Spondylo-epi-metaphyseal dysplasia with brain malformation: a milder form of *GPX4* gene related Sedaghatian skeletal dysplasia

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ABSTRACT
We were referred a male child aged 4 years and 9 months with intellectual disability due to microcephaly and brain malformation and severe short stature. After whole-exome sequencing, he was found to be homozygous for a likely pathogenic missense mutation p.R179H in the GPX4 gene. Sedaghatian skeletal dysplasia (SSD) (MIM250220) was described as a lethal neonatal disease with short limbs, platyspondyly, cardiac conduction defects and central nervous system abnormalities. Amorph mutations in the GPX4 gene have been identified in the severe lethal phenotype. The p.R179H mutation, a putative hypomorph has also been reported in two other living children of Indian origin with the diagnosis of Sedaghatian skeletal dysplasia. The patient was diagnosed to have a variant or milder form of Sedaghatian skeletal dysplasia. This case report thus expands the clinical spectrum of Sedaghatian skeletal dysplasia.

KEYWORDS: Sedaghatian skeletal dysplasia, *GPX4*, Spondylo-epi-metaphyseal dysplasia

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INTRODUCTION

Sedaghatian skeletal dysplasia (SSD) is a genetic disorder with spondylo-epi-metaphyseal skeletal dysplasia, with multisystem effects such as brain malformation, cardiac (myocarditis or arrhythmias), renal (hemorrhage) or pulmonary dysfunction (Sedaghatian, 1980; Aygun et al, 2012). The multisystem involvement rather than skeletal dysplasia is thought to be the cause of perinatal lethality in most patients. Indeed, several published neonates have near normal crown-heel length at birth (Kerr et al., 2000). Smith et al. (2014) identified truncating mutations in the GPX4 gene (locus 19p13.3) in two unrelated families with severe SSD. GPX4 gene encodes phospholipid hydroperoxide glutathione peroxidase, an anti-oxidative, selenocysteine containing enzyme that protects cell membranes by dissipating lipid peroxides (Scheerer et al, 2007). We present clinical, radiological and genetic data of a male child with known pathogenic homozygous missense mutation p.R179H in the GPX4 gene identified by whole-exome sequencing.

Clinical details

The patient was referred at the age of four years and nine months for genetic evaluation of developmental delay since birth. Around the third trimester, short limbs were noticed on ultrasound. He was born at term vaginally, birth weight was 3.2 kgs, with average size (exact measurements not available), cried well after birth. The floppiness and feeding problem were noticed on day 1 of life, initially fed through tube feeds but later on shifted to spoon feeds. He had later poor weight gain. His milestones were as follows: social smile at six months, stranger anxiety three and a half years, head control not achieved, speech in the form of cooing only since 2 years of age. He could recognize his parents but did not have stranger anxiety (social age around 5 to 6 months, motor age around 2 to 3 months). There was increased tone with intermittent tonic posturing, and he often bite his tongue, cheeks, lips. He had increased tolerance to pain. He enjoyed listening to music. His head circumference at presentation was 43.5 cm [minus 5.1 standard deviation (SD)], total length was 75 cm (minus 6.9 SD), upper segment: lower segment ratio was 1.5, weight was 7.1 kilo gram. The facial features noted were low anterior hairline, thick eyebrows, flat nasal bridge, broad nasal tip, long philtrum, thin lips with scars (bite marks), dental caries, gum hypertrophy, rounded cheeks, well defined squared chin, symmetric, low set ears. The microcephaly was not apparent. The neck was short. The sternal and rib prominences were seen. There was rhizomelia, bowed forearms, normal-sized hands and feet. Elbows could be extended till 150 degrees and knees could be extended till 160 degrees. Supination of the forearm was also restricted. The skin was wrinkled over the abdomen, and palms and soles showed thick fat pads with wrinkled skin over soles (see figure 1). He had spastic quadriparesis with intermittent decerebrate rigidity (posturing) and dystonia deep tendon reflexes were normal. He preferred to keep his fists clenched, with ~~the~~ cortical thumb. He could not approach objects. He had visual fixation, pupillary reflexes were normal, could smile/frown when spoken to. He was fed liquid or semi-solid diet/porridge and had no trouble swallowing. He had normal stool. He could not indicate bladder or bowel. He did not speak any meaningful word but smiled on being called his name. The following analysis was normal around one month of age: plasma acylcarnitine and amino acid analysis on tandem mass spectrometry, glucose-6-phosphate dehydrogenase, 17 hydroxyprogesterone, immunoreactive trypsinogen, total galactose, serum ammonia, plasma glucose and thyroid-stimulating hormone. At two to three months of age, serum

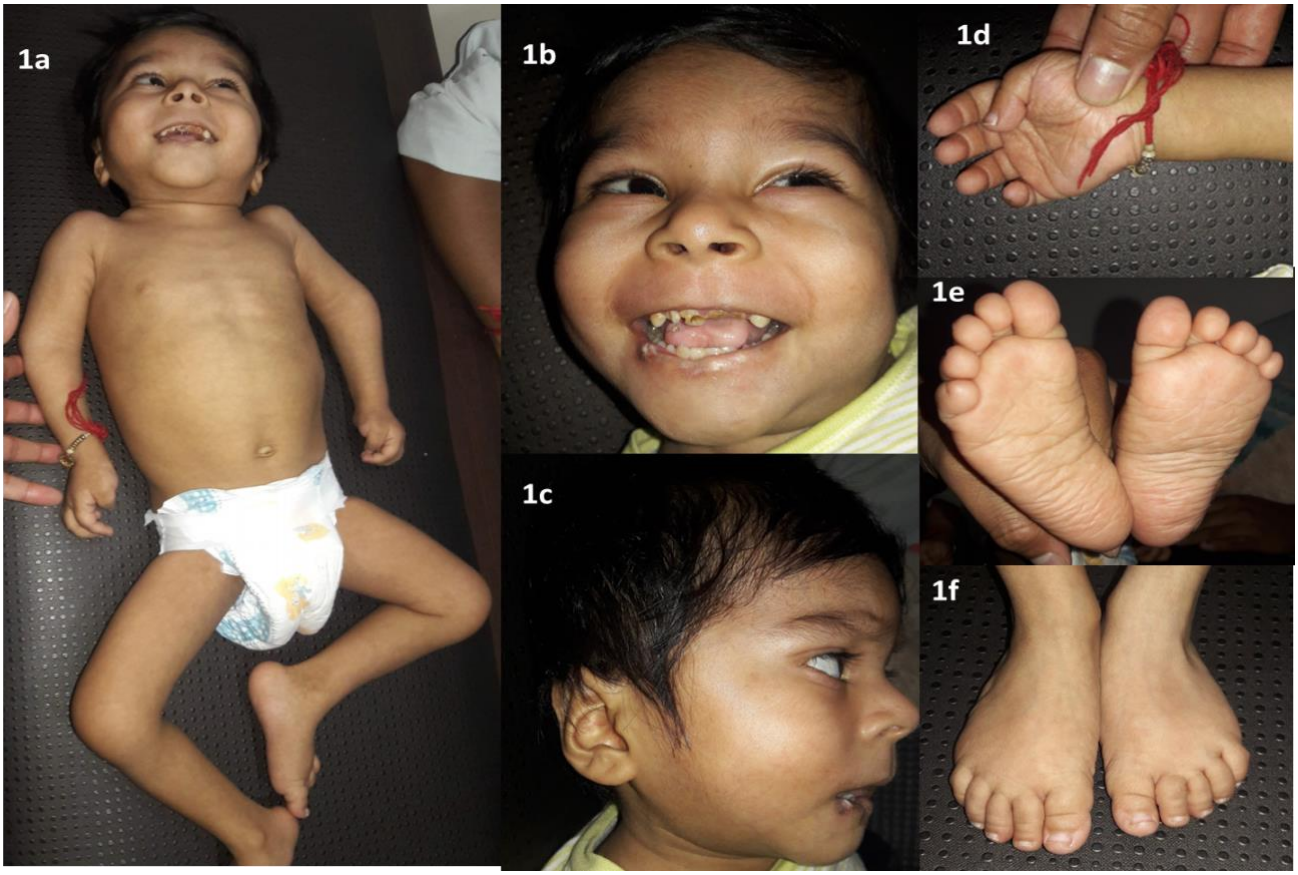

Figure 1: The clinical photos of the child showing rhizomelic short stature, coarse facies, dental dysplasia, wrinkled abdominal skin, thick plantar fat pads with wrinkled skin.

analysis reports were as follows: ammonia 73 micro gm/dl (range 15 – 50), lactate 47 mg/dl (4.5 – 20), creatine phosphokinase total 140 IU/l (24 – 190), creatine phosphokinase MB fraction 5.3 ng/ml (up to 4.94). The X ray findings at age of 4 years 10 months were thus (see figure 2 and figure 3): hand with wrist showed short broad metacarpals with thin cortices, proximal pointing was present in metacarpals except that of thumbs and the ends were irregular, there was no diaphyseal modeling defect, phalanges were bullet shaped with conical epiphyses, only 2 carpal bones were ossified (delayed bone age), the radius and ulna ends were convex, spine showed platyspondyly (bottom to top increasing gradient of severity) with over-faced pedicles, and scoliosis, the vertebral bodies have regular end plates (there was no beaking or scalloping), few of the ribs appeared oar shaped, pelvis with knees and ankle showing hyoplastic iliac bones, flat acetabular roof, small femoral capital epiphysis, broad femoral metaphyses, prominent lesser tubercle, cupping, flaying, spraying at long bone ends (femora, tibiae), hypoplastic, irregular epiphyses, upper limb and shoulder showed elbow, wrist joints showed rhizomelic shortening, bulbous lower end of humerus, irregular surfaces of scapula-humeral joints, small humeral epiphyses, foot showed hypoplastic middle and terminal phalanges, broad first metatarsal, irregular osteopenic tarsal bones (with prominent trabecular markings). The typical facial appearance, skeletal dysplasia suggested diagnosis of rhizomelic chondrodysplasia punctata, which was ruled out by normal red blood cell plasmalogen analysis. MRI

brain performed at 2 years showed neuronal migration abnormalities (cortical dysplasia, pachygyria, impaired grey-white difference, nodular heterotopia), hypoplastic splenium of corpus callosum, cerebellar atrophy (prominent foliae with hyperintensity on T2W) and the brainstem and spinal cord were thin (see figure 4). Refer to table 1 for comparison with reported cases.

Table 1: Clinical features present in our patient as compared to reported/published cases of Sedaghatian skeletal dysplasia.

S No	Clinical feature	Frequency in reported cases	Present/Absent in our case
1	Facial features	Hypertelorism 8/10 (80%) Flat nasal bridge: 5/10 (50%) Broad nose: 5/10 (50%) Low set ears: 7/10 (70%)	Hypertelorism, flat nasal bridge, broad nasal tip and low set ears present
2	Rhizomelic shortening of long bones	Present in 21/21 (100%)	Present
3	Vertebral involvement	Platyspondyly at fetal age/ perinatal age 21/21 (100%)	Mildly Present, in infancy, overfaced pedicles, scoliosis, present
4	Long Bone metaphyses involvement	Splaying and fraying of metaphyses present in fetal/ perinatal age 21/21 (100%)	Widened and irregular metaphyses in infancy, present
5	Long Bone epiphyses involvement	Irregular epiphyses, delayed ossification present	Irregular epiphyses, delayed ossification present
6	Bones of hands and feet	Short bones with irregular and cupped metaphyses 21/21 (100%) Irregular carpal and tarsal bones 21/21 (100%)	Short bones with irregular and cupped metaphyses present Irregular carpal and tarsal bones are present
7	Pelvic bone involvement	Grooved iliac crests: 10/16 (62.5%) Flat and horizontal acetabular roof- 10/16 (62.5%) Medial extension of iliac wings: snail like appearance: 3/10 (30%)	Iliac bone is small but crest is not grooved; Acetabular roof is flat and horizontal, Medial extension of iliac wing is mild
8	Cardiac conduction abnormality	Partial/ complete heart block 5/10 (50%)	Absent
9	Brain involvement	Simplified gyral pattern 5/7 (71 %) Lissencephaly – 2/7 (28.6 %) Agenesis of corpus callosum 2/10 (20%)	Simplified cerebral gyral pattern and hypoplastic splenium of corpus callosum; cerebellar atrophy, thin brainstem present

		Cerebellar hypoplasia: 2/10 (20%)	
10	Survival	Still birth to 161 days	Presently alive at more than 5 years, at time of writing the paper

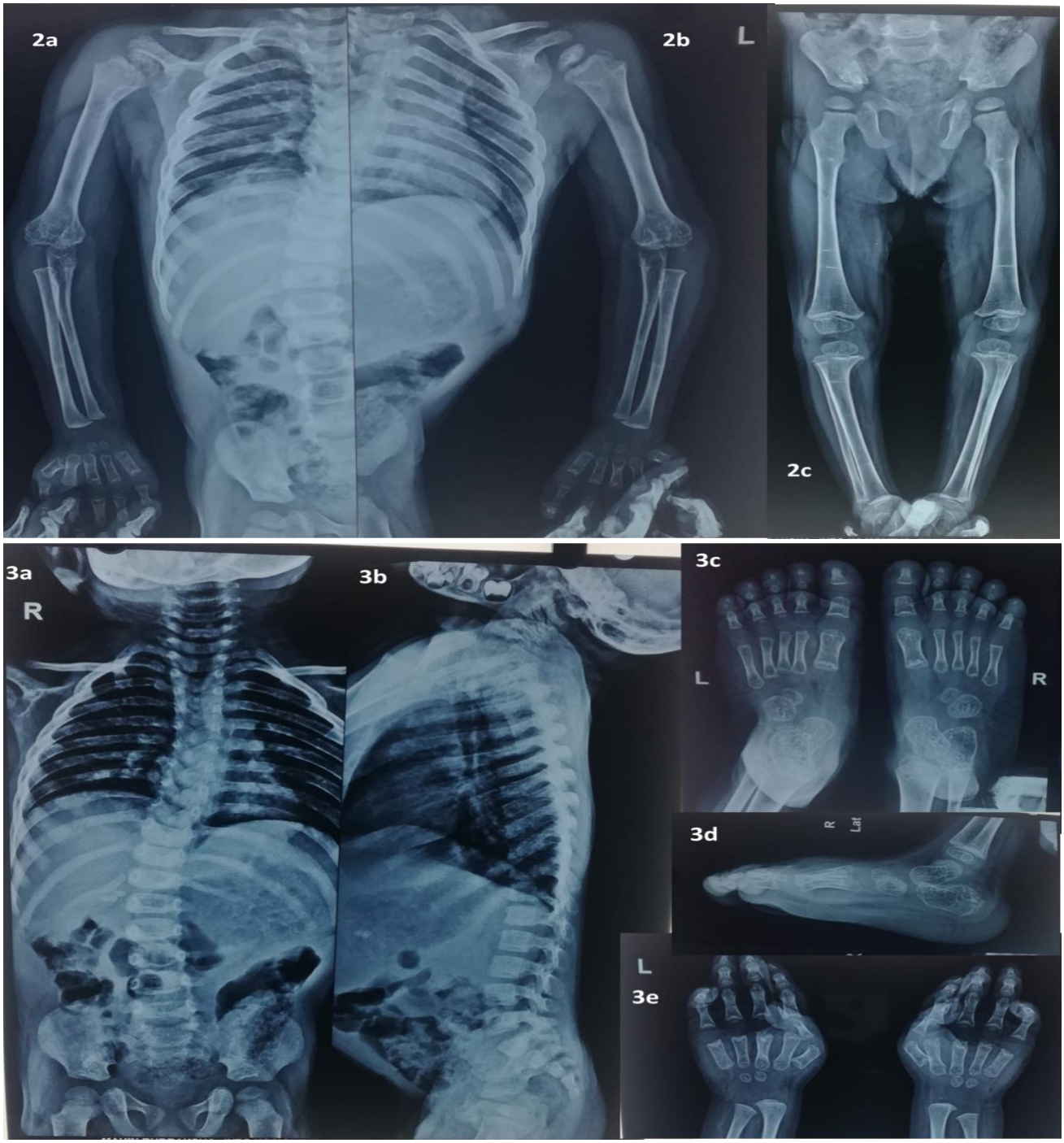

Figure 2 and 3: Skeletal dysplasia: spondlyo-epi-metaphyseal dysplasia hands with wrist showing short broad metacarpals with thin cortices, proximal pointing was present in metacarpals except that of thumbs and the ends were irregular, there was no diaphyseal modeling defect, phalanges were bullet shaped with conical

epiphyses, only 2 carpal bones were ossified (delayed bone age), the radius and ulna ends were convex, spine showed platyspondyly (bottom to top increasing gradient of severity) with overfaced pedicles, and scoliosis, the vertebral bodies have regular end plates (there was no beaking or scalloping), few of the ribs appear oar shaped, pelvis with knees and ankle showed hypoplastic iliac bones, flat acetabular roof, small femoral capital epiphysis, broad femoral metaphyses, prominent lesser tubercle (resembling "monkey wrench"), cupping, flaying, spraying at long bone ends (femora, tibiae), hypoplastic, irregular epiphyses, upper limb and shoulder showed elbow, wrist joints showed rhizomelic shortening, bulbous lower end of humerus, irregular surfaces of scapula-humeral joints, small humeral epiphyses, foot showed hypoplastic middle and terminal phalanges, broad first metatarsal, irregular osteopenic tarsal bones (with prominent trabecular markings).

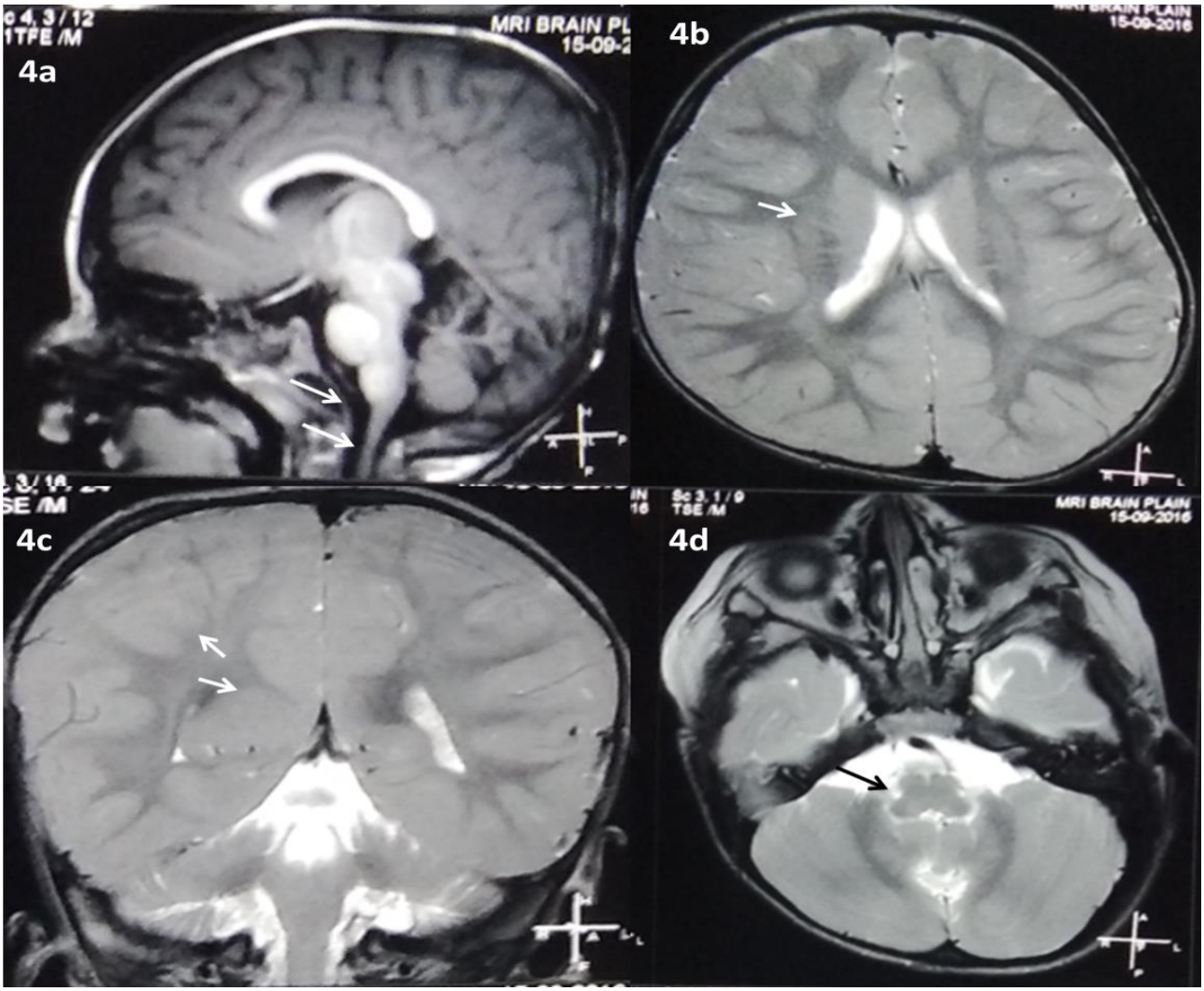

Figure 4: Neuronal migration abnormalities (cortical dysplasia, pachygyria, impaired grey-white difference: white arrow 4c, nodular heterotopia: white arrow, 4b), hypoplastic splenium of corpus callosum, cerebellar atrophy (prominent foliae with hyperintensity on T2W) and the brainstem (black arrow 4d) and spinal cord were thin (white arrows 4a).

Genetic analysis

Further, whole-exome analysis was done to identify the genetic etiology. Genomic DNA was extracted from peripheral blood. Libraries were prepared using SureselectXT Human All Exon (V5plusUTR version) to target 21522 genes. Indexed captured library DNA was sequenced on Illumina Hiseq series to generate 2x150bp sequence reads at an average of 80-100x sequencing depth. A minimum of 75 percent of sequenced bases were of Q30 value. The sequences were aligned to the human reference genome (GRCh37/hg19) and analysed using Picard and GATK-Lite toolkit to generate a variant call format (vcf) file. The variants were annotated using various softwares, such as Wannovar, MutationTaster, MutationDistiller and a set of variant databases including Clinvar, Online Mendelian Inheritance in Man, Exome Aggregation Consortium, GnomAD. The pathogenic mutation in the GPX4 gene, namely chr19:1106433:G>A or c.536G>A or p.R179H (transcript id: ENST00000354171.12 or NM_002085.5), was identified in the homozygous state, confirming the diagnosis of the variant as a milder form of SSD. The variant was confirmed in the proband and in a carrier state in parents by Sanger sequencing.

Discussion

Since, the first description of a case in 1980, a total of 21 infants from 15 families, all with perinatal lethality, have been described with Sedaghatian skeletal dysplasia, a severe spondylo-epi-metaphyseal dysplasia (Sedaghatian, 1980; Opitz et al., 1987; Peeden et al., 1992; Elcioglu and Hall, 1998; Koutouby et al., 2000; Kerr et al., 2000; Foulds et al., 2003; English et al., 2006; Mahendran et al., 2007; Aygun et al., 2012; Smith et al., 2014, Ipek and Akin, 2016). The longest survival reported is 161 days (Koutouby et al, 2000). Other systemic involvement includes neuronal migration disorder (lissencephaly, polymicrogyria, cortical dysplasia, nodular heterotopias, cerebellar hypoplasia), delayed myelination, myocarditis, cardiac arrhythmia (English et al, 2006; Smith et al, 2014). Smith et al., 2014 reported two infants from unrelated families with truncating mutations. The mutations were of splice site variety [compound heterozygous c.587+5G>A and c.588-8_588-4del: NM_001039848.1] in one family and premature termination in another (c.381C>A or p.Y127X: NM_001039848.1). The c.588-8_588-4del mutation occurred de novo, a rare occurrence in the recessive syndromes. RNA studies were possible but protein studies and animal model studies were not possible for these variants. Since, then no cases have been published. We were able to further confirm our diagnosis courtesy personal communication from a colleague Dr. Katta M Girisha who diagnosed an unrelated patient with a similar clinical, radiological findings and with the same mutation as our patient. Also, as per the website (http://gpx4.org) and (https://medium.com/gpx4/baby-with-rare-gpx4-genetic-mutation-7d250a42efd9) (accessed on 23rd Feb 2020) another unrelated child of Indian origin has been diagnosed as having SSD due to the same p.R179H mutation. His parents were confirmed as carriers for the same variant. Photos available on the website reveal a facial appearance and physical appearance similar to our patient. The child also had microcephaly, dysphagia, hypotonia, auditory neuropathy, mild optic nerve dysplasia, and developmental delay. This suggests that p.R179H is a recurrent mutation, located at a mutation hotspot or there could be founder effect.

On *in-silico* analysis, the variant p.R179H was predicted as disease-causing by Sorting Tolerant From Intolerant (SIFT), Polyphen2, MutationTaster, MutationAssessor but tolerant by FATHMM. (Ng et al., 2003; Adzhubei et al., 2010; Schwarz et al., 2010; Reva et al., 2011; Shihab et al., 2013). The variant p.R179H (rs763745871) has been identified in ExAC database with a minor allele frequency of 0.00004167, ExAC African database as 0.0001, not reported in ExAC- East Asian, and in ExAC South Asian as 0.0002. The crystal structure of cytosolic human GPX4 showed that the catalytic triad (C63, Q108, W163) is localized on a flat impression of the

protein surface which also constitutes positively charged amino acids K75, K162, R179) and the polar amino acid T166. The residue R179 is conserved in all isoforms (GPx1 to GPx7). As per Uniprot, the secondary structure of the protein shows that it has eight helical regions, nine beta-sheet regions and two turns. The R179 residue is present in the ninth beta-sheet region (residue 176 to 180) (https://www.uniprot.org/uniprot/O70325/protvista, accessed on 23rd Feb 2020). The enzyme is expressed in almost all cells. The various biological processes wherein GPX4 plays a role include: lipid peroxidation, protection from inflammation and atherogenesis, sperm maturation, apoptosis regulation, cerebral embryogenesis, cell-mediated immune defense and thyroid metabolism (Scheerer et al., 2007). The etiopathogenesis of skeletal dysplasia and myocardial involvement can be explained by drawing parallel with selenium deficiency manifestations which can present as Kashin-Beck disease, a disabling deformity of bones, cartilage, and joints leading to enlarged joints and restricted movements and Keshan disease associated with cardiomyopathy (Zha and Gao, 2019; Ren et al., 2004). Constitutive knockout of GPX4 gene in mice leads to embryonic lethality (Imai et al., 2003). During mouse embryogenesis, expression begins in neural tube followed by most organs and limbs (Borchert et al., 2006; Ufer and Wang, 2011).

As per the ACMG guidelines for interpretation of sequence variants, the variant R179H in the GPX4 gene is classified as likely pathogenic (rule 5: two moderate criteria and two or more than two supporting criteria) (criteria: PM1: located in a mutational hot spot and/or critical and well-established functional domain (e.g., the active site of an enzyme) without benign variation, PM2: absent from controls (or at extremely low frequency, if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium, PP3: multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc., PP4: patient's phenotype or family history is highly specific for a disease with a single gene etiology, PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation) (Kleinberger et al., 2016). Limitation of the study is that RNA, protein and enzyme studies were not available to us for further studies.

CONCLUSION

We describe a patient with the mild phenotype of Sedaghatian skeletal dysplasia due to hypomorphic mutation p.R179H in the GPX4 gene. This patient is the longest surviving of the reported patients with Sedaghatian skeletal dysplasia. This report thus expands the clinical spectrum of Sedaghatian skeletal dysplasia patients.

Ethical Compliance

The data contained was obtained through routine clinical care, counselling and patient management. The study complies with the ethical principles laid down in the Declaration of Helsinki. Signed informed consent for publication of clinical material was obtained from the patient's family.

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Conflict of interest statement

The authors have declared to have no conflict of interest.

Authors' contributions

PMT, SG, VPT performed clinical assessment of case, genetic counseling, PMT, VPT, SM performed genetic analysis, PMT, SG, VPT, LV, RK wrote the first draft and reviewed literature, all authors finalized the manuscript.

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Declaration of originality

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