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A Novel Missense Variant in the *CHST3* Underlies Spondyloepiphyseal Dysplasia with Congenital Joint Dislocations

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Keywords
Spondyloepiphyseal dysplasia with congenital joint dislocations · Exome sequencing · Sanger sequencing · *CHST3* · Novel variant

Abstract
Background: Spondyloepiphyseal dysplasia (SED) is characterized by skeletal dysplasia and multiple joint dislocations. SEDs encompass various types, such as SED congenita, SED tarda (SED-T), SED with congenital joint dislocations (SED-CJD), SED stanescu, and SED-T with progressive arthropathy. ***Methods and Results:*** In the present study, we clinically and genetically characterized a consanguineous Pakistani family with SED-CJD. The affected member showed large joint dislocation, spinal deformities, and previously unreported facial features. Exome sequencing followed by Sanger sequencing revealed a missense variant, [c.601T>A; p.(Tyr201Asn)], in the *CHST3*. ***Conclusion:*** This study has not only expended the mutation spectrum in the gene *CHST3* but also will facilitate diagnosis and genetic counseling of related features in the Pakistani population.

Introduction

Spondyloepiphyseal dysplasia (SED) with congenital joint dislocations (OMIM #143095) represents a distinctive type of skeletal dysplasia. It is distinguished by severe short stature, malformed and dislocated joints, along with progressive kyphosis. The cardinal features of the condition are dislocations of the hip, knee, and elbow joints, with equinovarus or equinovalgus foot deformities. Craniofacial anomalies include hypertelorism, prominence of the forehead, a depressed nasal bridge, and a flattened midface. Cleft palate and short stature are often associated features. Spinal anomalies include scoliosis and cervical kyphosis [1, 2].

SEDs are a group of hereditary skeletal deformities, primarily affecting cartilage maintenance and bone growth. One form of this condition, SED tarda (SED-T), exhibits a diverse genetic inheritance pattern, manifesting in three different forms. The autosomal dominant form is linked to variants in the *COL2A1* gene, while the autosomal recessive form is associated with abnormalities in the *CCN6* gene. Additionally, there is an X-linked-form of SED-T related to variants in the *TRAPPC2* gene. Beyond SED-T, there are other types of SEDs, such as SED type C

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and SED type S, which are either autosomal dominant or occur sporadically due to the variants in the *COL2A1* gene. Another distinct type, SED Omani, is specifically caused by variants in the *CHST3* gene.

Here, we present a case of a family with clinical features representing an SED with congenital joint dislocation (SED-CJD). Clinical examination revealed a few previously unreported facial features in the affected member. Exome sequencing followed by Sanger sequencing identified a novel missense variant, [c.601T>A; p.(Tyr201Asn)], in the *CHST3* gene.

Materials and Methods

Ethical approval, to conduct the study, was provided by the Institutional Review Board (IRB) of the Women University of Azad Jammu and Kashmir Bagh Pakistan (IRB-WUAJ&K-4560). Consent forms were signed by all those who participated in the clinical and genetic analysis of the family. Blood samples from affected and unaffected individuals of the family were collected in EDTA vacutainer tubes. Genomic DNA from the blood samples was extracted using the Sigma's GenElute™ Blood Genomic DNA Kit (Sigma Aldrich, CA, USA). Extracted DNA was quantified using the Nanodrop-1000 spectrophotometer (Titertek Berthold, Germany).

Exome Sequencing

Exome sequencing was performed on the genomic DNA of the affected individual (IV-1). Barcoded libraries were pooled and sequenced using the capture kit of the SureSelect v6 on Illumina HiSeq 2000 platform with 54× average on-target coverage (Macrogen Inc; Seoul, South Korea). Burrows-Wheeler Aligner software package was applied for the alignment to the reference human genome assembly (GRCh38/Hg38) [3]. Indel realignment, duplicate exclusion, quality recalibration, variant calling, and detection were conducted by means of the Picard and Genome Analysis Toolkit (GATK). ANNOVAR (version: 2019Oct24) was used to annotate the data set. Variant selection criteria included CADD-phred (≥13), minor allele frequency (MAF; <0.001), splice sites (±12bp), and exonic variants. Sanger sequencing was performed using a commercially available BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA, USA). Primers for the *CHST3* gene were designed using the Primer3 program (http://frodo.wi.mit.edu/primer3). Specificity of the primer pairs was analyzed using NCBI primer-BLAST (https://www.ncbi.nlm, nih.gov/tools/primer-blast/). Sequence variants were identified via the BIOEDIT sequence alignment editor, version 6.0.7 (Ibis Biosciences, Carlsbad, CA, USA). The pathogenicity score for the identified variant was determined using MutationTaster (http://www.mutationtaster.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/).

Structural Modeling

The amino acid sequence of CHST3 protein was obtained from the UniProt database (identifier: Q7LGC8). For in silico analysis, 3D structure of CHST3 was used for assessment of amino acid alteration in the variant p.(Tyr201). The altered region of reference structure was restored, and variation was generated via DynaMut 2 [4].

Results

The family, presented here, is recruited from AJ&K, Pakistan, a region contributing 3.04% of the overall mutational spectrum of the Pakistani population [5]. The family had one affected member born from two healthy Pakistani consanguineous parents (Fig. 1a). Patient had one sister who is reported to be healthy. The affected member was born with normal delivery with birth defects including club foot and joint dislocations. Birth weight of the affected individual was normal and cried immediately after birth and did not report to have any breathing difficulty or noisy breathing. At the time of clinical evaluation, the proband, a 20-year-old male, presented with distinct physical characteristics. His stature was notably below average with a height of 110 cm, and he weighed 35 kg. His head circumference was measured at 65 cm. Notable facial features included a broad, high forehead, a broad nasal tip, and hypertelorism, characterized by an outer intercanthal distance of 104 mm and an inner intercanthal distance of 40 mm. Additionally, a prominent philtrum was observed. Despite these physical manifestations, he was mentally normal, displaying no cognitive or developmental delays. The family history did not reveal any similar cases or relevant genetic conditions. Both parents, along with his younger sister, were reported to be in good health, with normal mental development and average stature.

The affected individual manifested short stature, congenital joint subluxations/dislocations with multiple joint deformities, and limited range of movement. The affected individual was using wheelchair at presentation. Broad/spatulate thumbs, broad big toes, and flat feet were observed in our patient. There was loss of normal curvature in the cervical spine. Excessive kyphosis and lordosis were seen in the dorsal spine and at lumbosacral junction, respectively. There was scoliosis of the dorsolumbar region (Fig. 1b–d). X-ray of the individual manifested dislocation at the elbow with cubitis valgus, accessory ossification centers seen at the elbow joint, and mild inferior subluxation at the hip joint; pelvic bones appear normal (Fig. 1e, f).

Complete blood count values examined were present in the normal range. The peripheral film revealed anisocytosis, hypochromic, and microcytic elliptical cells.

Genetic Analysis

The affected individual's (IV-1) genomic DNA was undergone for exome sequencing. Considering the pedigree, homozygous and compound heterozygous variants were prioritized for further analysis. The variant filtration criteria were based on CADD-phred (≥13), gnomAD frequency (≤0.005), exonic, and splice site

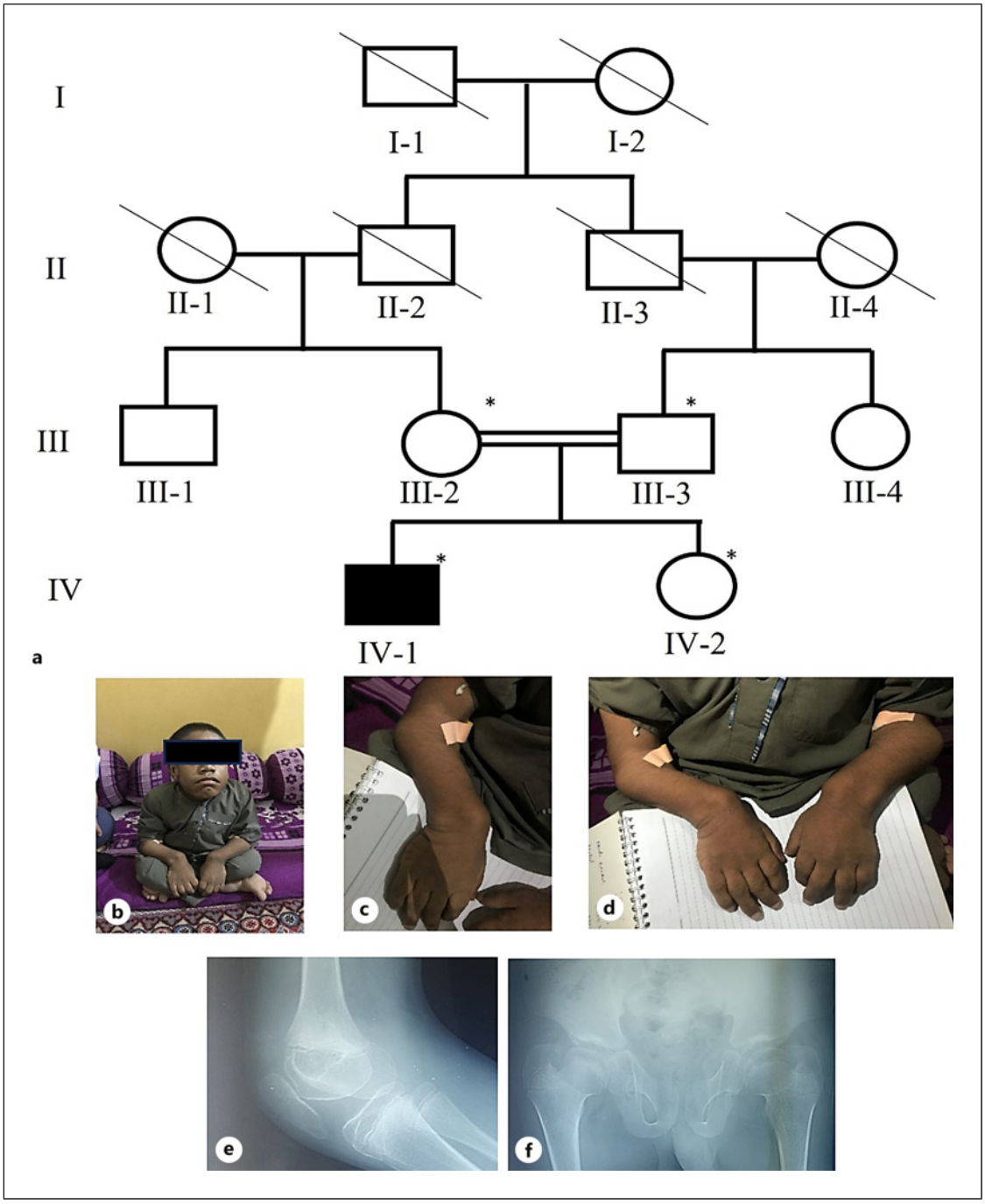

Fig. 1. a Pedigree drawing of a family segregating SED-CJD in autosomal recessive manner. Filled symbols represent affected and unfilled normal members in the family. Asterisks indicate the subjects who were clinically and genetically examined. **b**–**d** Pictures of the affected individual manifesting short stature and facial and limb anomalies. **e**, **f** X-ray of the affected individual manifesting elbow and hip dislocations.

(±12bp) variants. In our study, we identified a novel variant in the *CHST3* gene, [c.601T>A; p.(Tyr201Asn)]; Figure 2a. This variant is being associated for the first time with SED-CJD, while the *CHST3* gene has been previously linked to SED [6]. In the family, the affected individual was found to be homozygous for the condition, while both parents were carriers. This suggests that the variant is segregated within the family. The variant, found

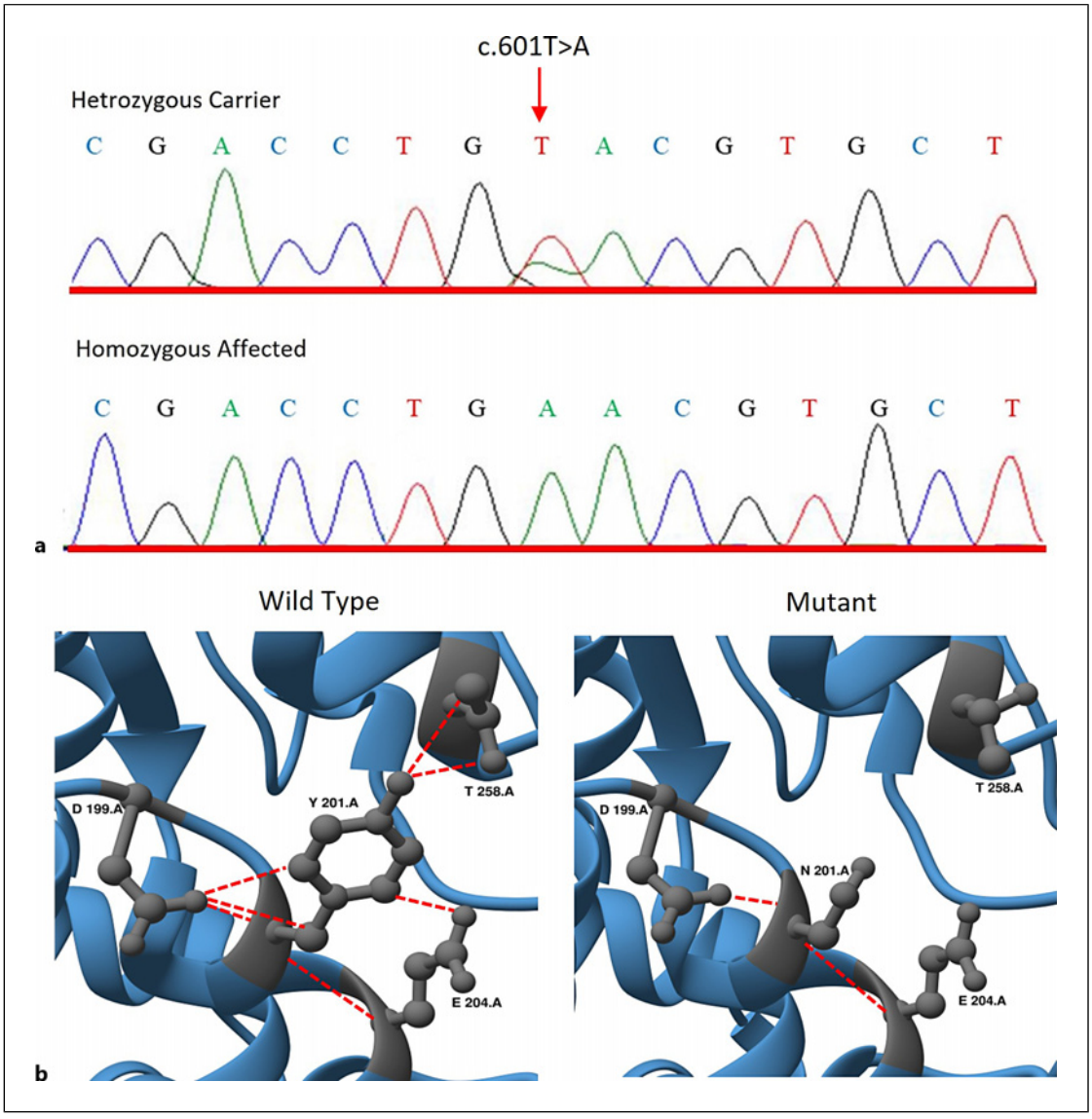

Fig. 2. a Sequencing chromatograms showing nucleotide sequence in the carrier individual (upper panel), affected (lower panel). **b** Homology modeling of wild-type and mutant protein showing bonding between different amino acids. Red lines represent hydrogen bonds.

in present study, was absent in the human variation databases, including 1000 Genomes, ExAC, gnomAD, and 100 ethnically matched control individuals.

Structural Analysis of Wild- and Mutant-type CHST3

In silico simulation of mutant- and wild-type CHST3 predicted stability change of −0.39 kcal/mol which is destabilizing. Wild-type Tyr201 makes a hydrogen bond with Glu204, Asp199, Thr258, shown in red dashes (Fig. 2b). Mutant Asn201 loses hydrogen bonds with Thr258 (Fig. 2b). It is highly likely all these loss of hydrogen bonding interactions may disrupt proper protein folding and interaction with other molecules which subsequently alter the function of CHST3.

Discussion

CHST3 encodes chondroitin 6-O-sulphotransferase, an enzyme pivotal in catalyzing sulfation within proteoglycans present in the extracellular matrix of cartilage. Sulfation is a crucial modification in several instances of

Table 1. Previous studies in *CHST3* with detailed phenotypes and mutation types

Studied by	Dislocations			Spinal deformity		Facial anomalies	Variant types
	elbow	hip	knee	scoliosis	kyphosis		
Hermanns et al. [2]	6/6	4/6	6/6	4/6	3/6	PFH	7 missense 1 deletion
Singh et al. [6]	9/9	5/9	2/9	8/9	8/9	–	4 missense 1 frameshift
Albuz et al. [12]	1/1	0/1	1/1	1/1	0/1	–	1 nonsense
Searle et al. [13]	0/1	1/1	1/1	0/1	0/1	–	Duplication
Tuysuz et al. [14]	1/3	0/3	0/3	3/3	1/3	–	Missense
Unger et al. [15]	15/21	14/21	19/21	5/21	9/21	PFH, DNB	14 missense 1 insertion 1 deletion
Kausar et al. [16]	0/8	4/8	3/8	2/8	2/8	–	2 missense 1 nonsense
Srivastava et al. [17]	2/3	2/3	2/3	2/3	2/3	–	1 missense 1 insertion
Waryah et al. [18]	0/10	7/10	0/10	3/10	6/10	–	Nonsense
Ranza et al. [19]	0/1	1/1	1/1	1/1	0/1	–	Deletion
Muys et al. [20]	1/1	0/1	1/1	0/1	0/1	–	Missense
Tanteles et al. [21]	0/2	0/2	1/2	1/2	1/2	–	Missense
van Roij et al. [22]	0/2	2/2	1/2	1/2	1/2	–	Missense
Rajab et al. [10], Thiele 2004 [23]	N/A	1/9	N/A	9/9	9/9	–	Missense
Song [24]	N/A	N/A	N/A	N/A	N/A	N/A	Regulatory
Duz and Topak [25]	6/8	6/8	8/8	7/8	4/8	–	3 missense 1 nonsense
Çetin et al. [26]	0/1	1/1	1/1	1/1	1/1	–	Deletion
Liang et al. [27]	0/1	1/1	0/1	1/1	1/1	–	Compound Het
Current study	1/1	1/1	N/A	1/1	1/1	HP, DNB, PFH	1 missense
Total	42/79	50/79	47/79	50/79	49/79		

HP, hypertelorism; DNB, depressed nasal bridge; PFH, prominent forehead; –, absent.

biological recognition. Carbohydrate sulfotransferases are the enzymatic agents that facilitate the transfer of sulfate to carbohydrate groups in glycoproteins. The carbohydrate sulfotransferase family plays a significant role in the sulfation process of keratan, dermatan, and chondroitin sulfate (CS) structures [7]. CS exhibits a linear polymer structure comprising repetitive, sulfated disaccharide units containing glucuronic acid and N-acetylgalactosamine (GalNAc). In mammalian tissues, the predominant CS carries sulfate groups positioned at either 4 or 6 of GalNAc residues. Specifically, chondroitin 6-sulfotransferase (C6ST) is responsible for transferring sulfate from 3-prime-phosphoadenosine 5-prime-phosphosulfate to the 6th position of the GalNAc residues [8, 9].

CHST3 has been associated with different skeletal disorders, including autosomal Larsen syndrome and SED-CJD. Both disorders have overlapping clinical features. Most of the features associated with SED-CJD include short stature, severe progressive kyphoscoliosis, severe arthritic changes with joint dislocations, rhizomelic limbs, genu valgum, cubitus valgus, mild brachydactyly, camptodactyly, and microdontia [10]. The

Larsen syndrome has been reported to be associated with the features, including joint hyperextensions and bifid humerus, clubbed feet, facial features such as depressed nasal bridge, widely separated eyes, prominent forehead, abnormalities in the hands and fingers, and polyhydramnios [11]. The main difference between the two conditions is the absence of typical facial features in SED-CJD. In the study of 79 probands with underlying SED, the most frequently observed joint dislocation is in the hip, followed by the knee and then the elbow. In terms of spinal deformities, kyphosis is reported in 49 out of the 79 cases, while scoliosis is observed in 50 out of the 79 cases (Table 1). In the Larson syndrome, facial features have been reported only in the dominant form caused by the variants in the *FLNB* gene [12, 13].

The novel missense variant [c.601T>A; p.(Tyr201-Asn)], detected in the present study, is located in the sulfotransferase domain. Sulfotransferase domain catalyzes the modifying step of CS synthesis by transferring sulfate to the C-6 position of the GalNAc of chondroitin [14]. The wild-type Tyr201 forms hydrogen bonds with Glu204, Asp199, and Thr258, maintaining structural integrity. Asn201 disrupts the bond with Thr258, as shown in Figure 2b, potentially leading to structural rearrangements. This loss of hydrogen bonding could result in improper protein folding and altered interactions with other molecules, impacting CHST3's function. Such structural and functional deviations are significant as they might contribute to disease pathogenesis, particularly in conditions where CHST3 plays a crucial role. In previous functional studies, missense variants detected within the sulfotransferase domain are implicated in disrupting the normal function of CHST3. These variants significantly contribute to a notable reduction in C6ST activity while maintaining normal levels of C4ST activity in fibroblast cells from homozygous patients compared to control groups [15]. In previous investigations, a stop gain variant denoted as Y201X was identified in the Spanish and Pakistani populations [2, 16]. This particular codon alteration is associated with and underlies SED. This suggests that the variant identified in the current study may indeed disrupt the proper function of the CHST3 enzyme.

In conclusion, our reported missense variant in the *CHST3* gene contributes to expanding the mutation spectrum. This expansion, in turn, enhances our ability to diagnose and provide effective genetic counseling for families displaying features associated with SED-CJD within the Pakistani population.

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Statement of Ethics

The IRB of the Women University of Azad Jammu and Kashmir Bagh Pakistan approved the research study (IRB-WUAJ&K-4560). Written informed consent was provided by all individuals who were over the age of 18 years. For individuals who were minors, informed consent was obtained from their parents.

Conflict of Interest Statement

We declare that we have no conflict of interest.

Funding Sources

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Author Contribution

Tafail Akbar Mughal, Muhammad Asim, and Syed Haseeb ul Hassan Gillani carried out the experimental work in the laboratory. Tafail Akbar Mughal wrote the manuscript. Nimra Obaid Chughtai, Syeda Afifa Batool, and Kulsoom Shujaat sampled the families, performed the clinicals, and analyzed the data. Syed Zohaib Tayyab Gilani designed the study, provided funds, and finalized the manuscript.

Data Availability Statement

Data will be available upon request.

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