MPS-1 and GD is evident from literature however mutational analysis of all enrolled GD families depicted segregation of a reported missense variant p.L483P of *GBA* gene with disease phenotype in all families. Our findings highlight importance of homeostatic role of lysosomes in neuronal development as twenty eight out of forty families had neurological manifestations. Furthermore, identification of same mutation in GD patients with or without neuronal involvement may be related to some unknown differences in the expression of genetic modifiers or exposure to environmental triggers.

Key words: Lysosomal Storage Disorders, Gaucher Disease, Neurological manifestation, *GBA* gene, Pakistan

INTRODUCTION

All functional cells of body have central organelle for degradation i.e lysosome. Functional lysosomes are important for degradation of cellular autophagic contents to regulate the quality of cytoplasm by eliminating cellular macromolecular aggregates (SERANOVA *et al.,* 2017). The substrate for the lysosome is obtained through multiple pathways like phagocytosis, autophagy and endocytosis. These substrates are degraded in lysosomes via lysosomal hydrolases thus causing disposal and recycling of cellular waste (BALLABIO *et al.,* 2020). If this homeostatic role of lysosomes is disturbed due to compromised or absent lysosomal enzyme/s it may lead to inappropriate storage of material interfering with normal cell function and causing multisystem disorders including lysosomal storage disorders (LSDs), neurodegenerative diseases and cancer (PLATT *et al.*, 2012). LSDs are a large group of inherited disorders causing multiple organ failure. Furthermore, about two third of LSD's have central nervous system involvement (PARÁ *et al.,* 2020). Most of these disorders are caused by recessively inherited Mendelian mutations in genes encoding lysosomal proteins (MARQUES *et al.*, 2019; PARÁ *et al.*, 2020). Frequency of all LSDs in different population is 1:7500 in live births (MEIKLE *et al.,* 1999; POUPETOVA *et al.,* 2010). However, exact prevalence in some populations may even be higher because many cases remain undiagnosed (PLOTEGHER *et al.,* 2017). In addition, in societies like Pakistan, due to high ratio of consanguinity, recessively inherited disorders are more common than in any randomly mating populations (PRADHAN *et al.*, 2011; SHAHID *et al.*, 2020).

LSDs related damages are progressive therefore patient management requires early precise diagnosis of disease subtype (ZHOU *et al.,* 2011) for therapeutic management, which is a serious problem. Widely used therapeutic option is enzyme replacement therapy (ERT) (AFROZE *et al.*, 2017). ERT limitations include high cost, lifelong treatment requirement, inability to stop progression of neuronal symptoms and possibility of immune response against an exogenous enzyme (PARENTI *et al.*, 2015; AFROZE *et al.*, 2017). Recently, pharmacological chaperon (PC) therapies, are being developed to use chaperones which increase the stability of mutated protein inside the cell by avoiding endoplasmic reticulum (ER) associated degradation. Interestingly, PCs are capable of crossing the blood-brain-barrier, thus showing promising results in stabilizing the mutant enzymes which otherwise get degraded by ER. However, PCs also showed some limitations due to type of mutations affecting the enzyme structure (PARENTI *et al.,* 2015; MOHAMED *et al.*, 2017). Other treatment options including bone marrow transplant or human

stem cell transplant are only possible after early precise diagnosis within the first few months of life. Thus, molecular genetics diagnosis of LSDs along with enzyme analysis for accurate early detection and patient management is required (MITTAL, 2015; MOKHTARIYE *et al.*, 2019).

LSDs are considered a major reason of neurodegeneration in childhood (VERITY *et al.*, 2010). LSDs including some forms of Gaucher disease, Niemann-Pick disease C, neuronal lipofuscinosis and mucopolysaccharidosis manifest mild to severe neurodegenerative symptoms (MEIKLE *et al.*, 1999; SHETH *et al.*, 2004; MARQUES *et al.*, 2019). Due to continuous spectrum of phenotypes among LSDs severe cases are presented at infancy or early childhood while mild cases are being observed at adult age (VERITY *et al.*, 2010; MARQUES *et al.*, 2019). This study is the first report aimed to access neurological manifestations in Pakistani LSDs affected cases.

MATERIALS AND METHODS

Approval of the study was taken from bioethical committee of Quaid-i-Azam University Islamabad, Pakistan. A total of forty five unrelated LSD affected Pakistani families were enrolled through local hospitals during January 2018 to December 2019 from local hospitals. All diagnosis were made on the basis of clinical and biochemical analysis by expert pediatricians. For diagnosis of each LSD subtype enzyme analysis by dry blood spot, radiological findings and blood complete picture was performed. For each enrolled family, detailed family/medical history, pedigree drawing and blood samples of available affected and unaffected family members were collected after informed consent in accordance with the tenets of the Declaration of Helsinki (ASSOCIATION, 2013). Demographic and clinical profile of proband of each Gaucher disease family is listed in table 1 (Table 1). Five Gaucher disease (GD) affected families (Family A-E) (Figure1; Table 2) were selected for mutational analysis of *GBA* gene based on definitive diagnosis and availability of blood samples of affected and unaffected family members. All GD patients were born to consanguineous couples (Table 1). The age of diagnosis was between 2 to 14 months (Table 1). Probands of family A and C had one affected deceased sibling each at the age of 1.5 years and 7 years, respectively.

Table 1. Demographic and clinical details of proband of each enrolled Gaucher Disease family

Family ID	Ethnicity	Family history/ No. of affected	Parental Consanguinity	Neuronal manifestations	Other clinical features
Family A	Punjabi	Yes/1	Yes	No	Chronic diarrhea, AD, HSM, anemia
Family B	KPK	Nil	Yes	Yes	Breathing problem, AD, HSM, weak muscles
Family C	Punjabi	Yes/1	Yes	Yes	AD, HSM, DD
Family D	Punjabi	Nil	Yes	Yes	DD, AD, HSM
Family E	KPK	Nil	Yes	Yes	AD, HSM, weak muscles

KPK = Khyber Pakhtunkhwa, Abdominal Distension=AD, hepatosplenomegaly=HSM, Developmental Delay=DD

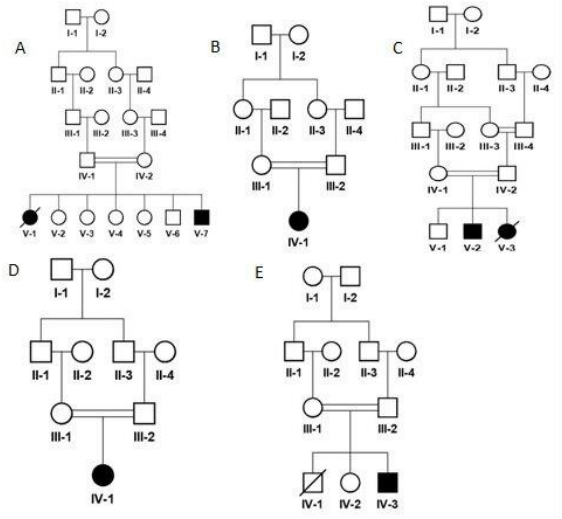

Figure 1. Pedigrees of Gaucher disease (GD) families (Family A-E) consistent with autosomal recessive inheritance pattern

Squares indicate males while circles indicate females. Filled and hollow symbols denote GD patients and normal individuals respectively. A double marriage line represents consanguinity.

Table 2. Clinical, biochemical and molecular genetics diagnosis details of proband of each Gaucher disease family.

Clinical data		Family A	Family B	Family C	Family D	Family E
Gender		M	F	M	M	M
Age at diagnosis/ age at examination		± 1 year/ 6.5 years	3 months/ 13 months	4 years/ 12 years	6 months/ 1 year	6 months/ 2 years
Treatment		ERT	N/A	ERT	ERT	ERT
Splenectomy		Yes	N/A	N/A	N/A	N/A
GCase activity		0.63 nmol/ml/h	N/A	0.2 µmol/L/h	0.5 µmol/L/h	0.1 µmol/L/h
RBCs		4.55 million/µL	3.22×10^{-6}/µL	5.62 10^6/uL	4.33 10^6/uL	3.87 million/µL
ALT		22 U/L	52U/L	20 U/L	16 U/L	N/A
WBCs		N/A	16.6×10^{-3}/µL	9.1 10^3/uL	4.8 10^3/uL	7000/mm^3
Age at diagnosis		5 months	3 months	6 months	14 months	3 months
Hemoglobin		11.9 g/dL	7.4g/dL	10.5 g/dL	13.4 g/dL	8.2g/dL
Platelets		220×10^{-3}/µL	16×10^{-3}/µL	531 10^3/uL	105 10^3/uL	154×10^{-3}/µL
Genotype	DNA mutation	c.1448T>C/ c.1448T>C	c.1448T>C/ c.1448T>C	c.1448T>C/ c.1448T>C	c.1448T>C/ c.1448T>C	c.1448T>C/ c.1448T>C
	Protein change	p.L483P/ p.L483P	p.L483P/ p.L483P	p.L483P/ p.L483P	p.L483P/ p.L483P	p.L483P/ p.L483P

M: Male, F: Female, N/A: Not available, ERT: Enzyme Replacement Therapy

Mutational analysis of GD families

Blood samples of enrolled affected and unaffected members of selected GD families were stored in 10 mL vacutainer tubes (BD vacutainer K2 EDTA 18 mg). Genomic DNA was extracted by a modified method as described by KAUL *et al.* (2010). Quantity and purity of extracted DNA was accessed using a μDrop Plate reader (Multiskan^{TM}, Thermo Fisher Scientific, and Waltham, MA, USA).The extracted DNA was stored at 4°C. Primers of *GBA* gene were designed by using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/) and are listed in table 3 (Table 3). Primers were further confirmed for binding and self ligation through BLAT and Primer stat respectively (https://www.bioinformatics.org/sms2/pcr_primer_stats.html) (https://genome.ucsc.edu/cgi-bin/hgBlat?command=start). Samples were amplified by polymerase chain reaction (PCR) using T100 thermal cycler (Bio-Rad, CA, USA) with a cycling program of 95°C for 5 min, followed by 10 cycles of 95°C for 45s, 69°C- 64°C (according to melting temperature of each primer pair) for 45s with an increment of -1°C in each subsequent cycle, 72°C for 45s again followed by 30 cycles of 95°C for 45s, 59°C- 54°C (according to melting temperature of each primer pair) for 45s, 72°C for 45s and a final extension at 72°C for 10 min followed by a final hold at 25°C. The amplified PCR products were loaded on the 1.5% agarose gel along with 1kb size ladder to evaluate product size and purified by using DNA purification Kit (*Wiz Bio* Solutions, Seongnam, Korea). The sequencing reaction was performed using Big Dye Terminator Ready reaction mix (Applied Biosystems) following manufacturer instructions. Sanger's sequencing was done on an automated ABI 3730 genetic analyzer by Macrogen, Korea.

The sequenced data was analyzed by using BioEdit (version 7.2). The variants were confirmed by using Mutation taster software (http://www.mutationtaster.org/). Further the effect of mutation on the structure and function of protein was studied by using a bioinformatics tool HOPE (https://www3.cmbi.umcn.nl/hope/input/).

Table 3. Primers used for PCR amplification of GBA gene in Gaucher disease affected families

S. No.	Primer ID	3'-5' sequence	GC contents	Melting temperature	Product size
1	1,2 F	GGGAAGCCGGAATTACTTG	52.63	59.51	850 bp
2	1,2 R	GAGCCAAAATTGCACCACT	47.37	58.71	
3	3,4 F	ACCGTGTTCAGTCTCTCCTAGC	54.5	62.9	652 bp
4	3,4 R	GACAGAATGGGCAGAGTGAG	55	59.9	
5	5,6 F	AGGAGCCCAAGTTCCCTTT	52.63	60	806 bp
6	5,6 R	CTGATGGAGTGGGCAAGATT	50	60	
7	7 F	AGGCTGTTCTCGAACTCCTG	55	59.6	496 bp
8	7 R	GGGAATGGTGCTCTAGGAATC	52.38	59.92	
9	8 F	GTT GCA TTC TTC CCG TCA CC	55	62.8	367 bp
10	8 R	CTG GAC AGG AAG GGC TTC TG	60	62.3	
11	9 F	CTCTCCCACATGTGACCCTTA	52.3	59.9	399 bp
12	9 R	GCCTCCATGGTGCAAAAGGGG	52.9	59.1	
13	10,11 F	GCAGAAAAGCAGGGTCAGTG	55	60.98	575 bp
14	10,11 R	TGCTGTGCCCTCTTTAGTCA	50	59.59	

RESULTS

A total of forty-five families suffering from Lysosomal Storage Disorders (LSDs) were recruited during January, 2018 to December, 2019 through expert pediatrics from local hospitals. Among enrolled families, affected individuals of twenty eight 62.2% (28/45) families had neurological involvement. Majority of the patients i.e., 68.8% (31/45) were from Punjab province of Pakistan whereas 22.2% (10/45) and 8.8% (4/45) belonged to Khyber Pakhtunkhwa and Azad Jammu and Kashmir (AJK) respectively. All patients belonged to inbred families and in forty three families affected cases were products of marriage between first cousins. Average age of proband of each enrolled family was 11.04 ± 8.10 years. Majority of families were affected with Mucopolysaccharidosis (35/45) followed by Gaucher disease (5/45), Niemann-Pick disease (2/45), Chédiak-Higashi syndrome (1/45), Pycnodysostosis (1/45) and Griscelli disease (1/45). All collected patients of same subtype had comparable phenotypes e.g., all Gaucher disease cases had abdominal distension and hepatosplenomegalies (Table 1 and 2).

Gaucher's disease (GD) cases were selected for mutational analysis of *GBA* gene due to: a) limited financial resources for sequencing, b) clinical confirmation of disease for selection of gene and c) availability of blood samples of affected and unaffected family members. Out of five GD probands, four were males and one was female. In family A, there is a five generations pedigree with consanguinity and two affected individuals (V-1, V-7), among them one is deceased at the age of one and half year. While second affected is of 7 years and is still alive. The neurological degeneration is not present in this subject. The dry blood spot (DBS) measurement for enzyme concentration in index case i.e., V-1 was found to be lower than cut off value (Table 2) i.e 2.3-14.1 nmol/mL/h. Also chitotriosidase level is elevated (285.99 nmol/h/mL, normal: <150.0) which is in alignment with the Gaucher Disease. The bone marrow aspiration report revealed hyperplastic erythropoiesis and myelopoiesis. Increased atypical histiocytes with fibrillary cytoplasm and eccentric nucleous were observed. Splenectomy explains abundant eosinophilic cytoplasm having a wrinkled tissue paper appearance. Patient also has chronic diarrhea in common, abdominal distention and hepatospleomegaly. In family B, there is one affected individual with abdominal distention, severe breathing problem and require continuous oxygen supply, jaundice, weak muscles, swelling in feet, inguinal hernia, repeat episodes of shocks, macrocephaly, hepatosplenomegaly with gross ascites, gum hypertrophy, respiratory dyspnea, wheezing, localized paralysis, seizures and neurological features. The patient also reported severe cough attacks and intermittent high fever. In family C and D both patients were having hepatosplenomegaly, abdominal distention, delay in achieving milestones, neuronal dysfunction, respiratory distress and muscle weakness. In family E, enzyme analysis in DBS is below the cut off value (> 1.5 μmol/L/h), indicating the GD. The patient has abdominal distention, neuronal dysfunction, hepatosplenomegaly, inguinal hernia and delayed milestones.

Mutational analysis of Gaucher disease families

Analysis of sequencing results revealed a previously reported missense variant p.L483P (c.1448T>C) in exon 10 (rs421016) (TSUJI *et al.*, 1987) of *GBA* gene in all five GD affected families segregating with disease phenotype. All the affected cases were homozygous for mutated allele whereas their parents were heterozygous carriers of mutation. In addition, we

performed a control sample sequencing of exon 10 of *GBA* gene in order to confirm the normal homozygous state (Figure 2B).

Here we used in-silico analysis tool i.e., Have Our Protein Explained (HOPE) for the first time to check impact of p.L483P substitution, which highlighted that the mutated residue is located in a domain that is important for the activity of the protein and in contact with residues in another domain. It is possible that this interaction is important for the correct function of the protein. The mutation can affect this interaction and subsequently normal protein function. The wild-type and mutant amino acids differ in size. The mutant residue is smaller than the wild-type residue. The mutation was predicted to cause an empty space in the core of the protein (Figure 2D).

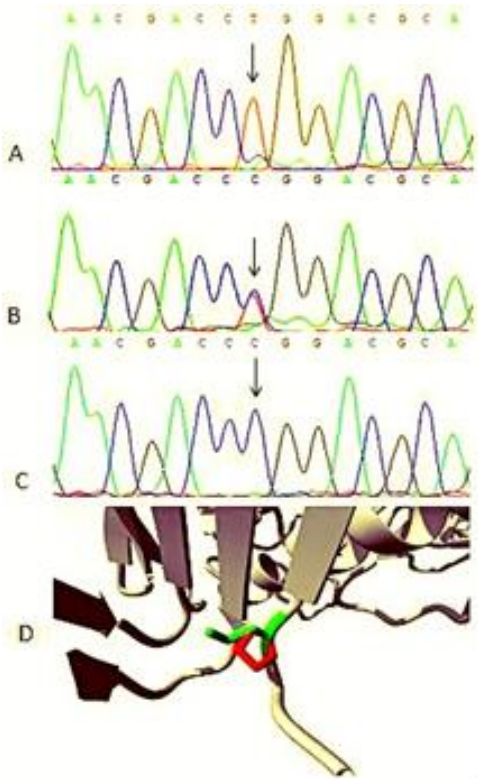

Figure 2. A: Sequence chromatogram of normal allelic sequence. B: Sequence chromatogram of heterozygous carrier for c.1448T>C. C: Sequence chromatogram of homozygous mutant for c.1448T>C in affected individuals of Gaucher affected families. D: Structural effect of the amino acid substitution as shown by HOPE analysis. Mutated amino acid is shown in red color while wild type amino acid is shown in green color.

DISCUSSION

Genetic defect/s leading to LSDs predisposes to progressive accumulation of material with growing age and show many devastating phenotypes including neurodegenerative diseases (PLATT *et al.*, 2012; SERANOVA *et al.*, 2017). Even most of the clinical phenotypes appear in later stages of life, but by then those damages are irreversible (COX *et al.*, 2012). Approximately 45% cases of neurodegenerations in the world are due to LSDs (VERITY *et al.*, 2010). However, central nervous system defects are reported in more than 70% cases of LSDs and rest ±30% cases may have no nervous involvement or have mild symptoms (JARDIM *et al.*, 2010; MARQUES *et al.*, 2019; SHETH *et al.*, 2019). Hence, understanding the pathophysiology of LSDs can be useful to unveil neurodegenerative mechanisms. Etiology of most LSDs predominantly involves recessively inherited Mendelian mutations. In societies like Pakistan, recessively inherited disorders are more common due to consanguinity (AFROZE *et al.*, 2017; GUL *et al.*, 2019; MARQUES *et al.*, 2019). Therefore present study was designed to study two aspects of enrolled LSD affected families from local population. Firstly, to identify neuronal manifestation in enrolled LSD subtypes and secondly to screen *GBA* gene in Gaucher's disease (GD) affected families.

Out of forty-five LSDs affected families enrolled during January, 2018 to December, 2019 twenty eight (62.2%) families had neurological involvement. All recruited cases of LSDs are novel and was not previously studied by any other group. Our data revealed that mucopolysaccharidosis (MPS) is the predominant LSD in our study with thirty five affected families segregating MPS-I, MPS-II, MPS-III and MPS IV phenotype in 19, 3, 6 and 7 enrolled families respectively . Our findings are consistent with Hutchesson et al., 1998 and Afroze et al., 2016 reports of increased frequency of MPS in Pakistani children (HUTCHESSON *et al.*, 1998; AFROZE *et al.*, 2016; AFROZE *et al.*, 2017); however data presented by CHEEMA *et al*. (2016) showed Gaucher disease as predominant LSD in cases presented at a tertiary care hospital Lahore, Pakistan (CHEEMA *et al.*, 2016). MPS-1 has further three subtypes including the most severe form Hurler (MPS I-H), Hurler-Scheie (MPS I-H/S) and the less severe Scheie (MPS I-S) (DE RU *et al.*, 2012). Previously, we reported a non-sense mutation (p.E486X) of IDUA gene in MPS-1H affected family displaying neuronopathy whereas another MPS1-H/S affected family was segregating a missense variant (p.L490P) with no neuronal involvement at early age (GUL *et al.*, 2019). Similarly, remaining seventeen unscreened MPS 1 families have different clinical manifestations but the underlying genetic mutations are yet to be clarified for exact molecular genetic subtyping of disease.

Unlike, MPS-1 all enrolled affected cases of MPS-II (Hunter syndrome) and MPS-III (Sanfillipo syndrome) displayed neuronal involvement. Multiple interviews of Sanfillipo affected families revealed that the neuronopathies got severe with age. Rest of the cases of Niemann-Pick, Griscelli and Chediak-Higashi disease also had severe neuronopathic symptoms. Previous reports suggest that among Gaucher disease (GD) cases, type 1 GD is most common which is non-neurological form of disease (MARQUES *et al.*, 2019; SHETH *et al.*, 2019). However, among our five GD affected families, affected members of all except Family A displayed neurological symptoms (Table 2). To unveil mutational spectrum of *GBA* in our enrolled GD families we performed Sanger's sequencing of coding exons of *GBA* gene. Data analysis showed a previously reported missense mutation p.L483P (rs421016), segregating with disease

phenotype in all five families. Recently, SHETH *et al*. (2019) identified p.L483P as the most common GD causing mutation in Indian population irrespective of ethnic groups (SHETH *et al.*, 2019) which is consistent with our findings. This mutation was also reported to be detected with 41% Japanese, 60% Thai and 2.84% Jewish GD cases (GRABOWSKI, 1997; ETO *et al.*, 1999; TAMMACHOTE *et al.*, 2013) but in this study we detected this mutation in 100% analyzed cases. Although p.L483P is reported to be associated with all subtypes of GD (KOPRIVICA *et al.*, 2000; TAMMACHOTE *et al.*, 2013; SHETH *et al.*, 2019) but KOPRIVICA *et al*. (2000) suggested that p.L483P is mainly associated with GD sub types showing neurological involvement which is also found in this study (KOPRIVICA *et al.*, 2000). Screening of *GBA* gene in our enrolled families and identification of same mutation in all of them suggest high prevalence of p.L483P allele in our population. Affected individual of one of our GD family (family A) segregating p.L483P has no neurological symptoms which is consistent with GD type 1, but the possibility of neuronal manifestations at the later stage of life could not be ruled out as reported by ALFONSO *et al*. (2007) Leucine at 483 position of GBA protein lies in β-barrel domain (residues 30–75 and 431–497) of Beta-Glucosidase which resembles an immunoglobulin fold (LIEBERMAN *et al.*, 2009). According to previous studies this substitution leads to alteration of secondary structure of protein also this variant lie close to the catalytic domain of enzyme thus results in loss of enzyme activity. This is supported by our HOPE data as shown in figure 2D, the mutated residue is located in a domain which is important for the activity of the protein and leucine 483 makes contact with residues in another domain (Figure 2D) further highlighting importance of this amino acid in wild type structure and function of GBA protein.

CONCLUSION

We concluded that presence of neuropathies in 62% of our enrolled LSD cases, involvement of lysosomal defects in age related neurodegenerative conditions, including Parkinson's and Alzheimer's (MARQUES *et al.*, 2019) highlights importance of studies on molecular basis of LSDs to unveil molecular networks and links between different autophagy associated neurodegenerative conditions. Additionally, identification of p.Leu483Pro in all of GD cases in our study, a high reported carrier frequency of this variant in Indian population (SHETH *et al.*, 2018) and reported association of carriers of this allele with an increased risk of Parkinson disease (PD) development (WANG *et al.*, 2012) necessitates screening of this mutation in all PD and GD cases from our population. As the recessive genes causing LSDs are predominantly common in consanguineous populations like Pakistan, and the Pakistani population is less explored for the genetic basis of these conditions, then further genetic studies are indispensible which may reveal novel variants for diagnostics and treatment regimes.

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NEUROLOŠKE MANIFESTACIJE POREMEĆAJA SKLADIŠTENJA LIZOZOMA I MOLEKULARNA KARAKTERIZACIJA GAUCHER -ove BOLESTI U PAKISTANU

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Izvod

Poremećaji skladištenja lizozoma (LSD) su velika grupa urođenih grešaka u metabolizmu, od kojih je svaka uzrokovana genetskim mutacijama određenog gena koji kodira lizozomalni protein. Ova nasledna stanja karakteriše lizozomalna disfunkcija sa velikim brojem uticaja na organe, a sa godinama ponekad i otkazivanje organa. Neurološke komplikacije u slučajevima LSD -a kreću se od teških neurodegeneracija u 70% slučajeva do blagih simptoma ili odsustva neuropatije u drugih. Svaki LSD je monogen, ali heterogen sa molekularnog stanovišta sa velikim brojem mutacija opisanih u odgovarajućem genu. Neke mutacije su specifične za određene populacije, odražavajući posledice efekta osnivača. Ova studija imala je za cilj da pristupi demografskim i kliničkim profilima četrdeset pet porodica pogođenih LSD-om upisanih u periodu od januara 2018. do decembra 2019. godine u lokalne bolnice kako bi se otkrili neurološki simptomi u pakistanskim slučajevima LSD-a. Štaviše, izvršena je molekularno - genetska analiza porodica pogođenih Gaucherovom bolešću kako bi se otkrile osnovne bolesti koje izazivaju mutacije. Neurološke manifestacije bile su prisutne u dvadeset osam porodica, uključujući jedanaest mukopolisaharidoza-1 (MPS-I), četiri Gaucherove bolesti (GD) i sve slučajeve MPS-II, MPS-III, Niemann-Pick, Griscelli i Chediak-Higashi. Neurološka uključenost nije pronađena u osam porodica pogođenih MPS-I, jednim GD-om, svim MPS-IV i piknodizostozom. Skrining GBA gena u GD porodicama otkrio je prijavljenu mutaciju p.L483P u svim analiziranim porodicama. Klinička heterogenost MPS-1 i GD evidentna je iz literature, međutim mutaciona analiza svih upisanih GD porodica pokazala je segregaciju prijavljene varijante p.L483P GBA gena sa fenotipom bolesti u svim porodicama. Klinička heterogenost MPS-1 i GD evidentna je iz literature, međutim mutaciona analiza svih upisanih GD porodica pokazala je segregaciju prijavljene varijante p.L483P GBA gena sa fenotipom bolesti u svim porodicama. Naši nalazi ukazuju na važnost homeostatske uloge lizozoma u razvoju neurona jer je dvadeset osam od četrdeset porodica imalo neurološke manifestacije. Štaviše, identifikacija iste mutacije kod pacijenata sa GD sa ili bez neuronske uključenosti može biti povezana sa nekim nepoznatim razlikama u ekspresiji genetskih modifikatora ili izloženosti okidačima iz okruženja.

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