A familial case of Pseudoachondroplasia in China, with a deletion-mutation in *COMP* gene identified by Next Generation Sequencing

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Abstract— Pseudoachondroplasia (PSACH) is a rare form of skeletal dysplasia, caused by mutation in the Cartilage Oligomeric Matrix Protein (COMP), which has an autosomal dominant (AD) inheritance pattern or can be from *de novo* mutation. Few cases have been reported in China. We report a family of two generations affected with PSACH. The Proband, male 38 years old, with short stature and obvious shortened upper and lower limbs attended our Genetic counseling center with his wife, normal physic and 11-weeks pregnant, with concern about the probability that the fetus may have PSACH phenotype. Peripheral venous blood sample and amniocytes were the sources of DNA extraction for proband and fetus respectively. Next generation sequencing was used to map any gene mutation as the cause of PSACH. DNA analysis revealed a heterozygous mutation *c1417_1419delGAC (p.Asp473del*) present in both proband and fetus. This deletion mutation has been described before worldwide but not as a familial case of PSACH in China. This case also emphasizes the importance of genetic molecular testing in prenatal diagnosis of rare diseases.

Index Terms— Pseudoachondroplasia, COMP gene, next generation sequencing,

1 Introduction

Pseudoachondroplasia (PSACH) is a rare inherited disorder of bone growth that is clinically characterized as short-limbed dwarfism and has a reported prevalence of approximately four per million individuals[1]. PSACH is inherited in an autosomal dominant (AD) pattern, meaning that only one copy of the mutated gene is sufficient to cause the condition and results in the characteristic phenotype. It may also occur as a result of *de novo* mutation(s) in patient with no prior family history of PSACH. Clinically all patients have short stature which becomes apparent in early childhood as the child growth rate falls below the standard growth curve. As the child grows, more features become evident such as short arms and legs; a waddling walk, laxity (hyperextensibility) of joints of arms, elbows, knees, ankles and hips. Some patients may present with lower limbs deformities (valgus or varus deformity). Spines abnormalities in PSACH patients include Scoliosis and Lordosis. Patients usually don't have facial deformities. They can perform normal daily activities, depending on degree of bone deformities, and have normal intelligence. Work-up for diagnosis includes clinical findings, radiographic features and a definitive diagnosis by molecular genetic indentification of mutation in the cartilage oligomeric matrix protein (COMP) gene, located on chromosome 19p13.1. A carrier of COMP gene when married to a normal partner therefore has a 50% chance of transmitting the mutation to next generation. There is no definitive treatment but supportive in daily activities of the carrier depending on degree of severity of deformity. Genetic molecular testing offers a crucial role in prenatal diagnosis of rare diseases. Here we report a familial case of pseudoachondroplasia in China.

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2 CASE REPORT

2.1 Patients

A 38 years old male Proband reported to our genetic and prenatal diagnosis center on November 2017 for short stature. Born to normal nonconsaguinous parents, his height was the same as the 50th percentile of a 3.5 years old child. He had short upper limbs, joint laxity at elbows and moderate lordosis. He has a 12 years-old daughter presenting with similar phenotype and a height of 72cm. His wife was pregnant at 11 weeks gestation age (calculated from her last menstrual cycle). The wife is physically normal. The Proband underwent surgery for bilateral lower limbs club feet in his early years. The Proband and his wife were worried about the probability that the fetus could present with similar deformities, which make them seek medical attention and counseling.

2.2 Next Generation Sequencing and Sanger Sequencing

Abdominal ultrasound was performed but provided no clear information to diagnose PSACH. After counseling the parents about genetic molecular testing, they agreed and opted for Next Generation Sequencing (NGS). Peripheral venous blood samples were extracted from the father by Tiangen Extraction kit (Beijing, China), while ultrasound guided amniocentesis was performed for fetus as source for DNA. Genomic DNA of 3ug was used for library preparation according to manufacturer's instruction (MyGenostics, Beijing, China). A panel of genes including *COMP, COL9A1, COL9A2, COL9A3, MATN3, and SLC26A, COL2A1* was captured with OncoCap Enrichment System (MyGenostics, Beijing, China) based on their establish procedures. After enrichment, libraries were sequenced on an Illumina Solexa HiSeq 2000 sequencer for paired reads of 100 bp followed by data retrieval using Solexa QA package and a cutadapt program. Expected coverage was 99% of the targeted genomic regions of interest, which achieved an average alignment performance of 98% across all samples. PCR and Sanger sequencing were performed to confirm the candidate mutation using an ABI 3130XL automatic genetic analyzer (Applied Biosystems, Foster City, CA).

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3 RESULTS

3.1 Clinical findings

Physical examinations of the proband revealed markedly reduced height measuring only 116 cm (Fig 1A), both the upper and lower limbs segments were disproportionately shortened. The upper limbs segments were 18 cm for forearn and 26cm for arms. In addition, there was limited elbow extension (up to 130° only). The size of hands was small and the length of each finger also appears shortened (Fig 1C). The lower limbs segment measured 16 cm for thigh and 26 cm tibial length with post surgery scar on tibia bilaterally. No genu valgum deformity awas present since he underwent surgery in his early years for bilateral club feet deformities (Fig 1F). There was exaggerated lumbar lordosis which was more evident when he was in the sitting position (Fig 1B). Systemic examination and intelligence were normal

Table 1 Clinical findings and measurements

Body part	Measurement/ cm
Head circumference	58
Height	116
Forearm	18
Arm	26
Chest circumference	84
Abdominal girth	88
Thigh	16
Tibial length	26

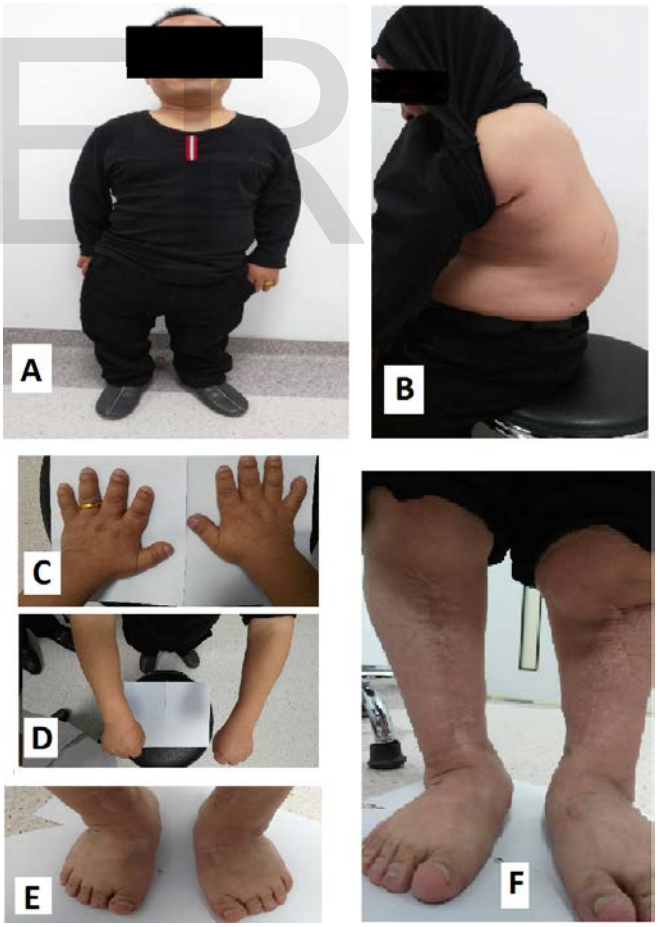

Figure 1 Clinical features of Proband (A) short stature, (B) lordosis (C) short hands and fingers, (D) short forearms, (E) short feet and (F) short tibia (post surgery)

3.2 Mutation identification by targeted NGS and Sanger Sequencing

In the present study, NGS identified a mutation in the *COMP* gene as the causative factor for the disorder in the proband and fetus. These findings were confirmed using Sanger methodology. The identified mutation *c1417_1419delGAC (p.Asp473del)* in the *COMP* gene has been reported in individuals sporadic cases but not as familial case in China. The proband and the fetus both harbored the same mutations which confirmed an AD inheritance pattern of this disorder. Thus the mutation in the *COMP* gene confirmed the diagnosis of PSACH in these two generations. The parents were informed of the condtion and given the choice for continuing or termination of pregnancy.

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4 Discussion

PSACH is an AD form of short limb dwarfism which can exist as mild, moderate and severe form and earliest feature appears as short stature in childhood [2]. Clinical features may suggest PSACH if interpreted by a skilled radiographer but a definitive diagnosis can only be made by genetic molecular testing. In this family, a heterozygous mutation *c1417_1419delGAC (p.Asp473del)* in *COMP* gene was identified as the cause of PSACH. Clinically the Proband had most of features of PSACH including short stature, short arms and legs, joint laxity and lordosis. An AD pattern of inheritance was concluded as the same mutation was identified by NGS in both Proband and fetus cDNA sequences. NGS proved useful in identifying the causative *COMP* gene mutation.

COMP is located at chromosome 19 and encodes for proteins that are essential for the normal development of cartilage and for its conversion to bone. *COMP* is a large extracellular pentameric glycoprotein found in the territorial matrix surrounding chondrocytes A mutation of *COMP* gene will therefore lead to a disorganized matrix in bone resulting in abnormal bone formation and growth ending in two skeletal dysplasia namely pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED) [3]. The mutation *c1417_1419delGAC (p.Asp473del*) in this study has beed reported worldwide as one of the most common cause of deletion--insertion mutation in the *COMP* gene to cause severe PSACH. Majority of deletion, insertion mutations lead to PSACH while only few instances lead to MED. In this mutation, *(p.Asp473del*), there is deletion of a single aspartic acid residue from a contiguous stretch of five aspartic acid codons (GAC) encoding residues 469 to 473 of COMP sequnence and similar findings have been described in numerous studies worldwide [4]. In china, few cases of PSACH have been described in literature and our findings add to another case of PSACH in the *c1417_1419delGAC (p.Asp473del*) mutation category. Here we described a rare case of familial pseudoachondroplasia affecting 3 members of a family, two of whom have same mutation (daughter was not included).

MED and PSACH can have overlapping features and involve *COMP* gene mutation. Autosomal dominant MED however can be caused by other genes including *COL9A1, COL9A2, COL9A3* and *MATN3* while recessive MED can involve *SCL26A2* gene. MED can be regarded as a milder form of *COMP* mutation and PSACH the severe form of COMP mutation. Clinically it is difficult to separate them based on clinical and radiographic findings. Therefore a panel of gene including *COMP* and the other genes of interest should be considered for a definitive diagnosis. The integration of NGS in prenatal diagnosis is fundamental as it allows identification of some rare disorders early in pregnancy. This provides a cutting edge in giving accurate diagnosis by identifying causative gene(s) quickly with greater certainty therby permitting parents-to-be to be informed early enough about the arrival of fetus with deformities. The parents can be counseled with much confidence and offered the choice for continuing or termination of pregnancy.

5 Conclusion

This is a familial case of PSACH in China, with two generations bearing similar mutations in the *COMP* genes. PSACH patients can lead normal life with limited daily activities. Due to its AD inheritance pattern, there is 50% chance of transmission to offsprings therefore genetic molecular testing allows for an early recognition and diagnostic precision which is essential for accurate prognostication, meaningful genetic counseling, and early therapy where possible.

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Conflict of Interest

The author has no conflict of interest.

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