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FULL LENGTH ARTICLE

A novel mutation in exon 11 of COMP gene in a Chinese family with pseudoachondroplasia

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Pseudo-achondroplasia;
Therapy

Abstract Pseudoachondroplasia (PSACH) is a relatively common skeletal dysplasia characterized by disproportionate short stature, joint laxity, early-onset osteoarthrosis, and dysplasia of the spine, epiphysis, and metaphysis. It is known as an autosomal dominant disease which results exclusively from mutations in the gene for Cartilage Oligomeric Matrix Protein (COMP). We have identified a five year old Chinese boy who was diagnosed as pseudoachondroplasia according to clinical manifestations and X-ray symptoms. His mother seems like another effected individual because of the apparent short stature. Genomic DNA was extracted from peripheral blood lymphocytes. DNA sequencing analysis of the COMP gene revealed a heterozygous mutation (c.1219 T > C,p.Cys407Arg) in the patient. His mother was also affected with the same genetic change. Mutations in COMP gene is proved to change the Cartilage Oligomeric Matrix Protein. This missense mutation (c.1219 T > C) has not been reported before and it is not belongs to polymorphism sites. Our results extend the spectrum of mutations in COMP gene leading to pseudoachondroplasia.

Introduction

Pseudoachondroplasia (PSACH) is a relatively common skeletal dysplasia characterized by disproportionate short stature, joint laxity, early-onset osteoarthritis, and dysplasia of the spine, epiphysis, and metaphysis, with normal craniofacial appearance and intelligence.[1,2]

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Multiple epiphyseal dysplasia (MED) is also a skeletal dysplasia quite similar to PSACH but with a milder severity. The biggest difference is that MED hardly leads to spine dysplasia. There are no clearly distinct boundaries between PSACH and MED, both of which are different phenotypes of the same disease. The length and facies of the patients are normal at birth.[3] Growth retardation appears approximately at the age of two years.[2] The most common symptom arousing medical attention is waddling gait or pain, recognized at the onset of walk. Radiological findings include irregular epiphyses and metaphyses of nearly all tubular bones with disproportionate short, and anterior beaking of vertebral bodies. The skull of the affected individuals is as normal as the unaffected.[4,5]

PSACH is known as an autosomal dominant disease which result exclusively from mutations in Cartilage Oligomeric Matrix Protein (COMP) gene.[2,6] But some scholars think that mutations in the CLO9A3 gene could also induce this disease.[7] There are some other reports indicating disorder resembling PSACH without COMP mutation.[8] The human COMP gene localizes on chromosome 19p13.1 and contains 18 introns and 19 exons. The COMP gene encodes cartilage oligomeric matrix protein (COMP; MIM#600310). COMP is a large secreted pentameric glycoprotein of the thrombospondin family: the molecular weight is 550 kDa. It expressed predominantly in the extracellular matrix (ECM) surrounding the cells that make up cartilages, ligaments and tendons.[9] The molecule of COMP consist of an amino-terminal domain, four type II epidermal growth like repeats (EGF-like), eight type III calmodulin-like repeats (CLRs), and a carboxyl terminal globular domain (CTD).[10] Numerous PSACH-related mutations have been found up to now, majority of which occur in the CLRs regions.[6] 182mutations of COMP gene have been identified to date, and 111 of them are referred to PSACH(http://www.hgmd.cf.ac.uk/ac/index.php) (Table 1). The majority of these mutations located in TSP type-3 repeats (87.4%; 97/111), while a small part in CTD (7.2%; 8/111), and very few in EGF-like domain (2.7%; 3/111). The three remaining mutations were gross deletions (2.7%; 3/111) crossing domains according to the literature. These mutations composed by missense mutation (79.3%; 88/111), splicing (0.9%; 1/111), small deletions (12.6%; 14/111), small insertions (2.7%; 3/111), small indels (1.8%; 2/111), and gross deletions (2.7%; 3/111). In this study, we researched the gene changes of a PSACH boy and made a systematic review of the literature on the COMP.

Material and methods

Clinical materials

The proband first came to our hospital because of short stature and joint pain. He was the younger of the two children of non-consanguineous parents. His father as well as his sister is healthy but his mother is short of stature. There is no other affected individuals in his family (Fig. 1). He was born at term with a birth weight of 3.6 kg and a height of 50 cm. His growth and development were normal before the age of 1.5years. Since then he was bothered by multi-joint pain and growth retardation with unknown causes. On physical exam, the patient has an apparent short stature with normal craniofacial appearance. The height was 96.2 cm (<3th,-3.8SD), weight 16 kg (3th～5th, −1.5SD), and a sitting height 61 cm (−1.125SD). Sitting height/leg length ratio was 1.73. Disproportionate short stature was noted. The laboratory tests including mucopolysaccharide of urine, thyroid function, and serum Ca, P and AKP, were normal.

Radiographic exam showed short tubular bones with irregular epiphyses and metaphyses, short and thick femoral neck of bilateral side, flattened femoral head, anterior tonguing or beaking of the vertebral bodies. The skull appears to be normal (Fig. 2). All these signs indicate PSACH.

Methods

DNA extraction

Genomic DNA was extracted from peripheral blood lymphocytes by standard procedures using QIAamp DNA Bloodmini kits (Qiagen, Germany). Thereafter, 3 ug genomic DNA was fragmented by Covaris sonicator (Covaris S2, USA) to sizes of 150−300 bp and then purified.

Library construction

The blunt ends of the purified DNA fragments were then repaired, and A-tailing was added. The fragments were ligated overnight using standard Illumina paired-end (PE) adapter. The ligated products were then amplified through 7-cycle polymerase chain reactions (PCRs) using PE primers containing 8 bp index tags.

Target region capture

The purified PCR products containing 0.003 mg DNA were hybridized to the GenCapTM probe solution (Mygenostics Co. Ltd.,China) at 65 °C for 22 h using a PCR thermocycler. The products were bound to a rotator for 1 h at room temperature using Dynal Myone Streptavidin C1 magnetic beads (Invitrogen, USA), which had been activated beforehand, and the products were then washed with buffer according to the kit manual. The captured DNA libraries were amplified using 15-cycle PCRs, purified, and subsequently eluted in a 0.03 ml volume and subjected to Agilent 2100 Bioanalyzer and quantitative PCR to estimate the magnitude of enrichment.

Next generation sequencing

The final captured DNA libraries were sequenced using the Illumina HiSeq2500 DNA Sequencer as PE 90 bp reads (following the manufacturer's standard cluster generation and sequencing protocols), providing an average coverage depth for each sample of at least 100-fold.

Data filtering and analysis

Image analysis, error estimation, and base calling were performed using the Illumina pipeline (version 1.3.4) with default parameters. Indexed primers were used to identify the different samples in the primary data. All unqualified reads (defined as reads either polluted by adapter, containing more than 10% nucleotides out of read length, having an average quality of less than 10, or having 50%

Table 1 COMP mutations in pseudoachondroplasia to date.

Exon	DNA change	Protein change	COMP domain	Reference
5	c.500G > A	Gly167Glu	EGF-like 4	Jackson,et al. Hum Mutat,33,144,2012
7	c.700C > T	Pro234Ser	EGF-like 4	Jackson,et al. Hum Mutat,33,144,2012
7	c.772G > C	Gly258Arg	EGF-like 4	Jackson,et al. Hum Mutat,33,144,2012
8	c.806 A > G	Asp269Gly	TSP type-3 1	Briggs,et al. Eur J Hum Genet,22,1278,2014
8	c.811G > C	Asp271His	TSP type-3 1	Deere,et al. Am J Med Genet,85,486,1999
8	c.812 A > T	Asp271Val	TSP type-3 1	Elliott,et al. Genet Mol Res,9,1785,2010
8	c.815 T > C	Leu272Pro	TSP type-3 1	Deere,et al. Am J Med Genet,85,486,1999
8	c.818 A > C	Asp273Ala	TSP type-3 1	Briggs,et al. Eur J Hum Genet,22,1278,2014
8	c.868G > A	Asp290Asn	TSP type-3 1	Ikegawa,et al. Hum Genet,103,633,1998
8	c.869 A > G	Asp290Gly	TSP type-3 1	Jackson,et al. Hum Mutat,33,144,2012
8	c.876C > G	Cys292Trp	TSP type-3 1	Deere,et al. Am J Med Genet,85,486,1999
8	c.893C > T	Ser298Leu	TSP type-3 1	Kennedy,et al. Eur J Hum Genet,13,547,2005
8	c.895G > A	Gly299Arg	TSP type-3 1	Ikegawa,et al. Hum Genet,103,633,1998
8	c.895G > C	Gly299Arg	TSP type-3 1	Jackson,et al. Hum Mutat,33,144,2012
9	c.925G > A	Gly309Arg	TSP type-3 2	Delot,et al. J Biol Chem,273,26692,1998
9	c.925G > C	Gly309Arg	TSP type-3 2	Nakayama,et al. Oncol Rep,10,871,2003
9	c.976G > A	Asp326Asn	TSP type-3 2	Yu,et al. Mol Med Rep,14,2180,2016
9	c.976G > T	Asp326Tyr	TSP type-3 2	Jackson,et al. Hum Mutat,33,144,2012
9	c.982 T > C	Cys328Arg	TSP type-3 2	Briggs,et al. Nat Genet,10,330,1995
10	c.1021_1026delGAGGAC	del 6 bp codon 341	TSP type-3 3	Kennedy,et al. Eur J Hum Genet,13,547,2005
10	c.1023_1025delGGA	del 3 bp codon 341	TSP type-3 3	Jung,et al. Int J Mol Med,26,885,2010
10	c.1024G > T	Asp342Tyr	TSP type-3 3	Briggs,et al. Nat Genet,10,330,1995
10	c.1042 T > C	Cys348Arg	TSP type-3 3	Unger,et al. Am J Med Genet,104,140,2001
10	c.1046 A > G	Asp349Gly	TSP type-3 3	Ikegawa,et al. Hum Genet,103,633,1998
10	c.1052G > A	Cys351Tyr	TSP type-3 3	Mabuchi,et al. Hum Genet,112,84,2003
10	c.1111 T > A	Cys371Ser	TSP type-3 4	Susic,et al. Clin Genet,51,219,1997
10	c.1120_1122delGAC	del 3 bp codon 373	TSP type-3 4	Briggs,et al. Nat Genet,10,330,1995
10	c.1127 A > T	Asp376Val	TSP type-3 4	Kennedy,et al. Eur J Hum Genet,13,547,2005
10	c.1133 A > T	Asp378Val	TSP type-3 4	Jackson,et al. Hum Mutat,33,144,2012
10	c.1159 T > C	Cys387Arg	TSP type-3 4	Jackson,et al. Hum Mutat,33,144,2012
10	c.1159 T > G	Cys387Gly	TSP type-3 4	Ikegawa,et al. Hum Genet,103,633,1998
10	c.1160_1162delGCC	del 3 bp codon 387	TSP type-3 4	Luo,et al. Hum Genome Var,3,,2016
10	c.1170_1181del ACCCAACTCAGAinsTGT	del 12 bp/ins 3 bp codon 390	TSP type-3 4	Loughlin,et al. Hum Mutat,S1,S10,1998
11	c.1183_1191del CAGAAGGAC	del 9 bp codon 395	TSP type-3 4	Deere,et al. Am J Med Genet,85,486,1999
11	c.1189G > T	Asp397Tyr	TSP type-3 5	Cao,et al. Genet Mol Res,10,955,2011
11	c.1205_1212del GTATAGGGinsTCTGT	del 8 bp/ins 5 bp codon 402	TSP type-3 5	Jackson,et al. Hum Mutat,33,144,2012
11	c.1220G > A	Cys407Tyr	TSP type-3 5	Cao,et al. Genet Mol Res,10,955,2011
11	c.1280G > A	Gly427Glu	TSP type-3 6	Deere,et al. Am J Med Genet,80,510,1998
12	c.1310 A > G	Asp437Gly	TSP type-3 6	Deere,et al. Am J Med Genet,85,486,1999
12	c.1318G > A	Gly440Arg	TSP type-3 6	Loughlin,et al. Hum Mutat,S1,S10,1998
12	c.1318G > C	Gly440Arg	TSP type-3 6	Cao,et al. Genet Mol Res,10,955,2011
12	c.1319G > A	Gly440Glu	TSP type-3 6	Briggs,et al. Am J Hum Genet,62,311,1998
12	c.1336G > A	Asp446Asn	TSP type-3 6	Maddox,et al. J Biol Chem,272,30993,1997
12	c.1336G > C	Asp446His	TSP type-3 6	Briggs,et al. Eur J Hum Genet,22,1278,2014
12	c.1343G > C	Cys448Ser	TSP type-3 6	Jackson,et al. Hum Mutat,33,144,2012
13	c.1345_1347delCCC	del 3 bp codon 449	TSP type-3 6	Shotelersuk,et al. Int J Mol Med,9,81,2002
13	c.1345C > A	Pro449Thr	TSP type-3 6	Deere,et al. Am J Med Genet,80,510,1998
13	c.1352_1353ins TGTCCCTGG	ins 9 bp codon 451	TSP type-3 6	Dai,et al. BMC Med Genet,12,,2011
13	c.1359C > A	Asn453Lys	TSP type-3 6	Briggs,et al. Eur J Hum Genet,22,1278,2014
13	c.1360 A > C	Ser454Arg	TSP type-3 6	Nakashima,et al. Am J Med Genet,132A,108,2005
13	c.1366_1368delCAG	del 3 bp codon 456	TSP type-3 6	Jung,et al. Int J Mol Med,26,885,2010
13	c.1371_1373delGGA	del 3 bp codon 457	TSP type-3 7	Newman,et al. J Med Genet,37,64,2000
13	c.1375_1377delTCA	del 3 bp codon 459	TSP type-3 7	Hecht,et al. Nat Genet,10,325,1995

(*continued on next page*)

Table 1 (*continued*)

Exon	DNA change	Protein change	COMP domain	Reference
13	c.1393G > A	Gly465Ser	TSP type-3 7	Briggs,et al. Eur J Hum Genet,22,1278,2014
13	c.1393G > C	Gly465Arg	TSP type-3 7	Kennedy,et al. Eur J Hum Genet,13,547,2005
13	c.1393G > T	Gly465Cys	TSP type-3 7	Newman,et al. J Med Genet,37,64,2000
13	c.1394G > A	Gly465Asp	TSP type-3 7	Briggs,et al. Eur J Hum Genet,22,1278,2014
13	c.1394G > T	Gly465Val	TSP type-3 7	Wang,et al. Hum Genet,125,350,2009
13	c.1403G > A	Cys468Tyr	TSP type-3 7	Hecht,et al. Nat Genet,10,325,1995
13	c.1411_1419del GACGACGAC	del 9 bp codon 471	TSP type-3 7	Liu,et al. Chin Med J (Engl),123,2181,2010
13	c.1412 A > C	Asp471Ala	TSP type-3 7	Nakashima,et al. Am J Med Genet,132A,108,2005
13	c.1412 A > G	Asp471Gly	TSP type-3 7	Kennedy,et al. Eur J Hum Genet,13,547,2005
13	c.1414_1419delGACGAC	del 6 bp codon 472	TSP type-3 7	Song,et al. J Hum Genet,48,222,2003
13	c.1414_1419dupGACGAC	ins 6 bp codon 472	TSP type-3 7	Delot,et al. Hum Mol Genet,8,123,1999
13	c.1414G > C	Asp472His	TSP type-3 7	Briggs,et al. Eur J Hum Genet,22,1278,2014
13	c.1414G > T	Asp472Tyr	TSP type-3 7	Hecht,et al. Nat Genet,10,325,1995
13	c.1417_1419delGAC	del 3 bp codon 471	TSP type-3 7	Hecht,et al. Nat Genet,10,325,1995
13	c.1417G > A	Asp473Asn	TSP type-3 7	Deere,et al. Am J Med Genet,80,510,1998
13	c.1417G > C	Asp473His	TSP type-3 7	Jackson,et al. Hum Mutat,33,144,2012
13	c.1417G > T	Asp473Tyr	TSP type-3 7	Song,et al. J Hum Genet,48,222,2003
13	c.1418 A > G	Asp473Gly	TSP type-3 7	Ikegawa,et al. Hum Genet,103,633,1998
13	c.1420_1425dupAATGAC	ins 6 bp codon 476	TSP type-3 7	Briggs,et al. Eur J Hum Genet,22,1278,2014
13	c.1423G > A	Asp475Asn	TSP type-3 7	Deere,et al. Am J Med Genet,80,510,1998
13	c.1423G > C	Asp475His	TSP type-3 7	Zhang,et al. J Hum Genet,60,769,2015
13	c.1435G > T	Asp479Tyr	TSP type-3 7	Kennedy,et al. Eur J Hum Genet,13,547,2005
13	c.1444G > A	Asp482Asn	TSP type-3 7	Jung,et al. Int J Mol Med,26,885,2010
13	c.1444G > C	Asp482His	TSP type-3 7	Song,et al. J Hum Genet,48,222,2003
13	c.1445 A > G	Asp482Gly	TSP type-3 7	Susic,et al. Hum Mutat,S1,S125,1998
13	c.1450 T > G	Cys484Gly	TSP type-3 7	Mabuchi,et al. Hum Genet,112,84,2003
13	c.1489 + 28G > A	IVS13 ds G-A +28	TSP type-3 7	Nakayama,et al. Oncol Rep,10,871,2003
13	c.1510 T > C	Cys504Arg	TSP type-3 8	Briggs,et al. Eur J Hum Genet,22,1278,2014
13	c.1511G > A	Cys504Tyr	TSP type-3 8	Xie,et al. Gene,522,102,2013
13	c.1511G > C	Cys504Ser	TSP type-3 8	Kennedy,et al. Eur J Hum Genet,13,547,2005
13	c.1520 A > G	Asp507Gly	TSP type-3 8	Deere,et al. Am J Med Genet,80,510,1998
13	c.1525G > A	Asp509Asn	TSP type-3 8	Jung,et al. Int J Mol Med,26,885,2010
14	c.1526 A > C	Asp509Ala	TSP type-3 8	Deere,et al. Am J Med Genet,80,510,1998
14	c.1526 A > G	Asp509Gly	TSP type-3 8	Deere,et al. Am J Med Genet,80,510,1998
14	c.1526 A > T	Asp509Val	TSP type-3 8	Zhang,et al. J Hum Genet,60,769,2015
14	c.1527 T > G	Asp509Glu	TSP type-3 8	Mabuchi,et al. Hum Genet,112,84,2003
14	c.1529_1540del CAGACAAGGTGG	del 12 bp codon 510	TSP type-3 8	Kennedy,et al. Eur J Hum Genet,13,547,2005
14	c.1531G > C	Asp511His	TSP type-3 8	Deere,et al. Am J Med Genet,80,510,1998
14	c.1531G > T	Asp511Tyr	TSP type-3 8	Hecht,et al. J Orthop Res,22,759,2004
14	c.1532 A > G	Asp511Gly	TSP type-3 8	Tufan,et al. Eur J Hum Genet,15,1023,2007
14	c.1533C > G	Asp511Glu	TSP type-3 8	Briggs,et al. Eur J Hum Genet,22,1278,2014
14	c.1537_1548del GTGGTAGACAAG	del 12 bp codon 513	TSP type-3 8	Susic,et al. Clin Genet,51,219,1997
14	c.1544 A > G	Asp515Gly	TSP type-3 8	Jackson,et al. Hum Mutat,33,144,2012
14	c.1552G > A	Asp518Asn	TSP type-3 8	Ikegawa,et al. Hum Genet,103,633,1998
14	c.1552G > C	Asp518His	TSP type-3 8	Deere,et al. Am J Med Genet,85,486,1999
14	c.1553 A > G	Asp518Gly	TSP type-3 8	Kennedy,et al. Eur J Hum Genet,13,547,2005
14	c.1579 A > G	Thr527Ala	TSP type-3 8	Hecht,et al. Matrix Biol,17,269,1998
14	c.1585 A > G	Thr529Ala	CTD	Zhang,et al. J Hum Genet,60,769,2015
14	c.1586C > T	Thr529Ile	CTD	Kennedy,et al. Eur J Hum Genet,13,547,2005
15	c.1747G > A	Glu583Lys	CTD	Deere,et al. Am J Med Genet,85,486,1999
16	c.1754C > A	Thr585Lys	CTD	Jackson,et al. Hum Mutat,33,144,2012
16	c.1754C > T	Thr585Met	CTD	Briggs,et al. Am J Hum Genet,62,311,1998
16	c.1760 A > G	His587Arg	CTD	Deere,et al. Am J Med Genet,80,510,1998
18	c.2155G > A	Gly719Ser	CTD	Kennedy,et al. Eur J Hum Genet,13,547,2005
18	c.2156G > A	Gly719Asp	CTD	Mabuchi,et al. Am J Med Genet,104,135,2001

Table 1 (*continued*)

Exon	DNA change	Protein change	COMP domain	Reference
Null	c.N	deletion c.1048_1116del69	Null	Jackson,et al. Hum Mutat,33,144,2012
Null	c.N	deletion 553 bp incl. ex. 9	Null	Mabuchi,et al. Hum Genet,112,84,2003
Null	c.N	deletion 21 bp nt 831−851, cd. 277−283	Null	Kennedy,et al. Eur J Hum Genet,13,547,2005

bases with a quality value less than 5) were removed using a local dynamic programming algorithm. The remaining reads were aligned to the reference human genome (UCSC hg19) using Burrows-Wheeler Alignment Tool (BWA-0.7.12-r1044). Next, SNPs and indels were identified using GATK software3.4-46 using the recommended parameters.

Large deletions/duplications analysis

The depths of each region of a gene in different samples within the same sequencing lane are significantly correlated ($r > 0.7$), and the depth of each capture region was therefore used to calculate a z-score equation.

The large deletions and duplications were identified using a predefined cut-off point (± 3) of derived z-score of each captured gene region. We used the cut-off value of 3 for absolute z-score, as it represents the 99.9th percentile of the normal samples set for one tailed region. Any region with a z-score above 3 was defined as either a deletion (<-3) or a duplication (>3).

Results

The proband was the younger of the two child of non-consanguineous parents, and he has an apparent disproportionate short stature according to the physical examination. Radiographic exam showed typical appearance of PSACH with irregular epiphyses and metaphyses, short and thick femoral neck of bilateral side, flattened femoral head, anterior tonguing or beaking of the vertebral bodies.

The genetic analysis indicated a novel heterozygous mutation c.1219 T > C in exon 11 of the COMP, which is located in the type-3 calcium-like repeat region of the COMP gene. As a consequence, the amnio acid cysteine was substituted by arginine. The same missense mutation was also found in his mother, while his father is normal at this locus (Fig. 3). This mutation do not belong to polymorphism sites. We can not found the mutation in 100 healthy controls. We did not find the mutation in the Human Gene Mutation Database professional. This mutation is predicted to be probably damaging with a score of 1.000 (sensitivity:0.00; specificity:1.00) using the Poly-phen2 both on HumDiv and HumVar models (Fig. 4). What's more, the substitution at position 407 from cysteine to arginine is predicted to affect protein function with a score of 0.00 with the Sorting Intolerant From Tolerant (SIFT) predictions (Fig. 5).

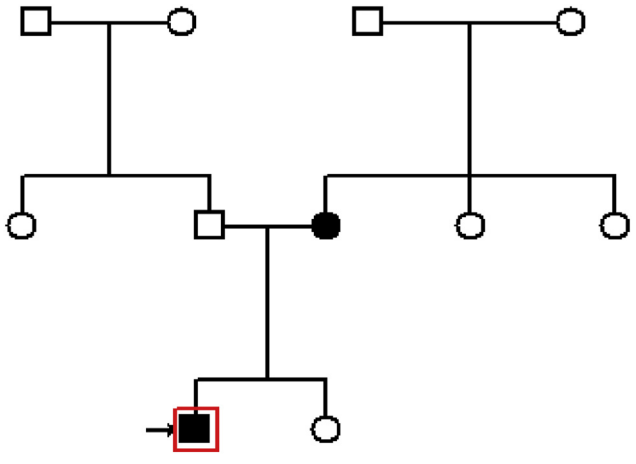

Figure 1 pedigree chart of the family.

Discussion

The prevalence of PSACH in a particular group of foreign countries is approximately 1/30,000 (www.orpha.net/consor/cgi-bin/home.php?Lng=GB),[9,11] but there is no definite investigation about prevalence of PSACH in China. We make the diagnosis of PSACH mainly depending on family history, clinical symptoms and radiological features.[12,13] It is necessary for us to differentiate this disease with MED or achondroplasia (ACH) especially for atypical cases. At this time, genic analysis can largely assist in diagnosis. Mutations of COMP gene are the cause for nearly all PSACH patients and most MED patients.[6,14,15] Mutations of MED still involving *MATN3*, type IX collagen (*COL9A1*,-*COL9A2*, and *COL9A3*), *SLC26A2*, *DTDST*.[6] The disease-causing gene of ACH is FGFR3.[16] In our case, we have indicated a novel missense mutation c.1219 T > C in exon 11, which result in the residue substitution from cysteine to arginine. The alteration of amino acid located in TSP type-3repeats, which was the most frequent mutation of the PSACH patients. Previously, there are reports that identified mutations in the same location of amino acid but with different nucleotide (PSACH:c.1220G > A,p.Cys407Tyr; MED:c.1220 G> T,p.Cys407Phe) and different amino acid substitution. It reminds us that this codon is a relatively susceptible loci of the COMP gene.[17,18] In Table 1, five mutations in exon 11 are cited, but one of them is base fragments insertion, one is deletion of base fragments, and with three point mutations. Although they are in the same exon, they can lead to different amino acid changes and different protein transformation.

Cartilage Oligomeric Matrix Protein is the only thrombospondins that has been associated with skeletal

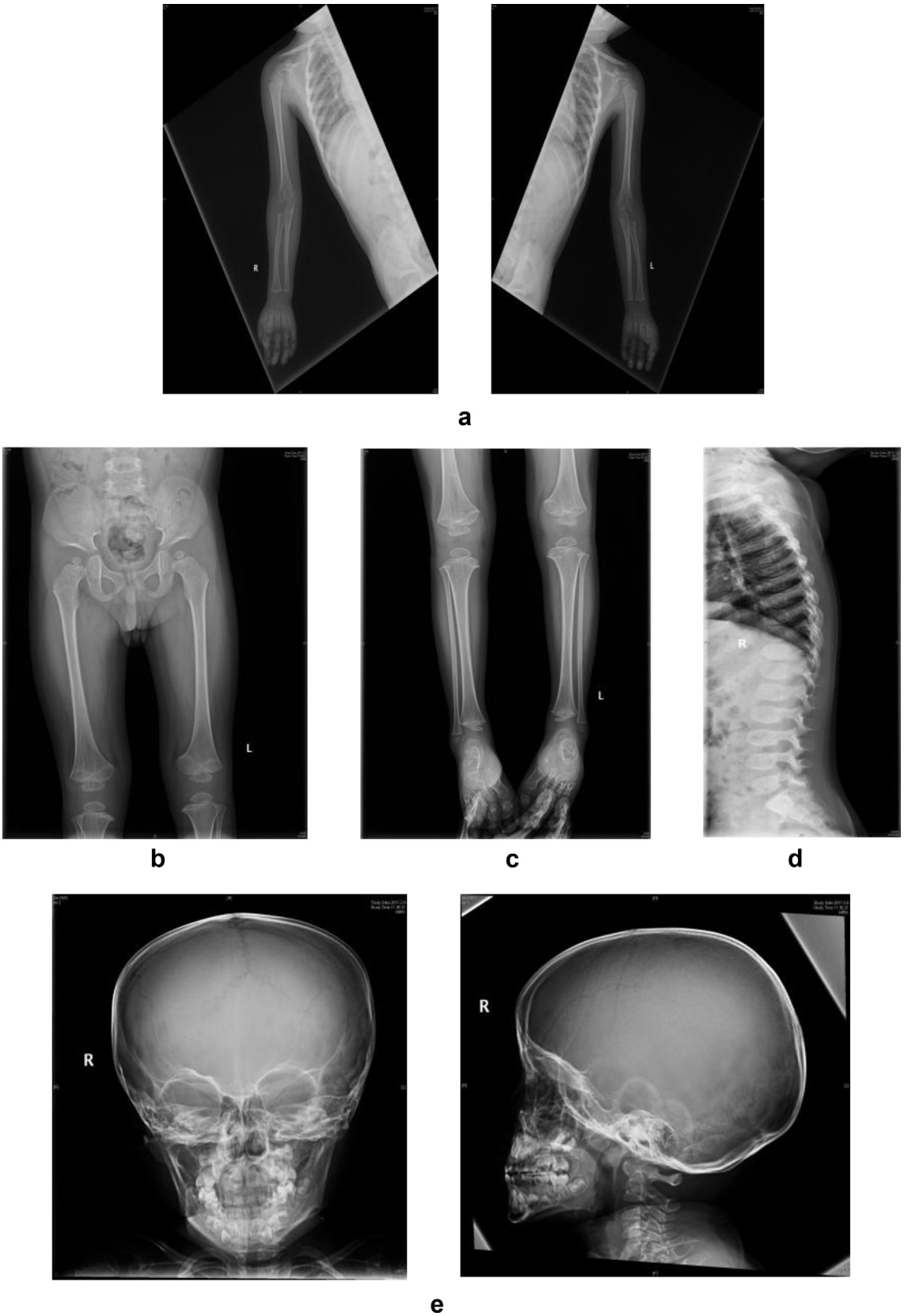

Figure 2 Radiographic findings of the patient: 1. Short tubular bones with irregular epiphyses and metaphyses (a,c); 2. Bilateral short and thick femoral neck, flattened femoral head (b); 3. Anterior tonguing or beaking of the vertebral bodies (d); 4. Normal skull(e).

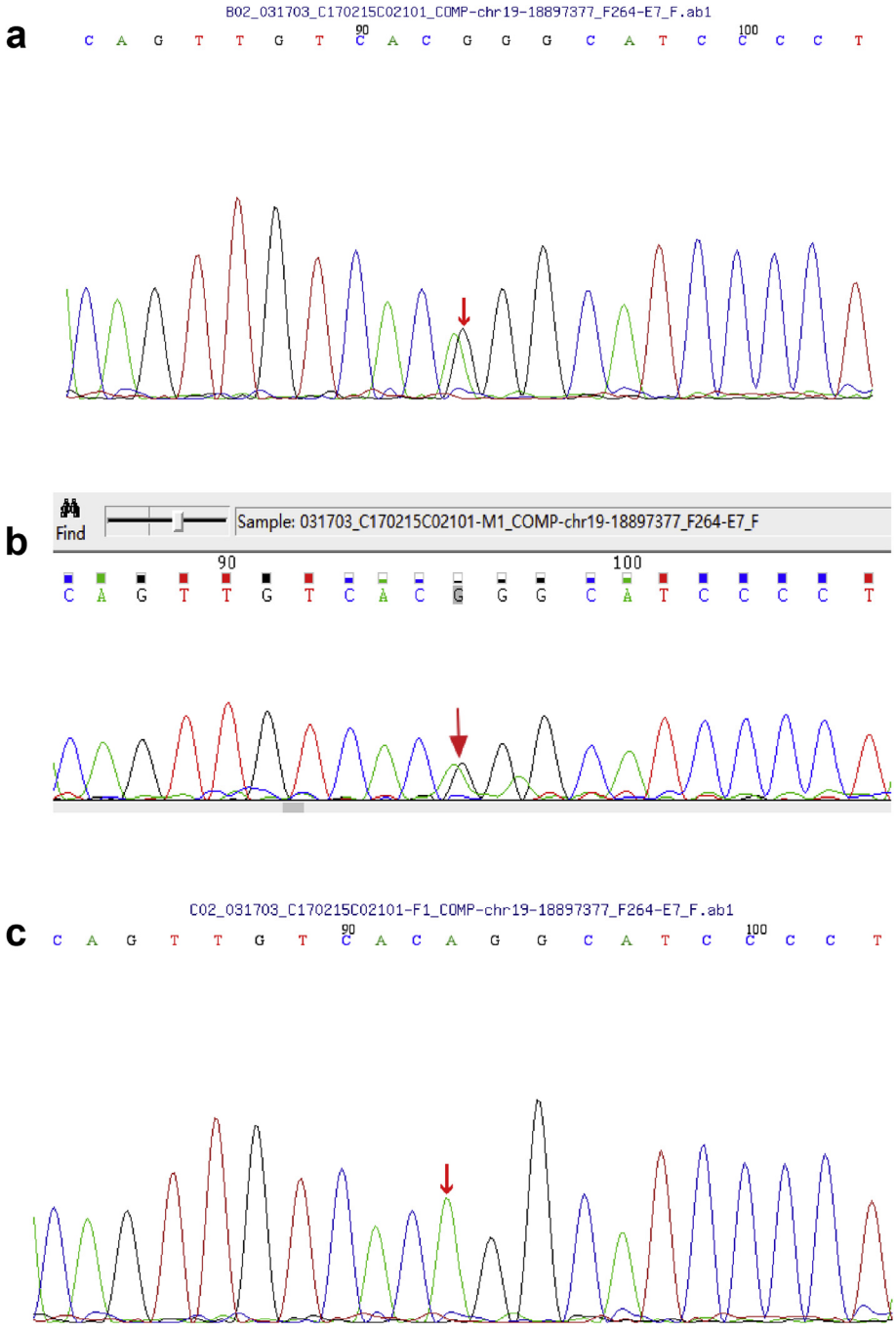

Figure 3 Consequence of DNA analysis. a. The proband: heterozygous mutation. b. His mother: heterozygous mutation. c. His father: normal.

disorders in humans.[19] And it is remarkably conserved protein among different mammalian species. So far there are many studies on animal models aiming at the mechanism of COMP.[20–24] COMP is abundantly expressed in extracellular matrix (ECM) of musculoskeletal tissues. In the ECM, COMP interacts with many other proteins such as collagen type II, collagen type IX, matrilin 3 and SPARG. In addition, variety of proteins such as MMPs could regulate the levels of COMP in different conditions. These interactions play an important role in maintaining the structural integrity of cartilage and in regulating cellular functions.[19] Mutations of COMP gene lead to misfolding of COMP, which makes massive intracellular retention of COMP and other ECM proteins in the endoplasmic reticulum (ER) of growth plate chondrocytes later on. The changes can result in activation of the unfolded protein response (UPR), which is related to variety of inflammatory and stress signaling pathways.[25] Inflammatory matters much in the pathology and may contribute to the all pain sequelae. Meanwhile, unregulated apoptosis of chondrocyte appears.[16] Skeletal dysplasia appears as a result of the aforementioned alteration. What's more, it

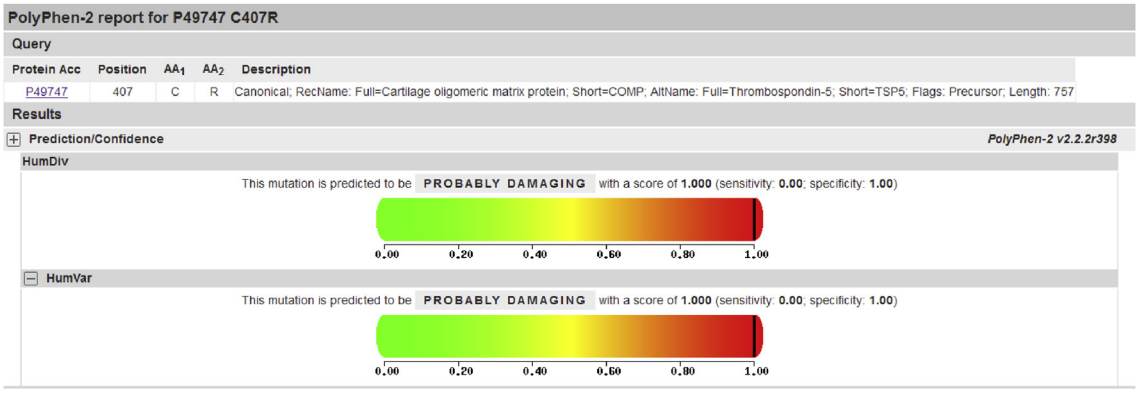

Figure 4 PolyPhen-2.

pos	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
101D 1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
102G 1.00	0.05	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.04	0.03	0.00	0.02	0.03	0.04	0.03	0.00	0.00	0.00	0.00	0.00
103I 1.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.17	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
104G 1.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
105D 1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
106A 1.00	1.00	0.12	0.16	0.27	0.46	0.24	0.53	0.32	0.22	0.62	0.18	0.22	0.14	0.36	0.25	0.29	0.27	0.62	0.12	0.65
107C 1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
108D 1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
109N 0.99	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.27	0.01	0.00	0.00	0.00
110C 0.99	0.00	1.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Figure 5 SIFT prediction: prediction of the protein function can be more accurate when the number of the amino acid ranges from 300 to 400, so we have deleted the first 300 amino acids and the last 157 amino acids of COMP.

seems that mutations in the type 3 thrombospondin-like domain of COMP cause severe phenotype of PSACH patients.[26] Interestingly, studies in mice showed normal phentype when the whole COMP gene was knocked-out.[27] It means that cartilage dysplasia of PSACH/MED is not a result of the reduced amount of COMP but dysfunctional mutated COMP. Actually, we know little about the exact molecular defects of skeletal dyspalsia, which limit the progress of effective therapies. Further experimentation on animals are demanded aiming at molecular mechanism and therapies.

So far there is no special therapy for this genetic disease, only symptomatic treatments have been available for the affected individuals.[28] Of course, if there are spinal cord compression, severe osteoarthritis or severe osteoarticular deformity, surgical operation required. It was confirmed that growth hormone can do nothing about the short stature of PSACH patients.[29] Antioxidant and anti-inflammatory agents can mitigate pathology by studies in a mouse model of pseudoachondroplasia. They found that both of the two kind of pharmaceutical preparations could improve the organization of MT-COMP growth plate, restore the chondrocyte proliferation, reduce intracellular retention of MT-COMP and decrease irregular apoptosis. It is meaningful that the study draws a conclusion that both antioxidant and anti-inflammatory agents can increase femoral length, which can be very critical for the therapy of disproportionate short stature.[30] While in one other mouse model experiment, researchers delivered antisense oligonucleotides to the growth plate. They concluded that it is clearly effective in reducing COMP mRNA, COMP intracellular retention and inflammation caused by MT-COMP expression.[31] It provide us an extra approach for the PSACH or MED.

In conclusion, we have identified a novel mutation in exon11 of COMP gene in a pseudoachondroplasia patient. His mother has the same mutation and apparent short stature. We can see that the little patient inherited the mutation from his mother. This novel mutation can expand the spectrum of COMP mutations. Although many mutations are identified, we know little about the exact mechanism and progression of the disease. More depth studies are demanded for the specific pathology of PSACH/MED and guidelines of therapy.

Conflict of interest

The authors declare no conflict of interests.

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