CLINICAL QUIZ (p50-51) ANSWER

What is the diagnosis?

Our patients presented with mesomelic short stature together with the classical X-ray findings of Madelung deformity over the wrists, *SHOX* related haploinsufficiency was highly suspected. Therefore multiplex ligation-dependent probe amplification (MLPA) testing of the *SHOX* gene was performed. A heterozygous interstitial deletion of at least 766.5 kb downstream of the *SHOX* gene at 594.7 kb from Xp-telomere was detected in both girls. The diagnosis of Léri-Weill dyschondrosteosis (LWD) was substantiated. The deletion was inherited from their father.

What is *SHOX*-related disorder?

SHOX related haploinsufficiency is one of the most common genetic causes for short stature. The estimated frequency is less than 1:2500,[1] even more common than growth hormone deficiency and Turner syndrome in female children presented with idiopathic short stature. The clinical expression is highly variable, ranging from idiopathic short stature (ISS; OMIM #604271), Turner syndrome, Léri-Weill dyschondrosteosis (LWD; IMIM #127300), and Langer mesomelic dysplasia (OMIM #249700). The estimated prevalence of *SHOX* mutations is about 2-15% for ISS, 50-90% in LWD and almost 100% in Turner syndrome.[2]

The *SHOX*-short stature homeobox-containing gene is located in the PAR 1 region on the short arm of both sex chromosomes at Xp22 and Yp11.3 and the genes in the PAR region escape the X inactivation. Two copies of genes are required for normal growth development and thus mutation of *SHOX* gene exhibit pseudoautosomal inheritance. *SHOX* gene encodes SHOX protein, which act as homeodomain transcription factor that is expressed in the developing limb buds and in the first and second pharyngeal arches. It mediates linear growth mainly by modifying the bone

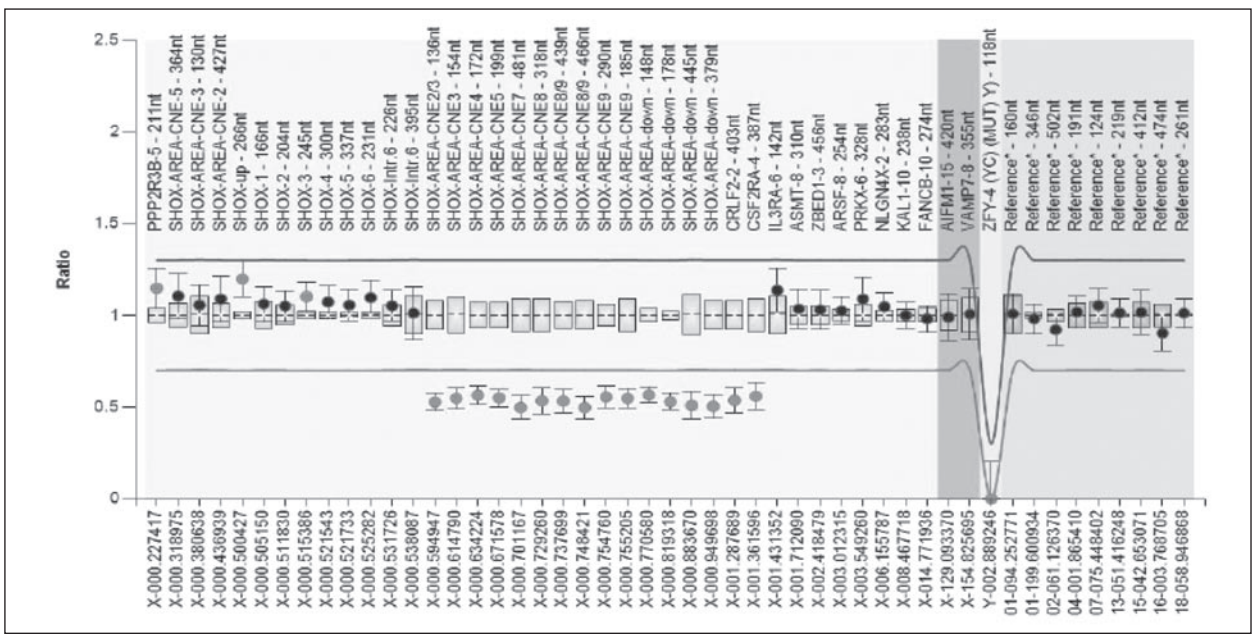

Figure 2 MLPA assay results of proband using Coffalyser programme. Map view locations (NCBI) are displayed on the x-axis and y-axis represent dosage quotient (DQ). Normal is between 0.8 to 1.2. Ratio between 0.4 to 0.65 suggestive of heterozygous deletion. Black dot means normal while grey dot means abnormal DQ. This showed a heterozygous deletion of downstream enhancer region of *SHOX* genes included ECR1/CNE7 loci.

marrow fibroblasts and hypertrophic chondrocytes at growth plate. *SHOX* gene mutations would lead to disorganisation of the proliferating chondrocytes and thus leading to short stature and other skeletal abnormalities specifically those developed from the first and second pharyngeal arches (bones of forearms and wrist and equivalent bones in lower limbs). There were more than 60 different mutations found in *SHOX* gene associated with short stature and those reported would be assembled in the *SHOX* gene mutation database (www.shox.uni-hd.de).[3] The most common mutation reported was deletion (around 80%) either in the *SHOX* gene or the regulatory enhancer region upstream or downstream of the coding genes.[2,4]

The phenotype for *SHOX* gene mutation is highly variable and the penetrance is incomplete. The size and types of mutation are not correlated with the severity of phenotypes. This has demonstrated in this family that father has normal height and phenotype, while both twin daughters had typical features of Léri-Weill dyschondrosteosis. In general, females would be more severely affected than affected males and the skeletal defects tend to be worsening with puberty with the proposed mechanism due to estrogen effect influencing the phenotype in *SHOX* haploinsufficiency.[5] Short stature is the most common presentation. It starts since infancy and the mean adult height could be -2.2 standard deviation (SD)[6] compared with mean height for normal adult. Because of the higher expression of SHOX protein in bones developed from the first and second pharyngeal arches, *SHOX* gene mutations would lead to other common clinical features included mesomelia (i.e. shortening of forearms and lower legs), Madelung deformity of wrists and cubitus valgus or genu valgus. Less specific signs including those from Turner syndrome, namely high-arched palate, shortening of the fourth and fifth metacarpal, increased carrying angle of elbow, scoliosis and micronagthia.

Clinical and diagnostic implication

Because of the high phenotypic variability, there is no uniform consensus for the indications of *SHOX* related haploinsfficiency. There are several scoring systems developed to identify children who should be screened for *SHOX* related haploinsufficiency including Binder[7] (based on anthropometric measurements or typical X-ray abnormalities) and Rappold score[8] (based on clinical signs and anthropometric measurements). However, the scoring system may not be able to identify some young children when the skeletal disproportion has not yet sufficiently developed. Wolters et al conducted a study in prepubertal short stature children in German to estimate the prevalence of *SHOX* mutations in idiopathic short stature and analyse the validity of different methods and diagnostic scores to identify children with *SHOX* deficiency.[9] From the study, different clinical signs are not significantly different between *SHOX* and non-*SHOX* deficient children. For anthropometric measurements, the ratios of forearm length to height, lower leg length to height, forearm circumference to height, and arm span to height were significantly decreased, while sitting height-to-height ratio and sitting height-to-height ratio's standard deviation score (SDS) were significantly increased in children with *SHOX* deficiency compared to their counterparts without *SHOX* deficiency. Typical radiological signs in children with *SHOX* deficiency, include: (1) triangularisation of distal radial epiphysis (due to hypoplasia of ulnar aspect of distal radial epiphysis); (2) pyramidalisation of lunate (due to rotated lunate wedging at the apex of narrowed radioulnar angle); and (3) lucency at ulnar aspect of distal radius. These signs are quite specific for *SHOX* deficiency, as none of the children without *SHOX* deficiency showed radiological signs typical of *SHOX* deficiency. Interestingly different groups, including Binder et al have found that the radiological signs typical of *SHOX* deficiency are not obvious until the children is or more than eight years old.[7,8] Therefore, radiographs of wrist or hand of younger patients have to be interpreted with cautions, and comparison with serial and follow-up radiographs have to be considered in all cases.

Wolters et al analysed different scoring systems for identification of children for screening of *SHOX* mutations. Rappold scoring system showed high sensitivity (73%) but low positive predictive value (9%). Binder score with extremities-to-trunk ratio and sitting height–to-height ratio showed the best specificities (91%) and positive predictive

value (25%). Therefore, children with increased sitting height-to-height ratio and/or reduced extremities-to-trunk ratio should prompt the clinician to obtain a molecular analysis of the *SHOX* gene.

As majority of *SHOX* related haploinsufficiency is caused by deletion of *SHOX* gene or its regulatory enhancer region, MLPA study should be the first line of genetic testing. If MLPA result is normal, *SHOX* gene sequencing should be pursued for clinically suspected *SHOX* related haploinsufficiency.

Management

Growth hormone (GH) therapy was proven to be effective in improving the growth pattern and final adult height in children with Turner syndrome and *SHOX* haploinsufficiency.[10] It was reported that the height gain can be reached up to 0.9 SDS over 2 years of GH treatment and final adult height would be increased by 1.1 SDS when compared with baseline. It has been approved in 2012 by the Hospital Authority in Hong Kong to include *SHOX* gene disorder as the indications for growth hormone therapy.[11] Gonadotrophin releasing hormone analogue (GnRHa) could also be used to delay puberty and to prolong the period for bone growth and mitigate the development of skeletal features.

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