Whole exome sequencing identifies a novel mutation in spermine synthase gene (SMS) associated with Snyder-Robinson Syndrome

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Abstract

Background: Mutations in the spermine synthase (*SMS*) gene have been reported to cause a rare x-linked intellectual disability known as Snyder-Robinson Syndrome (SRS). Besides intellectual disability, SRS is also characterized by reduced bone density, bone deformities, osteoporosis and facial dysmorphism. SRS phenotypes evolve with age from childhood to adulthood.

Methods: Whole exome sequencing was performed to know the causative gene/mutation. Later we confirmed the mutation through sanger sequencing. Furthermore, we also performed the mutational analysis through HOPE SERVER and SWISS-MODEL. Also, radiographs were also obtained for affected individual to confirm the disease features.

Results: In this article, we report the first Pakistani family consisting of three patients with SRS and a novel missense mutation in the *SMS* gene (c.905C >T: p.S302L). In addition to the typical phenotypes, one patient presented with epilepsy from an early age that was characterized by generalized seizures. The clinical, genetic and in-silico analysis, review of the literature links the affected patients of the family with Snyder-Robinson syndrome and mutation affects the spermine synthase activity

Conclusion: A novel missense mutation in the *SMS* gene (c.905C >T: p.S302L) causing Snyder-Robinson Syndrome (SRS) reported in Pakistan Family.

Background

Polyamines are organic compounds having more than two amino groups. At neutral pH, they exist as ammonium derivatives. These are polycations that can interact with negatively charged particles, i.e. DNA, RNA and some negatively charged proteins.

Polyamines play an essential role in cell growth, survival and proliferation. As adding half of the polyamines results in the activity of spermidine synthase to convert putrescine into spermidine and spermine synthase to convert spermidine into spermine [1–3].

Snyder-Robinson syndrome is a rare disorder with an unknown prevalence. Worldwide, around ten families, segregating this disorder, have been identified so far. Other names for this disorder include X-linked mental retardation syndrome, Snyder-Robinson type, X-linked mental retardation, SRS and spermine synthase deficiency (Genetics Home Reference). Snyder-Robinson syndrome (SRS) caused by a mutation in the gene (*SMS*) encoding for spermine synthase. This is an X-linked disorder first time identified in 1969 [5]. The phenotype was better defined in a re-evaluation of the original family, and linkage analysis localized the related gene to Xp21.3–p22.12 [6].

Intellectual disabilities (ID), muscle hypoplasia, dysmorphic faces, seizures, gait abnormalities, and osteoporosis are the symptoms and clinical features of SRS. In the affected individuals, *SMS* hemizygous mutation results in reduced activity of *SMS* activity and decreased spermine-spermidine ratio [7].The daily

life routine, of the individuals suffering from SRS, is significantly disturbed, also having atraumatic osteoporotic features. Osteoporosis which means "porous bones" is a disease in which density and quality of the bone are reduced. This arises when the equilibrium of osteoclastic bone reabsorption and osteoblastic bone with a missense mutation in *SMS* (gene).

In the present research, we report the investigations of a consanguineous family (SRS1) from Vehari District, Punjab Province, Pakistan, segregating SRS in a pattern, which is consistent with X-linked recessive inheritance. We report a missense mutation in this family. This is the first case reported from Asia.

Results

The results of the Sanger test show two patients (VI:1 and VI: 2) in the pedigree are hemizygous for NM_004595: exon9: c.905C>T: p.S302L; the mother and sister (VI:5, V:2) are heterozygous; the father (V:1) and grandmother (paternal side) (II:2) are normal. These results indicate that the rare SNV (NM_004595: exon9: c.905C>T: p.S302L) is co-segregates with the patients' phenotypes. Since only male carriers showed disease phenotypes, the inheritance pattern of this disease matches XLR.

Clinical details Patient (VI: 1)

The Proband (VI: 1) is a boy, who is 18-years-old born to healthy parents, with a consanguineous marriage, who don't have any family history of bone deformities and intellectual disability. His birth weight and occipitofrontal circumference (OFC) were 2.20 kg and 34 cm, respectively. He cannot stand and walk, only move by crawling. He has global developmental delay. He has bulging (pigeon-like chest) with no other facial dysmorphic features. The patient exhibited severe dysarthria but did not complain about any visual and auditory problems **(Table 1).**

Patient (VI: 2)

The patient (VI: 2) is the 8-year-old boy with the complaint of severe pain in bones, hypotonia, regression and lost motor skill in the first two years of life. An EEG at 14 months of age showed generalized slowing and later on, manifested seizures. He had walking problems at an early age. He has multiple a traumatic fracture in tibia, femur and humerus **(Table 1).**

Patient (VI: 3)

Unfortunately, this patient (VI: 3) died during the study. By the time of his death, he was 10-year-old, and brother of the patient (VI: 1 and VI: 2) and born after an uneventful pregnancy. His weight and OFC were 2.27 kg and 37 cm respectively at birth. He had facial dysmorphic features including a long oval, midface hypoplasia. He had been suffering from respiratory secretions. He had frequent seizures, hypotonia, decreased muscle bulk, and flexion contraction of the large and small joints. He was not able to stand independently and could only move by crawling. He had skeletal problems, including bone

fractures of his distal fibula and spine problem. An EEG of the patient manifested slowing background at 14 months of age with no other abnormalities (**Table 1).**

Genetic and Biochemical Analysis

A mutation, (NM_004595: exon9: c.905C>T: p.S302L), in the *SMS* gene in the index patient, was identified through the analysis of the data obtained by whole-exome sequencing. This mutation was later confirmed by Sanger sequencing, and we found that this mutation was also present in the carrier mother and a carrier sister **(Fig. 2).**

In-Silico Analysis

The 3D-structure of our protein of interest was already known. We were interested in the mutation of a Serine into a Leucine at position 302. The schematic structures of the WT (left) and the mutant (right) amino acids are shown in **figure 3.** The backbone is coloured red, which is the same for each amino acid. The side chain is black coloured, which is unique for each amino acid.

Each amino acid is unique because of its specific charge, size, and the hydrophobicity-value. Regarding these properties, the original (WT) residue and the newly introduced mutant residue are often different. Mutation results in the formation of a bigger residue compared to the WT residue. The mutant residue has more hydrophobicity than the WT residue. The effects of the mutation were evaluated based on the contacts made by the mutated residue, structural domains where the residue is located, modifications in this residue and known variants for this residue.

The WT residue forms a hydrogen bond with Isoleucine at position 298. The disparity in size between the WT and mutant residue results in the new residue not being in the correct position to make the same hydrogen bond as the original WT does. The hydrophobicity difference may influence hydrogen bond formation.

The mutation lies within a domain, annotated as PABS in UniProt. The amino acid, which is introduced as a result of mutation, has different properties, which can abolish the domain's function.

The mutated residue is found in a domain, which is essential for protein activity and interactions with other domains that is also a vital part of the activity. Possible mechanisms for the disruption of normal activity are 1. There is a variation in size of WT and mutant amino acid; 2. The mutant residue has a larger size than the original residue; 3. The WT residue was buried in the protein core, but as the mutant residue is bigger so, it will not fit probably; 4. The hydrophobicity of the WT and the mutant residue is varied; 5. Loss of hydrogen bonds in the protein core will occur because of this mutation, and thus the proper folding will be disturbed.

Discussion

Only ten mutations have been related to Snyder-Robinson syndrome so far [6, 14, 15]. Here we identify the first Pakistani family with a novel mutation in the *SMS* gene, which expands the phenotypes and focuses on the characteristics of SRS. The mutation was tested in controlled samples (N=100) and not presented in population.

In this family, patients presented all the clinical features previously described in SRS [6], such as ID, facial dysmorphic features, including long oval midface hypoplasia and bone deformities **(Fig. 4).** The normal function of the *SMS* gene provides instruction for making an enzyme called spermine synthase, and this enzyme function s in the production of spermine. Dimerization is essential for the normal function of the spermine synthase, and the N-terminal domain plays an important role in the dimerization process.

The reported mutation p.S302L is the substitution of amino acid residue serine by a residue leucine. The mutation site S302 is buried in the protein interior. As it is shown in **Fig. 3D** and **Fig. 3F,** the structure around the S302 mutation site is present in a very packed and conserved area, and there is no space to adjust the amino acid leucine. This mutation affects protein stability, resulting in the loss of hydrogen bonding in the protein core and affects the dimerization process that can reduce the normal level of spermine synthase in the body.

The overall study revealed the molecular mechanism of the *SMS* gene, causing mutation. As most of the SUMOylation sites follow a canonical consensus motif of ψ-K-X-E/D (ψ, a hydrophobic amino acid, such as A, I, L, M, P, F, V or W; X, any amino acid residue); this protein has the motif (ψ-K- X-E/D), it may be the target of SUMOylation. The motif is an amino acid sequence, and interestingly, *SMS* has that motif, and the mutant site is located very close to the motif. (297-LILDLS/LMKV**LKQD**-309, the S of S/L is WT type, L is the mutant, the bold is the SUMOylated candidate motif) [16, 17]. Moreover, the mutation site is highly conserved among the species **(Fig. 3F).** Therefore, it can be said that this mutation can significantly affect spermine stability. The described mutation causes the structural integrity of the protein and halts the protein folding, which leads to protein degradation. The degradation of *SMS* proteins leads to the X-linked recessive Snyder-Robinson Syndrome.

Conclusion

In conclusion, only few mutations have been reported to Snyder-Robinson syndrome. we identify the first Pakistani family with a novel variant in the *SMS* gene, which expands the phenotypes.

Abbreviations

SRS: Snyder-Robinson Syndrome

ID: Intellectual Disability

WES: Whole Exome Sequencing

SMS: Spermine Synthase

WT: Wild Type

PDB: Protein Data Bank

Declarations

Ethics approval and consent to participate

The study was approved from research and ethical committee, International Islamic University, Islamabad, Pakistan, under the case No. IIU (BI & BT) FBAS-2017 and all the experiments were performed after taking the written informed consent of the subjected family and written consents were obtained from the parents or legal guardians of participant under the age of 16.

Consent for publication

Written informed consent for publication of clinical details and/or clinical images was obtained from all of the participants. Individuals younger than the age of 18, consent for publication were obtained from their parents or legal guardians.

Availability of data and material

The patients' data are available from the corresponding authors on request.

Competing interests

The authors declare that they have no competing interests.

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Authors contribution

TJQ, JY and HMJH designed the research and QW and JY performed the experiments; AA and DL provided reagents and physical help; TJQ and AM wrote the manuscript and analysed the data; IB helped in the family recruitment, phenotypic findings and getting the X-ray images; HQ approved the draft and supervised the overall research. All the authors read, revised and approved the final version of the article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial interest. So, the authors declare no conflict of interest

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Table 1

Table 1 Clinical representation of affected individuals in family

Clinical features	Patient 1 (VI:1)	Patient 2 (VI:2)	Patient 3 (VI:3)
Age	18	8	10
Intellectual disability	+ (mild)	+ (mild)	+ (mild)
Bone abnormality	+	+	+
Prominent lower lip	-	+	+
Speech abnormalities	Echolalia	Slow	Slow
marfanoid habitus	-	-	-
Ambulatory difficulties	limited	limited	limited
Low muscle mass	+	-	-
Kyphscoliosis	+	-	-
High narrow or cleft palate	+	+	+
Facial asymmetry	-	-	-
Unsteady gait	-	-	-
Long toes	+	-	-
hypotonia	-	-	-
Nonspecific movement disorder	-	-	-
Seizures	+	+	+
Long hands with large fingers	+	-	-

Figures

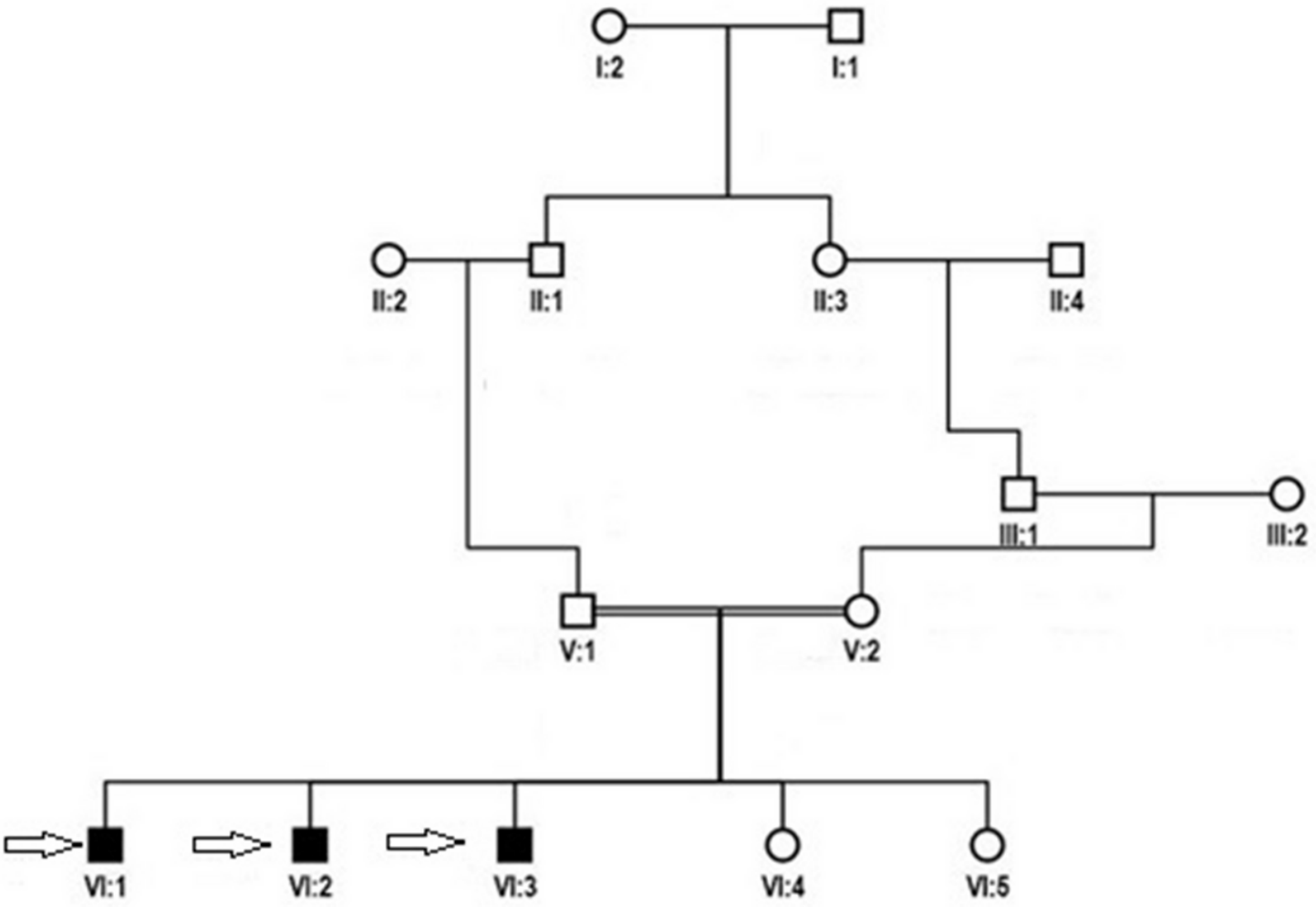

Figure 1

Family pedigree and Sanger sequencing confirmation of the novel c.905C>T SNV variant. Black symbols represent affected individuals. The index patients are indicated with an arrow.

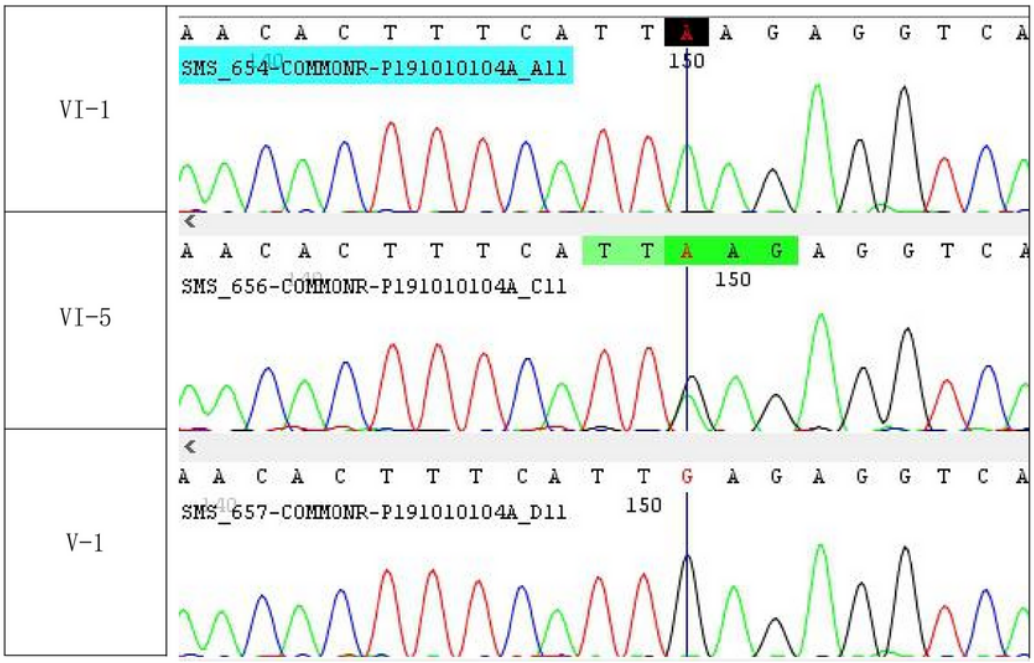

Figure 2

Sequence chromatograms of SMS from the family contains normal individuals (V:1), an obligate carrier (VI:5) and an affected individual (VI:1). A straight line indicates the position of the mutation (c.905C>T) on chromatogram.

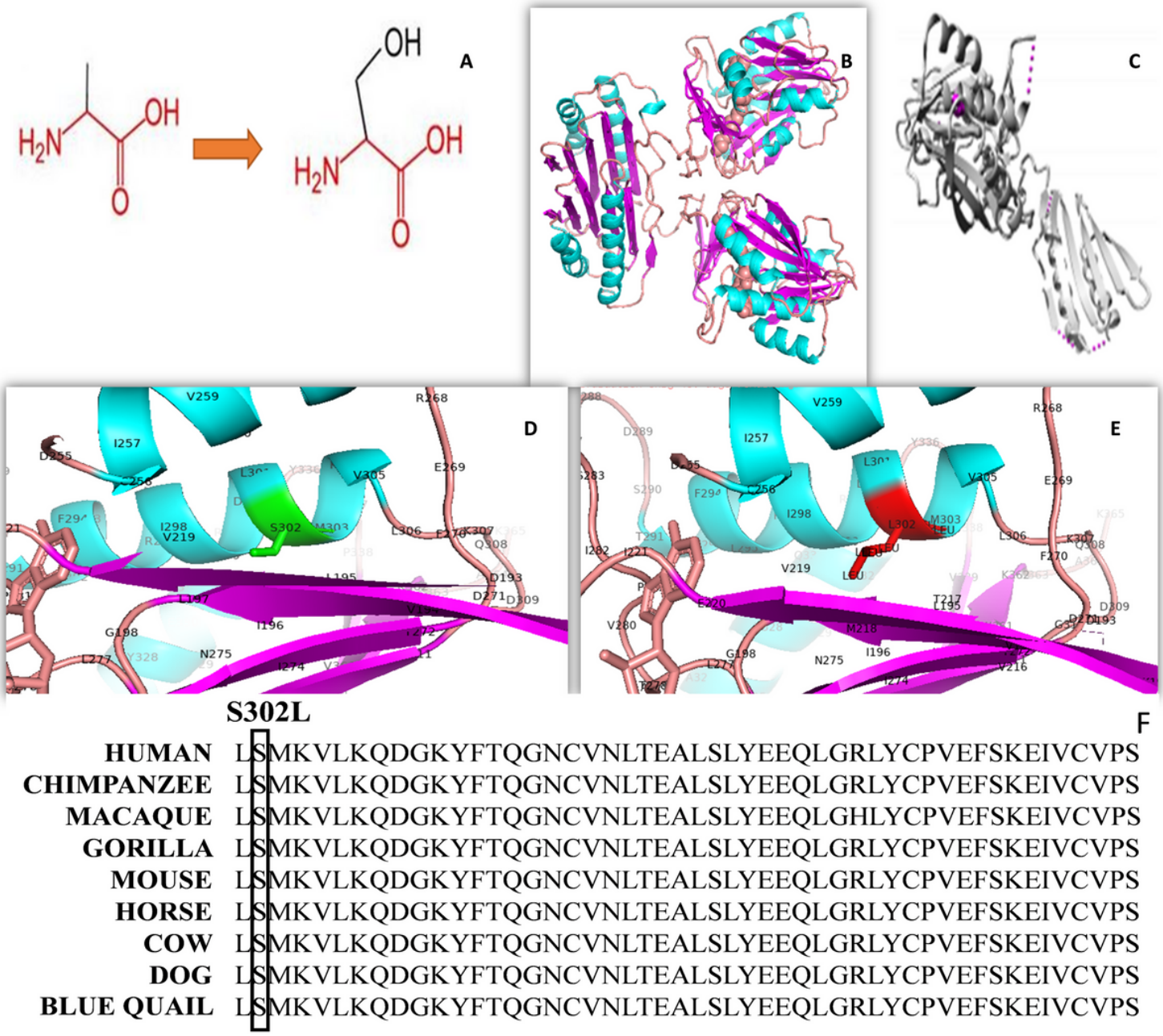

Figure 3

Structural information of SMS protein. (A) The mutant residue is bigger than the wild-type residue. (B) 3D structure of wild-type of Human SMS protein in ribbon presentation. Helices (shown in cyan color), Sheets (shown in magenta color) and loops (shown in orange color). (C) Overview of the protein in ribbon-presentation. The protein is colored grey, the side chain of the mutated residue is colored magenta and shown as small balls. (D) Zoomed 3D structure of wild-type of human SMS in ribbon presentation Helices (shown in cyan color), Sheets (shown in magenta color) and loops (shown in orange color). Serine is present at position 302 shown in green color. (E) Zoomed 3D structure of mutant SMS of human in ribbon presentation Helices (shown in cyan color), Sheets (shown in magenta color) and loops (shown in orange color). Serine is replaced by Leucine at position 302 shown in red color (F) Sequence alignment of SMS gene among different species. In the human sequence, amino acids from 301-360 are shown. The

mutation site considered in this study was showing complete conservation among different species. Multiple sequence alignment is performed with Clustal Omega protein alignment tool.

Figure 4

Frontal radiograph of the pelvis shows increased bone density, trabecular thickening and ossification of the sacrotuberous ligament. Mild flattening of the acetabular roof is noted giving rise to champagne glass deformity of the pelvis. (B) Both lung fields are clear Bilateral CP angles are sharp. Mediastinal contours appear unremarkable. Scoliotic deformity of the spine is noted with convexity towards right side. (C) Outward bowing of bilateral femur is noted. (D) Bilateral fibula shows marked thinning and outward bowing (E, F) outward bowing of bilateral humerus bone is also noted. (G) Long oval face hypoplasia and bone deformities.