A case of Raine syndrome presenting with facial dysmorry and review of literature

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Abstract

Background: Raine syndrome (RS) – an extremely rare autosomal recessive genetic disorder, is caused by a biallelic mutation in the FAM20C gene. Some of the most common clinical features include generalized osteosclerosis with a periosteal bone formation, dysmorphic face, and thoracic hypoplasia. Many cases have also been reported with oro-dental abnormalities, and developmental delay. Most of the cases result in neonatal death. However, a few non-lethal RS cases have been reported where patients survive till adulthood and exhibits a heterogeneous clinical phenotype. Clinical diagnosis of RS has been done through facial appearance and radiological findings, while confirmatory diagnosis has been conducted through a molecular study of the FAM20C gene.

Case presentation: A 6-year-old girl was born to healthy third degree consanguineous parents. She presented with facial dysmory, delayed speech, and delayed cognition. Radiography showed small sclerotic areas in the lower part of the right femur, and an abnormally-shaped skull with minimal sclerosis in the lower occipital region. Computer tomography scan of the brain revealed mild cortical atrophy, and MRI scan of the brain showed a normal female karyotype. No quantitative genomic imbalance was detected by aCGH. Further study conducted using Clinical Exome Sequencing identified a homozygous missense variation c.1228 T > A (p.Ser410Thr) in the exon 6 of FAM20C gene – a likely pathogenic variant that confirmed the clinical diagnosis of RS. The variant was confirmed in the proband and her parents using Sanger sequencing. Prenatal diagnosis during subsequent pregnancy revealed heterozygous status of the fetus, and a normal carrier child was delivered at term.

Conclusions: The syndrome revealed markedly variable presentations such as facial dysmory and developmental delay, and was localized to diffuse bone osteosclerosis. Clinical indications, striking radiological findings and molecular testing of FAM20C gene confirmed the diagnosis of RS. A rarity of the disorder and inconsistent phenotype hindered the establishment of genotype-phenotype correlations in RS. Therefore, reporting more cases and conducting further research would be crucial in defining the variable radiologic and molecular defects of the lethal and non-lethal forms of this syndrome.

Keywords: Case report, Developmental delay, Facial dysmory, FAM20C gene, Osteosclerosis, Raine syndrome

Background

Raine syndrome (OMIM #259775) is also known as osteosclerotic bone dysplasia. With the estimated prevalence of < 1 in 1,000,000, the disease is categorized as a rare autosomal recessive disorder. A mutation in the FAM20C (Family with sequence similarity 20, member C) gene (OMIM* 611061) located on chromosome 7p22.3 is responsible for the disease [1]. This gene encodes a member, which is a Golgi casein kinase and has an S-x-E/pS consensus motif (where S is serine, x is any amino acid and E/pS can be Glutamic acid or phosphoserine) that phosphorylates the serine residues of the extracellular proteins called several secretory calcium-binding-phosphoproteins (SCPP). The bio-mineralization of bones and teeth is carried out by a small integrin-binding ligand – N-linked glycoproteins (SIBLINGs), an SCPP due to its high affinity for calcium [2]. Also, FAM20C phosphorylates the C-terminal dentin matrix protein 1 (Dmp1) and makes it a highly negatively charged domain, which in turn recruits...
third-degree consanguinity. This family of Indian origin
A 6-year-old girl, was born to healthy parents with
Clinical features
Case presentation
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periosteal reaction was noted. CT scan of the brain revealed prominent sulcal pattern with enlarged cisternal spaces in the brain parenchyma. These changes were prominently observed in the frontoparietal region. In addition, mild cortical atrophy was also detected. There was no evidence of focal hypo- or hyperdense lesion. Moreover, subdural, extradural or intracerebral hemorrhage, or subarachnoid bleeding was not observed. The pons, midbrain, both cerebellar hemisphere, and brain stem appeared normal. No mass lesion was observed in the posterior fossa.

MRI revealed a significant hypoplastic appearance of posterior part of the brain. It also showed corpus callosal dysgenesis with the absence of rostral area. Moreover, a mild paucity of the peritrigonal white matter was seen surrounding the trigones of bilateral lateral ventricles. However, no obvious gliosis, extra-axial collection or lesions were noted. Her echocardiogram showed normal systemic and pulmonary venous drainage, normal valves, and normal sized cardiac chambers. The Ophthalmological evaluation revealed bilateral intact vision with healthy macula.

Molecular genetics investigations

Chromosome banding study showed a normal female karyotype i.e. 46, XX at 550-bands resolution [20]. The fluorescence in situ hybridization (FISH) study was performed using LSI ELN (7q11) (orange) /LSI 7q22 (green) (Kreatech dual color probe). Since two signals were observed in all the cells, clinical prediction of Williams syndrome was ruled out. The proband DNA was further investigated with array comparative genomic hybridization (aCGH) using [Agilent 60 K], with an average resolution of 150 kb. No copy number variations i.e. deletions and/or duplications of pathogenic significance were detected [arr(1–22,X)× 2]. Thus, cytogenetic aberrations with resolution of 150 kb or greater were unlikely to be responsible for the clinical features in the proband.

The genomic DNA (gDNA) of the proband was isolated from the peripheral blood using the salting-out technique [31]. This DNA sample was processed for clinical exome sequencing (CES) on Illumina NextSeq 500. A detailed protocol is mentioned in the Additional file 1. Sequencing detected a homozygous missense variation c.1228 T > A in the exon 6 of the FAM20C gene (OMIM*611061) (GenBank accession number NM.020223.3; coding sequences NP_064608). This variation resulted in the amino acid substitution of threonine for serine at codon 410 (p.Ser410Thr; ENST00000313766), confirming the clinical diagnosis of RS (OMIM # 259775).

The functional effect of this variant was studied using the in silico analysis tools. The variant was predicted to be disease-causing by Mutation Taster (http://www.mutationtaster.org/), with a score of 58. The impact of the substitution of serine to threonine was predicted to be probably damaging (score of 0.972) by Polyphen2 (PolymorphismPhenotypingV2) (http://genetics.bwh.harvard.edu/pph2/). Scale-invariant feature transform (SIFT) (http://sift.jcvi.org/) predicted that the variant was tolerated with a score of 0.51, and PROVEAN anticipated that the variant had a deleterious effect on the protein function. This variant was reported as a likely pathogenic allele (SCV000583504.1) and benign (SCV000343602.2) in ClinVar. Its reference SNP number is rs148276213. The minor allele frequency of the rs148276213 variant is 0.0034 in the 1000 genomes database and 0.006108 in the ExAC database for the South Asian population.

CES also revealed another compound heterozygous variant in the tubulin gamma complex associated with protein 6 (TUBGCP6) gene (OMIM*610053) in the

Fig. 1 a Clinical picture of the proband showing features of microcephaly, narrow bifrontal diameter, flat forehead, epicanthal folds, hypertelorism, depressed and low nasal bridge with bulbous nasal tip, flaring nares, prominent philtrum and pointed chin. b X-ray of extremities of the proband (Left): the arrow indicates small sclerotic areas observed in lower part of the right femur; X-ray of the skull (Right): abnormal skull shape skull; the arrow indicates minimal sclerosis of lower occipital region-bones.
proband. The compound heterozygous variant in exon 24 [c.5327C > G (p.Ser1776Cys)] and exon 16 [c.3383G > A (p.Arg1128Lys)] is associated with microcephaly and chorioretinopathy-1 (OMIM#251270). In silico analysis predicted the first variant (c.5327C > G/p.Ser1776Cys) to be disease-causing using the Mutation Taster, tolerated by SIFT (score 0.07) and probably damaging by PolyPhen (score 1.00). While second variant (c.3383G > A/p.Arg1128Lys) found to be a polymorphism by Mutation Taster, tolerated by SIFT (score 0.65), and benign by PolyPhen (score 0.023). Second variant was dismissed as the cause of disease in the proband, because this genotypic variation did not correlate with the phenotypic features of the proband, and also because various bioinformatics prediction softwares could not predict it as damaging.

The variant has an autosomal recessive mode of inheritance. Its confirmation in the proband and her parents was carried out using bi-directional Sanger sequencing with primers covering both the exon and the intron-exon boundary of exon 6 of the FAM20C gene (Additional file 2). Sequencing confirmed both the parents to be heterozygous, and the proband to be homozygous for c.1228 T > A (p.Ser410Thr) variant in exon 6 of FAM20C gene (Fig. 2a-d). This variant was submitted to ClinVar database (accession ID is SCV000583504.1). During a subsequent pregnancy, the
same family approached again for genetic counseling. The prenatal diagnosis revealed a heterozygous state of the fetus for the c.1228 T > A (p.Ser410Thr) variant, and a normal carrier child was delivered (data not shown).

Homology modeling, structure validation and protein stability due to c.1228 T > A (p.Ser410Thr) variant
Using NCBI Basic Local Alignment Search Tool (BLAST), the native and mutated sequences of the FAM20C gene were studied to understand the effect of the variant, and also to predict the protein structure against PDB with default parameters [32]. The template PDBID: 5WRR was considered for modeling the protein structure. Additional details regarding the homological modeling is mentioned in the Additional file 3 [33–41].

A root mean sequence deviation (RMSD) of 0.3 Å was observed between native and mutant structure, indicating changes in the loop regions of the superimposed structure. A decrease instability was predicted by iStable with a confidence score of 0.605. Furthermore, I-Mutant predicted a large decrease in protein stability due to the variant p.Ser410Thr with ΔΔG value of −1.37 Kcal/mol (Fig. 3).

Conservation of the FAM20C p.Ser410Thr residue in orthologs
The protein sequence of Homo sapiens (NP_064608) was aligned along with other species using an online multiple sequence alignment program known as Clastal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). It is observed that the orthologs protein sequences of the FAM20C gene were highly identical to H. sapiens FAM20C gene protein sequence suggesting a highly conserved locus (Fig. 4a, b; Additional file 4).

Population screening for c.1228T>A (p.Ser410Thr) variant by ARMS-PCR
Using PCR technique of amplification-refractory mutation system (ARMS), 200 unrelated control samples (100 males and 100 females) were studied to demonstrate the pathogenicity of c.1228T>A (p.Ser410Thr) variant. Amplification of the PCR product was confirmed by 2.5% agarose gel electrophoresis. From the 200 subjects screened for the above genotype, no carriers for the c.1228T>A (p.Ser410Thr) variant were found (Additional file 5).

Discussion and conclusions
Most of the RS cases initially reported died at an infantile age. The prevalence of non-lethal RS has been reported in few cases over time. The most striking features of RS are osteosclerosis and facial anomalies [42]. The case presented here exhibited facial dysmorphism such as a flat forehead, epicanthal folds, hypertelorism, depressed and low nasal bridge with bulbous nasal tip, flaring nares, prominent philtrum, and pointed chin. Such phenotypical appearances have also been observed in other lethal and non-lethal RS cases [7, 8, 20]. These developmental delays was reported in two siblings affected with RS from two different families [8, 28]. Developmental delay is a very rare phenomenon

![Image](https://example.com/image1.png)

![Image](https://example.com/image2.png)
| Sr No. | Mutation(s) in FAM20C gene | Exon/Intron of mutation | Pattern of mutation | Sex | Consanguinity | Fatality | Origin (Ethnicity) | Clinical Indications of the patients | Reference |
|-------|-----------------------------|-------------------------|--------------------|-----|---------------|---------|-----------------|------------------------------------|-----------|
| 1     | c.1603C > T (p.Arg535Trp)   | Exon 10                 | Homozygous missense mutation | M   | Yes           | Neonatal death | Caucasian       | Periosteal bone formation, Osteosclerosis, Thoracic Hypoplasia, brachycephaly with very large anterior and posterior fontanelles, prenatal features of both clavicles and some ribs | Kingston et al., 1991 [9] Simpson et al., 2007 [21] |
| 2     | c.915-3C>G Intron 4/Exon 5 acceptor splice-site change | Homozygous splice-site mutation | M | No | Neonatal death | Arab | Periosteal bone formation, Osteosclerosis, Thoracic Hypoplasia, and marked bowing of the femurs, tibiae, ulnae, short limbs | Prominent forehead, short neck, proptosis, midfacial hypoplasia, depressed nasal bridge, wide anterior fontanelle, exophthalmos, bilateral choanal atresia, large protruding tongue | Not Reported Not Reported Al-Gazali et al., 2003 [24] Simpson et al., 2007 [21] |
| 3     | c.1121T>G (p.Leu374Arg)     | Exon 6                  | Homozygous missense mutation | F   | Yes           | Neonatal death | Turkish         | Bi-temporally narrowed forehead, short neck, proptosis, midfacial hypoplasia, depressed nasal bridge, carp-shaped mouth with narrow lips, large low set ears, posteriorly rotated ears, smooth and prominent philtrum | Gingival hyperplasia Diffused intracerebral calcifications, microcephaly | Hulskamp et al., 2003 [14] Simpson et al., 2007 [21] |
| 4     | 45, XY pseudodiploid (7;7) (p22;p22) | NA                      | Chromosome 7 rearrangement and microdeletion, FAM20C gene is located within the deleted region | M   | No            | Neonatal death (died 2 h after birth) | Unknown         | Lethal osteosclerotic bone dysplasia, irregular periosteal bone formation along the clavicles and ribs; skull had wide cranial sutures with evidence of premature closure, base of the skull showed an increased thickness of all bony landmarks, pulmonary Hypoplasia, Thoracic Hypoplasia | Prominent forehead, short neck, proptosis, midfacial hypoplasia, and a depressed nasal bridge | Not Reported Not Reported Simpson et al., 2007 [21] |
### Table 1: Genotype and Phenotypic Variability in Some Reported Cases of Raine syndrome (Continued)

| Sr No | Mutation(s) in FAM20C gene | Exon/ intron of mutation | Pattern of mutation | Sex | consanguinity | Fatality | Origin (Ethnicity) | Clinical Indications of the patients | Bone anomalies | Facial dysmorphic appearance | Orodental abnormalities | Developmental delay | Reference [Reference number in text] |
|-------|----------------------------|--------------------------|---------------------|-----|---------------|----------|-------------------|--------------------------------------|----------------|-------------------------------|---------------------|----------------------|-------------------------------|
| 5     | c.1093G>A (p.Gly365Arg)    | Exon 6                   | Homozygous          | M   | Yes           | neonatal death | Unknown          | Prominent forehead, short neck, proptosis, midfacial hypoplasia, depressed nasal bridge | Not Reported | Not Reported                  | Simpson et al., 2007 [21]     |
| 6     | c.1094G>A (p.Gly365Glu)    | Exon 6                   | Compound heterozygous mutation | F   | No            | neonatal death | Unknown          | Periosteal bone formation, Osteosclerosis | Unknown | Cerebral Calcifications       | Simpson et al., 2007 [21]     |
| 7     | c.1322-2A>G                | Intron 7 / Exon 8        | Intron 7 / Exon 8 acceptor splice site mutation | F   | No            | neonatal death | Unknown          | Prominent forehead, short neck, proptosis, midfacial hypoplasia, depressed nasal bridge | Unknown | Cerebral Calcifications       | Simpson et al., 2007 [21]     |
| 8     | c.914+5G>C                 | Exon 4 / Intron 4        | Exon 4 / Intron 4 donor splice site mutation | F   | No            | neonatal death | Unknown          | Periosteal bone formation, Osteosclerosis | Unknown | Cerebral Calcifications       | Simpson et al., 2007 [21]     |
| 9     | c.1404-1G>A                | Intron 8 / Exon 9        | Intron 8 / Exon 9 acceptor splice site mutation | F   | No            | neonatal death | Unknown          | Periosteal bone formation, Osteosclerosis | Unknown | Cerebral Calcifications       | Simpson et al., 2007 [21]     |
| 10    | c.1309G>A (p.Asp437Asn)    | Exon 7                   | Homozygous missense mutation | M   | Yes           | (Age 8 years at the time of investigation) The details of death is unavailable | Unknown          | Sclerosing bone dysplasia, metaphyseal sclerosis of the long bones, diffuse abnormalities of the skull, thickening and coarse trabeculation, prominent mastoid bulges, short stature | Brachycephaly, downsized eyes, hypoplastic nose, small downturned mouth, proptosis, turribrachycephaly, plagiocephaly, downsizing palpebral fissures, proptosis, depressed nasal bridge, small nose, protruding tongue, thick alveolar margins, low-set ears | High palate, abnormal teeth | Hydrocephalus, impaired early development, with an increase in age severe developmental delay observed | Simpson et al., 2009 [11] |
| Sr No. | Mutation(s) in FAM20C gene | Exon/Intron of mutation | Pattern of mutation | Sex | Consanguinity | Fatality | Origin (Ethnicity) | Clinical Indications of the patients | Reference |
|-------|---------------------------|-------------------------|---------------------|-----|---------------|----------|-------------------|-------------------------------------|-----------|
| 11    | c.796G>A (p.Gly266Arg)    | Exon 2                  | Compound heterozygous missense mutation | M   | No            | Unknown | Indian subcontinent | Sclerosing bone dysplasia, pectus excavatum, bulbous fingertips, thick fingers, large halluces, short stature, Turbinobrachycephaly, hypertelorism, arched eyebrows, an inferiorly placed right eye, low-set and protuberent ears, flat nasal bridge with rounded and bulbous nasal tip and prominent alae nasi, sunken midface, wide mouth with large tongue, relative prognathism | Simpson et al., 2009 [11] |
| 12    | c.796G>A (p.Gly266Arg)    | Exon 3                  | Homozygous missense mutation | M   | Yes           | died 38 days after birth | Algierian | Cleft palate, long philtrum, open mouth appearance, midfacial hypoplasia, flat face, depressed nasal bridge, small nose, choanal atresia, entropion of eyelids, proptosis, low set ears, prominent forehead, clover leaf skull | Kochar et al., 2010 [20] |
| 13    | c.1630C>T (p.Arg544Trp)   | Exon 10                 | Homozygous missense mutation | M   | Yes           | Indian subcontinent | Algerian | Case 1: Osteosclerosis. Case 2: cerebral calcifications within parieto-occipital and periventricular white matter, increased density of vertebral bodies and calcifications of several intervertebral disks, presence of chain-like calcifications. Case 1: High forehead, hypertelorism with bilateral epicanthal folds and slightly downslanting palpebral fissures, nasal root hypoplasia, antverted nares, dysplastic and posteriorly angulated ears with prominent lobule. Case 2: brachycephaly, bilateral epicanthal folds, midface and nasal root hypoplasia with absence of nasal crest and micrognathia | Fradin et al., 2011 [13] |
| Sr No. | Mutation(s) in FAM20C gene | Exon/ intron of mutation | Pattern of mutation | Sex | consanguinity | Fatality | Origin (Ethnicity) | Clinical Indications of the patients | Bone anomalies | Facial dysmorphic appearance | Oro-dental abnormalities | Developmental delay | Reference |
|-------|---------------------------|--------------------------|---------------------|-----|---------------|----------|-------------------|-----------------------------------|----------------|-----------------------------|---------------------|-------------------|-----------|
| 15    | c.803C>T (p.Thr268Met)    | Exon 3                   | Compound heterozygous missense mutation | M (n = 2) | No | (Age 18 years at the time of investigation). The details of death is unavailable | Norway | Osteosclerosis, short distal phalanges | Dolicocephaly, a narrow face with a narrow malar region, prominent forehead, depressed nasal bridge, low set eyes, hypertelorism, proptosis, a high arched palate with a midline ridge, small mouth, flat malar area | Tooth decay (evident by approximately 18 months of age) | Periapical abscesses, enlarged pulp chambers, elongated pulp horns up to the enamel-dentin junction, globular defects in the dentine, gingival hyperplasia | Slightly delayed language and fine motor skills at age 11 years | Rafaelsen et al., 2013 [28] |
| 16    | c.915C>A (p.Y305X)        | Exon 4                   | Age 16 years at the time of investigation. The details of death is unavailable | | | | | | | | | | |
| 17    | 46,XY,del[46,XY] 7p22.3 (36,480-52,373) | chromosome 7p22.3 | Homozygous Complex rearrangement of chromosome and deletion of 487-kb at chromosomal location 7p22.3 that contains FAM20C gene | Unknown | Unknown | The details of death is unavailable | Unknown | Diffuse osteosclerosis, appositional new bone formation, the obliteration of the medullary cavities, narrow thorax, pseudorib fractures, small distal phalanges | Prominent forehead, eye proptosis, severe depression of the nasal bridge with short upturned nose, midface hypoplasia, micrognathia, protruding tongue, | Unknown | Unknown | Ababneh et al., 2013 [43] |
| 18    | c.1222G>T (p.Arg408Trp)  | Exon 6                   | Homozygous mutation | M | Yes | (Age 61 years at the time of investigation). The details of death is unavailable | Japanese | Hypophosphatemic osteomalacia, periosteal bone formation in the long bones, bone mineral density in the femoral neck, ossification of the posterior longitudinal ligament | Not reported | Worn out teeth, dental demineralization, loss of all teeth by the age of 17 years | | Takeyari et al., 2014 [29] |
| 19    | c.784+5G>C* (p.Trp262Cys*) | After Exon 2 (three siblings) | Homozygous donor splice site mutation | F (n = 1) & M (n = 2) | Yes | (Age 21 years, 22 years and 27 years at the time of investigation). The details of death is unavailable | Brazilian | Case 2: Short fingers | Case 1,2,3: Dysplastic ears, midface hypoplasia, exophthalmos, | Case 1,2,3: Dental caries, calculus, severe gingivitis, dental plaque, open bite malocclusion, abnormal enamel, high arched and narrow palate, micrognathia, periapical abscesses, yellow brownish discoloration of teeth | | Case 1: Intacranial calcifications, Case 2: microcephaly, Case 3: microcephaly, | Acevedo et al., 2015 [8] |
| Sr No. | Mutation(s) in FAM20C gene | Exon/ intron of mutation | Pattern of mutation | Sex | consanguinity | Fatality | Origin (Ethnicity) | Clinical Indications of the patients | Reference |
|-------|-----------------------------|--------------------------|--------------------|-----|---------------|----------|-------------------|-------------------------------------|-----------|
| 20    | c.1487C>T (p.Pro496Leu)     | Exon 9                   | Homozygous mutation | M   | (n = 2)       | Yes      | Brazilian         | Bone anomalies: small hands with bulbous fingertips and clinodactyly of the fifth fingers, under-mineralized distal phalanges, shaft and growth plate under-mineralization, bowing of the radius bones. Facial dysmorphic appearance: choanal atresia, low set ears, high arched and narrow palate, enlarged gingival and palatal mucosa, unerupted permanent teeth, hypoplastic AL. Developmental delay: delay in psychomotor development, microcephaly. | [Acevedo et al., 2015](#) |
| 21    | c.1228T>A (p.Ser410Thr)     | Exon 6                   | Homozygous mutation | unknown | unknown         | unknown | Unknown          | Unknown                            | Unknown   |
| 22    | c.676T > A (p.Trp226Arg)    | Exon 2                   | Homozygous mutation | F   | (n = 2) M (n = 1) | Yes      | Moroccan         | Not Reported                        | Learning disabilities, seizures [Elaloui et al., 2016](#) |
| 23    | c.1135G>A (p.Gly379Arg)     | Exon 6                   | Homozygous mutation | M   | Yes           | The details of death is unavailable | South East Asian                      | Not Reported | Abnormal/dystonic movements, hyperreflexia [Mahmood et al., 2017](#) |
| 24    | c.1228T>A (p.Ser410Thr)     | Exon 6                   | Homozygous mutation | F   | Yes           | Age 6 years at the time of investigation. The details of death is unavailable | Indian                             | Osteosclerosis, hallux valgus, sandal gap deformity, clinodactyly of toes, pes planus Flat forehead, epicanthal folds, hypertelorism, depressed and low nasal bridge with bulbous nasal tip, flaring nares, prominent philtrum, pointed chin. No orodental anomalies were observed. | Psychomotor developmental delay Present case |
observed amongst RS patients. Additionally, bone abnormalities like osteosclerosis, hallux valgus, sandal gap deformity, clinodactyly of toes, and pes planus were observed. Several cases of RS reported so far, have shown a broad range of bone anomalies including thoracic hypoplasia, metaphyseal flaring and under-mineralized distal phalanges [14, 43]. Thus, it can be concluded that there is an association of RS with heterogeneous clinical phenotypes.

Several mutations in the FAM20C gene have consistently been reported in RS patients (Table 1). In our case, homozygous missense variant in exon 6 of FAM20C gene was identified. The identified variant was also reported as benign by Emory University. Mutations in orthologs of the FAM20C gene have also exhibited a similar effect in different species. A group of Border Collies represented the canine model for human Raine syndrome. A recessive transmission of c.899C>T (p.A300V) mutation in FAM20C gene resulted in mineralization defect in these canines [44]. In a murine model, specific ablation of the FAM20C gene in cells expressing type I collagen led to skeletal defects and hypophosphatemia [45]. This suggests that FAM20C gene function is highly conserved amongst orthologs.

Another compound heterozygous variation in the TUBGCP6 gene (OMIM#610053) was observed in the current proband. Mutation in this gene caused microcephaly and chorioretinopathy-1 (OMIM#251270). The prominent clinical feature for this disease was microcephaly, retinal pigmentary abnormalities, and early onset of visual impairment. The proband in the present case had microcephaly with intact vision, ruling out retinopathy. There was a lack of genotypic and phenotypic co-relation in the present variant, and the variant was not considered to be predisposing for the clinical features of the proband.

One of the most prominent clinical indications in RS are skeletal defects. Mutation/s in FAM20C lead to loss of function, resulting in hypophosphatemia. The tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) substrates together with inorganic pyrophosphate gets accumulated in the extracellular niche due to mutation/s in FAM20C gene. This causes hindrance in the normal function of mineralization. An excess of pyrophosphate accumulation results in tooth loss, osteomalacia, and calcification of bones. Asfotase alfa, a hydroxyapatite-targeted recombinant TNSALP is being used as an enzyme replacement therapy to get rid of excess pyrophosphate [46]. However, the enzyme replacement therapy is expensive and may not be affordable to many families. In such a scenario, precise genetic counseling and prenatal diagnosis remains the preferred choice.

Though RS is synonymously read as lethal osteosclerotic bone dysplasia, several reported cases including ours show the non-lethal clinical phenotype associated with the FAM20C mutation. In these mild RS cases, the patient survives beyond infancy. RS reflects marked variability in presentations ranging from severe to localized, non-diffuse, and mild bone osteosclerosis. Facial appearance and striking radiological findings prompt the clinical diagnosis of RS. More number of such cases is crucial to define the variable radiologic, metabolic, and molecular defects of this seemingly lethal and non-lethal autosomal recessive syndrome.

Additional files

Additional file 1: Molecular investigations. The method describes the method used in isolation of genomic DNA, NGS and bioinformatics tools used during analysis. (DOCX 16 kb)

Additional file 2: Sanger sequencing (Variant Confirmation Test). It describes the details of primer’s used during Sanger sequencing of the proband and parents (DOCX 15 kb)

Additional file 3: Homology modeling, structure validation and protein stability due to c.1228T>A (p.Ser410Thr) variant. File describes the influence of variant change on the protein structure (DOCX 16 kb)

Additional file 4: Conservation of the FAM20C p.Ser410Thr residue in orthologs. Conservation of the variant in orthologs and Homo sapiens. (DOCX 15 kb)

Additional file 5: Population screening for c.1228T>A (p.Ser410Thr) variant by ARMS-PCR. The file provides details of normal healthy individuals studied for variant (c.1228T>A (p.Ser410Thr)) by ARMS-PCR. (DOCX 15 kb)

Abbreviations

1,25(OH)2D: 1,25-Dihydroxy vitamin D; aCGH: Array Comparative Genomic Hybridization; ADHD: Attention Deficit Hyperactivity Disorder; ARMS: Amplification-refractory mutation system; CES: Clinical Exome Sequencing; CNS: Central Nervous System; Dmp1: Dentin matrix protein 1; FAM20C: Family with sequence similarity 20, member C; FISH: Fluorescence in Situ Hybridization; FGFR3: Fibroblast growth factor 23; FISH: Fluorescence in Situ Hybridization; IUGR: Intrauterine Growth Retardation; LVEF: Left ventricular ejection fraction; NS: Not specified; OECD: Organisation for Economic Co-operation and Development; OI: Osteogenesis imperfecta; OI: Osteogenesis imperfecta; OMIM: Online Mendelian Inheritance in Man; PCR: Polymerase chain reaction; PDB: Protein Data Base; PKD: polycystic kidney disease; RBM5: RNA binding motif protein 5; SIBLINGs: Small integrin-binding ligand, N-linked glycoproteins; SIFT: Scale-invariant feature transform; TNSALP: Tissue-nonspecific isoenzyme of alkaline phosphatase; TUBGCP6: Tubulin gamma complex associated protein 6; YASARA: Yet Another Scientific Artificial Reality Application

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Availability of data and materials

The dataset generated and/or analyzed during the current study is available in the ClinVar repository. [https://www.ncbi.nlm.nih.gov/clinvar/variation/289269/](https://www.ncbi.nlm.nih.gov/clinvar/variation/289269/).

ClinVar Accession ID for c.1228T>A (p.Ser410Thr) variant in Exon 6 of FAM20C gene: SCV000833504.1 [47].

Authors’ contributions

Conceived and designed the experiments: JS. Clinical analysis: JS and AG.

Wrote the revised draft of the manuscript: DP. Laboratory workup: DP and RB. Made critical revisions and approved final version: JS and FS. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

The present case report has been approved by the institutional ethics committee (FRIGE’s Institute of Human Genetics) with approval number FRIGE/IEC/14/2016 dated 19th November 2016. This process is in accordance with the declaration of Helsinki.

- An informed consent for investigation and publication was obtained from the proband father at the time of enrollment for the study. [This was in accordance with the requirement of the institutional ethics committee].

Consent for publication

Informed written consent was obtained from parents on behalf of the minor child for publication of their clinical details and/or clinical images. A copy of the written consent is available for review.

Competing interests

The authors declare that they have no competing interests (financial or non-financial) in the present study.

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