ABSTRACT

Tay-Sachs disease (TSD) is an autosomal recessive neurodegenrative disorder that arises due to impaired activity of the lysosomal enzyme β-Hexosaminidase-A (EC 3.2.1.52) caused by the mutation in *HEXA* gene. Due to overlapping phenotype and limitations of carrier screening by enzymes study, combined approach of lysosomal enzyme study from leucocytes followed by mutation identification is globally advocated.

In the present study, we have identified 46 cases confirmed with classical TSD due to deficient activity of Hex-A in leucocytes and 2 cases with AB variant form of TSD (GM2 activator protein deficiency) due normal activity of Hex-A in leucocytes but they are clinically indistinguishable from classical TSD. A carrier detection (%Hex A) was also carried in 8 couples where proband was not alive but had confirmed TSD. Molecular analysis of *HEXA* gene was carried out in all 46 affected cases with classical TSD and 8 carrier couples with TSD followed by common mutation screening. We have found c.1278insTATC (p.Y427IfsX5) variant homozygously in five cases and one case was found to be heterozygous for the same variant by screening of common variants. These were further confirmed by bidirectional sequencing of *HEXA* gene. Complete sequencing analysis followed by MLPA analysis revealed 34 variants in 51 unrelated families, 21 of which were novel, including 13 missense variants [c.340 G>A (p.E114K), c.524A>C (p.D175A), c.616G>C (p.V206L), c.637T>C (p.Y213H), c.788C>T (p.T263I), c.805G>C (p.G269R), c.964G>T (p.D322Y), c.964G>A (p.D322N), c.1121A>C (p.Q374P), c.1178C>G (p.R393P), c.1360 G>C (p.G454R), c.1385A>T (p.E462V) andc.1432G>A (p.G478R)], 2 nonsense variants [c.1421G>A (p.W474X) and c.1454G>A (p.W485X)], 2 small deletion [c.426delT (p.F142LfsX57) and c.898_905TTCATGAG (p.F300HfsX21)], 2 splice site variants [c.459+4 A>C and c.460-1G>A] and 2 large deletion [Exon 2-3 deletion and Exon 1 deletion]. Previously known 5 missense variants [c.2T>C (p.M1T), c.508C>T (p.R170W), c.509 G>A (p.R170Q), c.532C>T (p.R178C) and c.1495C>T (p.R499C)], 3 nonsense variants [c.316C>T (p.Q106X), c.709C>T (p.Q237X) and c.1528C>T (p.R510X)], 4 splice site variants [c.459+*5 G*>A, c.805+1G>C, c.1073+1 G>A and c.672+30

T>G] and 4bp insertion [c.1278insTATC (p.Y427IfsX5)] were also identified. Disease causing variant allele was not identified in 3 cases of classical TSD. In addition, molecular analysis of *GM2A* gene was also carried out in 2 cases of AB variant form of TSD. Exon sequencing analysis revealed 2 novel variants in 2 cases including 1 nonsense variants c.472G>T (p.E158X) and 1 splice site mutation c.243-2 A>T.

All novel missense variants were confirmed to be pathogenic using various bioinformatics tools like SIFT, Polyphen2 and Mutation Taster algorithms while splice site mutations were confirmed by Mutation taster and NNSplice 0.9 algorithms. Protein homology modeling study was carried out to further establish the effect of novel variants that occurred at highly evolutionarily conserved and functionally active domain residues in the protein leading to conformational changes or mRNA producing truncated protein. The sequence alignment of *HEXA* gene from various species reveals that all residues are highly conserved.

The variants responsible for classical TSD in Indian population are unique. Variants c.1385A>T (p.E462V), c.964G>T (p.D322Y), and c.1278insTATC (p.Y427IfsX5) are found to be the commonest one (~43%) and can be used as a part of common mutation screening program for *HEXA* gene. The pathogenic variants responsible for TSD in Indian patients are mostly novel with nearly 62% variants clustered between exons 5 to 12. In addition, patients from Gujarat origin seems to have founder mutation c.1385A>T (p.E462V) which is not present in normal population. Moreover, two of our cases have also shown deficiency in GM2 activator protein which is very rare and only 9 cases so far reported in the world literature. It can be concluded from these cases that children with global developmental delay, hyperacusis, cherry red spot in macula and unmyelinated white matter with normal β-Hexosaminidase (Hex-A and total-Hex) study should be further investigated for GM2 activator protein deficiency.