



Identification and *In Silico* Analysis of a Novel Mutation of *MAN2B1* Gene in Congenital Family with Alpha-Mannosidosis from Pakistan

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ABSTRACT

Alpha-mannosidosis is a devastating metabolic disorder characterized by intellectual disability, facial dysmorphism, musculoskeletal and immune abnormalities. This study identifies a genetic mutation in a complex consanguineous Pakistani family of Punjabi language group. The family presented with rare complex neurological symptoms including mental retardation, facial dysmorphism, skeletal and muscle abnormalities. Five affected family members were recruited along with unaffected members, and whole blood was collected for genomic DNA extraction. A genome-wide scan was carried out on four affected members to establish homozygosity linkage. The analysis for genotype assessment was carried out, and three homozygous regions were identified. Whole exome sequencing was carried out to identify the mutation in putative gene/s. The data was arranged, and *MAN2B1* was selected for the DNA sequencing. Protein homology was analyzed by the pymol tool to predict the effect on the mutant protein. A novel missense mutation c. 2710A>T; p.904Tyr>Ser was detected, and co-segregation analysis was established in the complex neurological family. The pymol analysis detected the loss of hydrogen bonding between Thr at 904 with Arg at 916 in mutant *MAN2B1*. It is concluded that a novel mutation is identified in *MAN2B1* associated with a complex neurological disease. The results indicate huge heterogeneity in the Pakistani population due to consanguinity. The combination of next-generation sequencing has facilitated the identification of genes in complex disorders like alpha-mannosidosis.

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INTRODUCTION

Alpha-mannosidosis (MIM #248500) is a rare complex and progressive metabolic storage defect due to the loss of activity of alpha-mannosidase (*MAN2B1*; EC3.2.1.24). It is originated as an autosomal recessive pattern due to *MAN2B1* mutations. It is a rare disease (1:300,000-1:500,000) but is revealed to be universally prevalent. The disorder mainly characterizes intellectual disabilities and reduced intelligent quotient (IQ), facial coarsening and dysmorphism, loss of motor functions, musculoskeletal defects, and immune deficiencies (Beck *et al.*, 2013; Malm *et al.*, 2014; Cathey *et al.*, 2019). The manifestations are variable and comprised of three different spectra (type 1-mild, type 2- moderate and type 3-severe), of which type 3

is the most devastating (Cathey *et al.*, 2019). Regarding the treatment of alpha-mannosidosis, there is no clear evident management, but there is a promising outcome of enzyme replacement therapy in these patients (Borgwardt *et al.*, 2013; Ceccarini *et al.*, 2018; Lund *et al.*, 2018). Also, bone marrow transplantation can improve some patients' clinical spectrum, but it can develop remarkable complications in other patients (Mynarek *et al.*, 2012).

The *MAN2B1* gene is physically localized at chromosome 19p13.2 and is causative of lysosomal storage disease (Menéndez-Sainz *et al.*, 2012). The *MAN2B1* gene contains a 21.5 kbp nucleotide sequence with 24 exons which encodes a polypeptide of 1011 amino acids (Riise *et al.*, 2012). The deficiency of alpha-mannosidase due to the *MAN2B1* mutations result in *MAN2B1* mutant proteins that have been found in lysosomes and endoplasmic reticulum (ER) as sub-cellular constituents. Therefore, the blockage in the degradation and deposition of glycoprotein occurs in lysosomes due to the deficiency of enzymes. Consequently, the process can impair cellular function and apoptosis (Hansen *et al.*, 2004; Kuokkanen *et al.*, 2011).

In alpha-mannosidosis, about 162 causative mutations of *MAN2B1* are documented in different ethnic groups worldwide (HGMD Professional 2020.4). The *MAN2B1* mutations are reported entirely in the gene, and

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these include missense, point mutation/insertions, splice-site, deletions, nonsense, and duplications. Although *MAN2B1* mutations are investigated widely nevertheless, there are three frequent mutations associated with the Caucasian population, which constitute 35% of alleles of alpha-mannosidosis (Riise-Stensland *et al.*, 2012). The correlation genotype-phenotype of *MAN2B1* has been reported with characteristics including localization of subgroups of genotypic/sub-cellular and scrutinizing the link between the subgroups and outcomes of biochemical and clinical measurements (Borgwardt *et al.*, 2015).

A Pakistani consanguineous family associated with broad phenotype spectrum was collected for genetic analysis in the present study. Autozygosity mapping by SNP-microarray detected three homozygous regions in the affected individuals of the family. The combination of whole exome sequencing (WES) and termination sequencing mapped a new missense mutation in *MAN2B1* gene. Co-segregation analysis established the mutation impeding in alpha-mannosidosis phenotype.

MATERIALS AND METHODS

Prior to starting the study, ethical approval was obtained from Quaid-i-Azam University and Shifa International Hospital Islamabad, Pakistan. The recruitment of human subjects followed the amended Helsinki Declaration (2013). Written informed consent was taken from each participant.

Recruitment of congenital family

A Punjabi family with neurological phenotypes was collected, and clinical examination was diagnosed with the neurological syndrome. This was a two-generation consanguineous family with five affected siblings along with unaffected family members. The pedigree was drawn, and the disease was consistent with an autosomal recessive pattern. There are two loops, one with consanguinity link, and other loop did not show consanguinity. The mother (III:2) of proband (IV:4) was 46 years has siblings (III:3 and III:4) with disease phenotype. The parents (III:1) of proband were paternal cousin of blood relation. The age of male siblings IV:1 and IV:4 was 21 and 12 years respectively, while female siblings IV:2, IV:3 and IV:5 were 18, 15 and 9 years, respectively. This family belongs to the Punjabi language-speaking group. Whole blood in EDTA vacutainers was obtained from 5 affected and 7 unaffected family members.

Genome wide linkage by microarray

Total genomic DNA extraction was carried out according to standard phenol-chloroform methods (Lázaro-Silva *et al.*, 2015). The genetic mapping was carried out

by single nucleotide polymorphism (SNP) genotyping and linkage analysis using CytoSNP-12v2.1 Illumina Human microarray. Data of genotype was analyzed by software Illumina Genome Studio Integrated Informatics Platform v3.10. Generally, SNPs data retrieved through CytoSNP-12v2.1 carries enormous SNPs, and thus consanguineous families can genotype of non-informative state. Initially, data were analyzed with block length by default, but it presented various negative results. Then block length was kept at around 700, reducing the false negative SNPs and scrutinizing the homozygous regions (affected individuals III:3, III:4, IV:4, and IV:5 shared the homozygous region) were mapped on chromosomes 16 and 19 (Fig. 1).

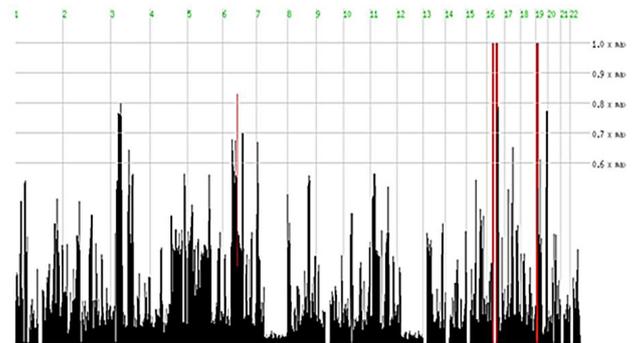


Fig. 1. SNP homozygosity mapping of alpha-mannosidosis family. The image is demonstrating three shared homozygous regions (red bars) which is a homozygosity by descent (HBD) on chromosome 16 and 19.

Whole exome sequencing in alpha-mannosidosis family

In the alpha-mannosidosis-affected family, the whole exome sequencing (WES) was carried out at the Wellcome Trust Sanger Institute or OtoGenetics Corporation (Agilent Sure Select V4 (51 Mbp) exome enrichment kit). Gene coding regions were captured using Sure-Select. Using Illumina HiSeq, the sequencing of the targeted genome sequence was done of all exons (50Mb), including a scalable platform, an automatable and scalable platform, and target enrichment solutions. In WES data, *MAN2B1* variant was considered for scrutinizing due to the relevant phenotype in the family. This was selected from the stratification of candidate variants by a web-based tools like Mutation Taster (<https://www.mutationtaster.org/>), which identifies the potential pathogenic variants along with other bioinformatics tools like SIFT (<https://sift.bii.a-star.edu.sg/>) and Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/index.shtml>). To confirm the variant identified by WES, the *MAN2B1* specific oligonucleotides of exon 22 (F-5'- CTCTCTCCCGCAGTTCTCAG-3'; R-5'- TCAATTTGCCCTTCTCACC-3') were used for PCR amplification. The PCR products were purified, and

sequencing analysis was done by sequencer ABI 3130XLA (Applied Biosystems). The Bioedit and CLC Viewer was used for sequence analysis.

In silico analysis of *MAN2B1* protein

The pathogenic effect of this mutation was determined by using Polyphen-2, SIFT, Mutation Taster and Provean tools. Protein homology by PYMOL analysis was carried out to determine the conformation change and function between the *MAN2B1* wild-type protein and mutated protein.

RESULTS

Clinical presentation

A boy of age 12 years (IV:4) showed features of intellectual disability and movement dysfunction. At birth and during the early developmental stage, the individual was presented as normal. Facial dysmorphism and musculoskeletal abnormalities were documented at the age of eight years. In this family, two sisters of the proband also showed comparable phenotypes. Familial history was also observed on the maternal side. The proband's parents were blood-related cousins, and the consanguinity was established in an autosomal recessive manner in one loop of the pedigree.

Physical examination showed that the affected boy was alert and oriented. He presented with movement sprints, problems in learning, thinking, feeling, hurdles in communication, and unusual behavior. He was also presented with moderate dysostotic multiplex with multiple infections. The characteristics of facial dysmorphism, including rounded eyebrows, flattened nasal bridge, and prominent forehead, were observed. No history of visual and hearing anomalies was available. Neurological assessments demonstrated delayed speech with progressive motor function loss. Other observed clinical features include ataxia, muscular weakness, and joint deformities. Altogether, these findings are suggestive of a complex syndrome pronounced to be cerebral palsy. Further, genetic analysis was recommended to point out the differential diagnosis.

Genome wide linkage analysis

SNP linkage analysis identified three homozygous regions shared by the four kindred of the family. These

regions include a 9.6 Mb region on physical location on chromosome 19p13.2, which was marked between SNPs rs4807526- rs8113506 SNP, while a 2.6 Mb region localized on chromosome 16p11.2 mapped between rs9926100-rs28483813 SNPs and a 21.0 Mb region was homozygous between SNPs rs8060373-rs13337095 localized on chromosome 16q11.2 (Table I).

In accordance with the phenotype of alpha mannosidosis, the homozygous segment on chromosome 19p13.2 (hg38, chr19: 3906868-13519929) carries an important *MAN2B1* gene (Fig. 2).

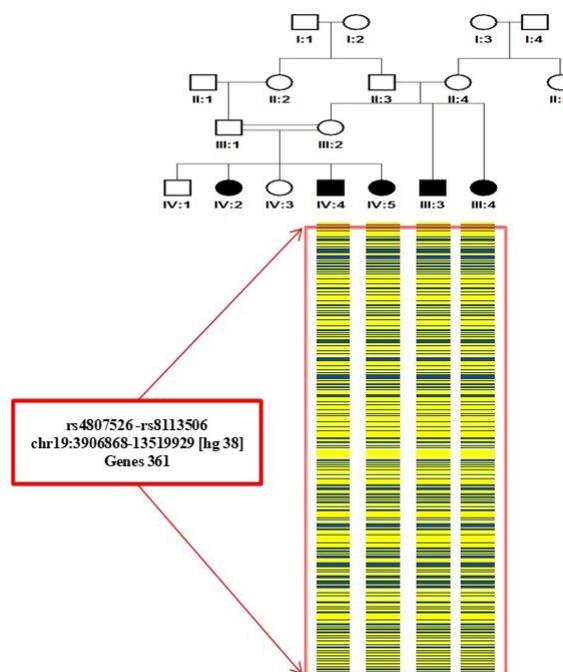


Fig. 2. Alpha-mannosidosis family pedigree presenting the homozygous region in affected individuals (III:3, III:4, IV:4 and IV:5). Representation of multi-generation pedigree of alpha-mannosidosis phenotype showing the autosomal pattern of the probands and siblings. Pakistani pedigree with alpha-mannosidosis, presented with pictorial genotypes which cover a nucleotide region of 9.4 Mb at chromosome 19 encompassing the disease locus (dashed blue boxed region).

Table I. In family with alpha-mannosidosis, the homozygous regions and gene information.

| Homozygous regions | Physical and band location on chromosome | Range of SNP region | | Genes covered in the region |
|--------------------|--|---------------------|------------|-----------------------------|
| | | Start | End | |
| 21 Mb | Chromosome 16; 16q11.2 | rs8060373 | rs13337095 | ~ 457 |
| 2.6 Mb | Chromosome 16; 16p11.2 | rs9926100 | rs28483813 | ~ 37 |
| 9.6 Mb | Chromosome 19; 19p13.2 | rs4807526 | rs8113506 | ~ 361 |

Whole exome sequencing and co-segregation analysis

WES and DNA sequencing identified a new missense nucleotide variation (c. 2710A>T; p.904Tyr>Ser) co-segregated precisely in the pedigree. To confirm the mutation, the ethnic matched ancestry of 50 chromosomes was analyzed and found negative for this mutation (Fig. 3). *In silico* tools like Polyphen-2, SIFT, Mutation Taster and Provean predicted the mutation (c. 2710A>T; p.904Tyr>Ser) as a pathogenic and having damaging effect.

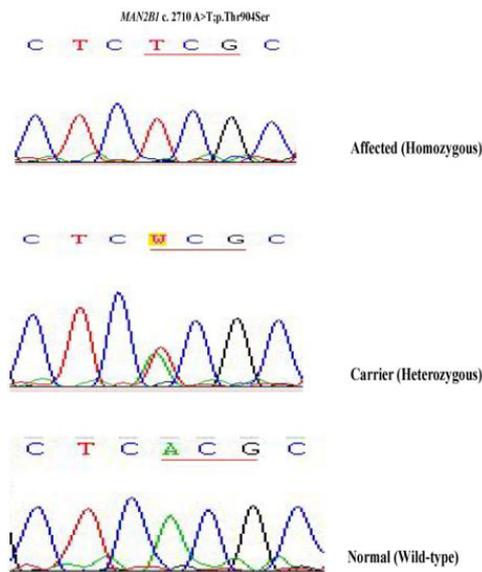


Fig. 3. DNA sequencing shows mutation in alpha-mannosidosis family. Electropherograms of sequencing represents (a) affected individuals (III:3, III:4, IV:4 and IV:5) were displayed to be homozygous for the *MAN2B1* variant c.2710A>T; p.904Thr>Ser (indicated), (b) heterozygous parents for *MAN2B1* variant c.2710A>T; p.904Thr>Ser, while and (c) unaffected sibling were wild-type.

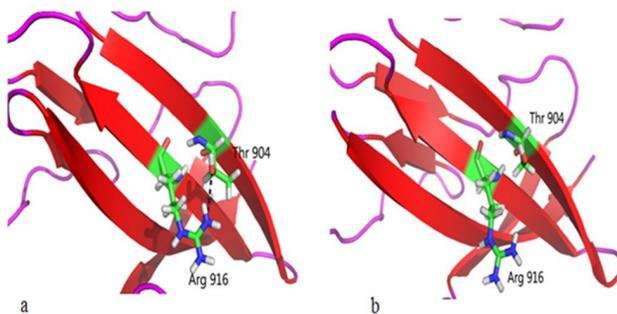


Fig. 4. The characterization of *MAN2B1* protein for *MAN2B1* mutation. In wild-type protein sequence, the amino acid Thr 904 established a hydrogen bond to amino acid Arg 916 residue in the same vicinity (a) while, the mutant sequence has loss the hydrogen bonding (b).

Protein homology analysis

The PYMOL software was run to compare the effect of *MAN2B1* protein between wild-type and mutant protein. In the wild-type protein, the amino acid Thr 904 forms a hydrogen bond with Arg 916 residue present in the vicinity. In the mutant type, there is a loss of hydrogen bonding between Thr 904 and Arg 916, resulting in a loss of function of *MAN2B1* (Fig. 4).

DISCUSSION

Alpha-mannosidosis is a rare metabolic storage defect due to α -mannosidase enzyme deficiency. It has diverse clinical characteristics which intellectual disability, motor activities dysfunction, hearing impairment, cerebral palsy, facial coarsening, immune incompetency, and facial musculoskeletal abnormalities. Other clinical presentations linked to this anomaly include macrocephaly and neuromuscular fault, cataract, and hepatomegaly (Borgwardt *et al.*, 2014; Kniffen, 2016).

The present study detected a novel *MAN2B1* (c.2710A>T; p.904Thr>Ser) in a complex family with two-generation-affected individuals, presented with diverse neurological features. In Pakistani population, there are very few studies investigated the identification of causative gene mutations in lysosomal storage disease like alpha-mannosidosis (Rafiq *et al.*, 2011). Due to the novelty of the mutation, the protein homology analyses may suggest the impact on alteration of protein function. The bioinformatics tools like Polyphen-2, SIFT, Mutation Taster and Provean confirmed the prediction of pathogenicity and deleterious effect. Protein functional analysis predicted that threonine (Thr904) develops hydrogen bonding in the same vicinity as arginine in a wild-type sequence (Arg916). On the other hand, in the mutant type of protein sequence, Thr904 with Arg916 carries a loss of hydrogen interactions consequences in conformational changes which lead to either diminished or partial protein function. Previous study also supports the result of current study where the molecular and structural analysis of novel mutations depicted the impact of variations at protein level. The common variant c.2248; p.T745R was studied for molecular docking and it was found that there is inefficient folding of protein due to the variant (Kuokkanen *et al.*, 2011).

A database of alpha-mannosidosis, more than 162 mutations associated with *MAN2B1* gene, almost 27% missense and 16% nonsense mutations are documented (<https://apex.jupiter.no/apex/f?p=101:5:4143948242291161::NO>). The most frequent mutation is c.2248C>T (p.Arg750Trp) documented in 74 cases in different ethnicities (Riise-Stensland *et al.*, 2012, 2015; Cathey *et al.*, 2013). Due to widespread distribution, analysis for

founder mutation was carried out in all positive c.2248T alleles (Borgwardt *et al.*, 2015), and haplotype analysis identified the phase-determined allele frequency of 72% similar genotype. The reduced association was possibly due to the high mutation frequency of microsatellite markers, resulting in different recombination events. Initially, the c.2248C>T allele was possibly associated with few ancestral haplotype origins, which spread as major haplotype by founder effects due to migration events throughout Europe and subsequently into the New World (Australia, Chile, and United States). By studying the disease alleles of Russian ancestry, the c.2248T allele frequency among the alpha-mannosidosis alleles reduces to other regional populations (Poland, France, Germany, The Netherlands, and Great Britain. This represents the declining gradient from East to West through Europe, suggesting that the c.2248T allele arose in Eastern Europe. The mutation identified in the Pakistani family is the second report, and five cases with *MAN2B1* mutations have been already reported from this population (Rafiq *et al.*, 2011; Riise-Stensland *et al.*, 2012).

In alpha-mannosidosis patients, the correlation of phenotype-genotype has been investigated, but no relationship has been established between the genetic mutations and the severity of the phenotype. There has been huge heterogeneity between genotype and phenotype, showing that clinical presentations are variable even with similar genetic mutations (Ara *et al.*, 1999; Malm and Nilssen, 2008; Govender and Mubaiwa, 2014; Wu *et al.*, 2014). However, a systemic study has been conducted to investigate the relationship between phenotype and the molecular basis of alpha-mannosidase deficiency. Interestingly, the considerable results indicated a link between the *MAN2B1* genotypes and cognitive function, forced vital capacity, balance, upper limb coordination, and CSF containing the oligosaccharides. This phenomenon integrated into the correlation is contingent on the localization of *MAN2B1* protein at the sub-cellular level (Borgwardt *et al.*, 2015; Riise Stensland *et al.*, 2016).

CONCLUSION

It is concluded that SNP-microarray and whole exome sequencing in combination identified a novel homozygous *MAN2B1* missense mutation (c.2710A>T; p.904Thr>Ser) in a complex neurological disease in a Pakistani family. The protein homology suggested the loss of hydrogen bonding interactions in the mutant protein. Although huge consanguinity has existed, the identification of *MAN2B1* mutations in inbred Pakistan. It may suggest that the diagnosis of complex neurological disorders is inappropriate due to lacking facilities in developing countries. Identification of mutation will be helpful for

the genetic counseling of the families and contribute to molecular diagnosis in the future.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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