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PAPSS2-related brachyolmia: clinical and radiological phenotype in 18 new cases

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Abstract

Brachyolmia is a skeletal dysplasia characterised by short spine-short stature, platyspondyly and minor long bone abnormalities. We describe 18 patients, from different ethnic backgrounds and ages ranging from infancy to 19 years, with the autosomal recessive form, associated with *PAPSS2*. The main clinical features include disproportionate short stature with short spine associated with variable symptoms of pain, stiffness and spinal deformity. Eight patients presented prenatally with short femora, whereas later in childhood their short-spine phenotype emerged. We observed the same pattern of changing skeletal proportion in other patients. The radiological findings included platyspondyly, irregular end plates of the elongated vertebral bodies, narrow disc spaces and short over-faced pedicles. In the limbs, there was mild shortening of femoral necks and tibiae in some patients, whereas others had minor epiphyseal or metaphyseal changes. In all patients, exome and Sanger sequencing identified homozygous or compound heterozygous *PAPSS2* variants, including c.809G>A, common to white European patients. Bi-parental inheritance was established where possible. Low serum DHEAS, but not overt androgen excess was identified. Our study indicates that autosomal recessive brachyolmia occurs across continents and may be under-recognised in infancy. This condition should be considered in the differential diagnosis of short femora presenting in the second trimester.

Introduction

Brachyolmia is a genetically heterogeneous skeletal dysplasia characterised by disproportionate short stature with a short trunk, platyspondyly and often, mild long bone abnormalities. Clinical and radiological descriptions of this condition have led to several recognised entities. Currently two genes are associated with brachyolmia: *TRPV4* (Transient Receptor Potential Cation Channel 4) with the autosomal dominant type, brachyolmia type 3 (#113500) (Rock et al., 2008) and *PAPSS2* (3'PhosphoAdenosine 5'PhosphoSulphate Synthetase 2) with the recessive forms brachyolmia types 1 (#271530) and 4 (#612847), (Miyake et al., 2012; Handa et al., 2016). Brachyolmia type 2 (#613678), which has minor phenotypic variation and is also recessive, may also be linked to *PAPSS2*, although it is possible that further genes may account for this form and other rare cases. *PAPSS2* encodes 3'phosphoadenosine 5'phophosulphate synthetase 2, one of two isoforms of PAPSS (3'PhosphoAdenosine 5'PhosphoSulphate Synthetase), which convert inorganic sulphate and ATP into PAPS (3'phosphoadenosine 5'phosphosulphate). As PAPS is a sulphate donor for many intrinsic metabolic pathways, including the sulphonation of proteoglycans in cartilage and the conversion of DeHydroEpiAndrosterone (DHEA) to DHEAS, perturbations of this pathway might be expected to have an impact on skeletal and endocrine systems. Recent studies have shown that recessive mutations in *PAPSS2* caused brachyolmia in a total of 20 patients in 3 separate studies (Miyake et al., 2012; Lida et al., 2013; Handa et al., 2016) as well as spondyloepimetaphyseal dysplasia Pakistani Type, a more severe dysplasia (Ahmad et al., 1999), in 21 patients (Tuysuz et al., 2013). *PAPSS2* variants have also been described in an alternative context: in one patient with premature puberty (Noordam et al., 2009), and two others with androgen excess (Oostdijk et al., 2015). *PAPSS2*-related recessive brachyolmia has previously been reported in patients from Turkey, Syria, Lebanon, Kurdistan, Japan and Korea, but not in the European population.

Here, we describe 18 new patients from 15 families from the UK and Europe, from different ethnic backgrounds, with *PAPSS2*-related brachyolmia. We review their clinical features and evolving radiological phenotype from childhood to 19 years, in the context of the *PAPSS2* variants we identified. We compare our findings with those of previously published patients and further delineate the phenotype of this condition.

Materials and Methods

Patients

Eighteen patients were identified through the Deciphering Developmental Disorders (DDD) Study as previously described (Wright et al., 2015) and European Clinical Genetics networks. A standard proforma was sent to each clinician to collate the clinical, radiological and molecular genetic findings for each patient. The anonymised radiographs for each patient were collected and systematically reviewed by three expert paediatric radiologists in consensus. Written consent for review of clinical data and inclusion in publication was provided from all patients where identifiable images are shown.

Z-scores for height and weight were calculated using British 1990 reference growth data. Bone age was assessed by a single observer (AC) using the Tanner-Whitehouse 3 (TW3) method, excluding the carpal bones, with separate TW3 assessment of the carpals and by another observer (ACO) using the Greulich and Pyle (G&P) method.

Laboratory methods

DNA for molecular analysis was extracted from peripheral blood leukocytes. The molecular genetic diagnosis was made through the Deciphering Developmental Disorders study, or through an independent whole exome sequencing approach or via targeted next generation sequencing using the Illumina TRUSight One Sequencing Panel. *PAPSS2* variants were confirmed by Sanger sequencing in all patients and bi-parental inheritance was confirmed with Sanger methods where samples were available. Variant nomenclature uses the accession number NM_001015880.1.

Results

Pattern of growth and stature

All patients presented with either short long bones prenatally or with short stature in infancy or childhood. Eight out of the 18 patients were identified to have short long bones on prenatal scans around 20 weeks' gestation. The 10 patients who did not present prenatally presented between 1 year 4 months and 14 years, due to short stature (Table 1). All patients had short stature compared with that expected for parental height. The four patients who are aged 16 years or older all had a height below the 0.4th centile (z-scores -4.53, -3.94, -3.44, -4.83). For those patients where sequential growth data are available, we observed a changing pattern of growth over time (Fig. 1), demonstrating that whilst the limbs are relatively short initially, spine shortening becomes more obvious later. The z scores for body mass index (BMI) were positive in all patients where measured (Table 1). Longitudinal growth data is available in supplementary materials.

Spine

Spinal deformity was recognised in 10 of the patients, including thoracic kyphosis, scoliosis and lumbar lordosis (Fig. 2). Four patients had limitation on flexion of the spine. Only one patient (P17) required surgical spinal intervention - a lumbar decompression for spinal stenosis at the age of 15 years, which was repeated at 18 years.

Limbs

Pain, usually but not always mild, affecting the back, hips or knees was reported in eight of the 18 patients. Of those who have not reported pain, six are under the age of 10 years old. Seven patients had abnormal lower limb alignment, genu varum in three patients and genu valgum in four. This was mild in the majority of cases, although one patient (P8) had medical hemiepiphysiodesis with 8-plates at 10 years of age. In one patient (P18) enlargement of the knee and ankle joints occurred and another (P8) had recurrent patella dislocations associated with swelling and also had restricted flexion of the metacarpophalangeal joints.

Endocrine

Eight patients in our study have had endocrine investigations (Table 2). All eight had low plasma DHEAS, three patients (P14, P16 and P17) had raised testosterone and two (P14 and P16) had raised androstenedione. Two patients (P16 and P17) reported clinical features that are consistent with androgen excess (Table 2). We note that P17 was taking a hormonal preparation (cyproterone-ethinyl estradiol) at the time of her endocrine investigations.

Additional findings

Motor development for all patients is normal. No consistent dysmorphic features were identified (facial features are described in Table 3). Neurodevelopment was normal in the majority of patients, although two had some additional educational needs requiring support within their mainstream school. One of these (P4), has an additional genetic diagnosis, a paternally inherited deletion of chromosome 10q11.22q11.23, which may be a contributing factor. Patient 4 is myopic, but no ophthalmological features or audiological abnormalities were seen in other patients. Apart from one patient (P5) with dental overcrowding, no patients have significant dental anomalies.

Radiological findings

Seventeen patients had one or more radiological examinations, selected data are shown in Fig. 2. One patient (P7, the younger sibling of P6) who was 8 months old at the time of our study, did not have radiographs as the diagnosis was made after the observation of short limbs prenatally and later confirmed by identifying biallelic *PAPSS2* variants, in the context of having an affected older sibling.

The radiological characteristics of *PAPSS2*-brachyolmia are shown in Figure 2 and Table 4 with comparison to those patients previously described in the literature (Miyake et al., 2012; Lida et al., 2013; Handa et al., 2016). Two patients without rectangular vertebrae, irregular vertebral end plates, narrow disc spaces and platyspondyly were under the age of 3 years at the time of assessment. Patient 12, who had only elongated vertebrae but did not have the other features of irregular end plates, rectangular vertebrae, platyspondyly or narrow disc spaces, was the exception. Three of the five patients with short metacarpals had coned metacarpal metaphyses. None of the patients assessed had broad metacarpals, in contrast to a previous study (Lida et al., 2013). Broad ilia, seen by Lida et al., were not identified in our patients. Metaphyseal striations at different metaphyseal sites were present in nine out of 16 patients. Relatively flat epiphyses were present in seven out of 16 patients, affecting variably the femur, knee and ankle. No patient had precocious ossification of costal cartilage. Bone age (TW3 RUS) was assessed in 12 patients. Whilst there were some minor variations, no patient had a general bone age that was more than two years advanced or two years delayed. Carpal bone age was assessed in 13 patients and was more than two years advanced in three.

Genetic studies

Genetic testing identified biallelic recessive mutations in *PAPSS2*: two patients were compound heterozygotes and 16 were homozygotes (Table 5). Twelve patients shared the common missense variant c.809G>A p.(Gly270Asp), ten patients from eight families were homozygous and a further two patients compound heterozygous for this variant. The ethnicities of patients with the c.809G>A variants were White British, White Norwegian and White French. Seven further variants were identified: two nonsense, two frameshift and three missense. Three of these variants have been previously described in association with brachyolmia (ul Haque 1998 et al., 1998; Tuyusz et al., 2013; Oostdijik et al., 2015). Six of the variants are reported in the gnomAD database (http://gnomad.broadinstitute.org) in the heterozygous, but not the homozygous state.

Evidence for pathogenicity of *PAPSS2* variants

Variant analysis conformed to ACMG guidelines (Richards et al., 2015). The premature termination codon variants and the frameshift changes were all classified as pathogenic. The c.809G>A p.(Gly270Asp) variant was also classified as pathogenic, it was not present in the homozygous state in the gnomAD DNA sequence variation database of clinically normal individuals (http://gnomad.broadinstitute.org/), it had been shown to functionally reduce the catalytic activity of the PAPS synthase 2 protein (Oostdijk et al.,

2015) and was demonstrated to be biallelic homozygous in many individuals with the disorder. The c.386G>C p.(Arg129Pro) variant only appears once as a heterozygote in over 250,000 alleles sequenced in the gnomAD DNA variant database, it was inherited in trans with the c.809G>A p.(Gly270Asp) variant and in silico analysis suggests that it is damaging. Similarly, neither the c.847T>G p.(Phe283Val), nor the c.1478C>T p.(Ala493Val) variant appear in the gnomAD database. These factors suggest that these 3 variants are likely to be relevant, although irrefutable evidence of pathogenicity has not been established.

Discussion

This study of patients with *PAPSS2*-related brachyolmia almost doubles the number reported, amounting to a total of 38. In this study, we found that short stature was present at birth, with either a short limb pattern or with normal skeletal proportions. Short and bowed long bones as a prenatal finding has only been described once previously (Handa et al., 2016). Interestingly, a significant proportion (8/18) of our patients had short femora at the second trimester anomaly scan, suggesting that brachyolmia should be considered in the differential diagnosis of dysplasias that present at this gestation. These 8 patients did not all have the same genotype, so the early presentation *in utero* cannot be predicted on the basis of current knowledge of specific *PAPSS2* variants.

Other aspects of the phenotype of patients in our study are in keeping with those previously reported (Lida et al., 2013; Handa et al., 2016, Miyake et al., 2012), especially the relative progressive shortening of the spine during childhood resulting in a final height below the normal range. For most patients, the complications of brachyolmia were manageable and did not preclude normal activities, but the most significant symptoms were of pain and stiffness in the spine. There was only one instance where surgical treatment for spinal stenosis was required. Similarly, in the limbs, there was no evidence of severe joint dysfunction and only mild deformity was encountered. As with the spinal complications, only one patient required surgical intervention.

Our patient series is consistent with previous studies in that patients with brachyolmia due to *PAPSS2* mutations do not have significant intellectual disability, nor is there a typical facial gestalt associated with this condition. Of those patients who had endocrine assessments, all had low serum DHEAS, but no consistent clinical abnormalities were encountered. Only one patient (P4) experienced delayed puberty and had a low serum testosterone. He had a paternally inherited microdeletion of chromosome 10q11.22q11.23 which was not obviously relevant. It is interesting that, given the role of *PAPSS2* in generating a ubiquitous sulphate donor, PAPS, to allow the conversion of DHEA to DHEAS, patients with *PAPSS2*-brachyolmia do not have major disorders of sexual differentiation and for the majority, there are only biochemical rather than clinical manifestations. This suggests that adequate sources of sulphate are generated from the activity of the isoform, *PAPSS1*, in the relevant tissues.

Review of the radiographs of affected patients at different ages demonstrates the evolution of the platyspondyly during childhood. The relatively non-specific radiological findings in infancy are likely to contribute to this diagnosis being under-recognised at this stage. Although the platyspondyly is significant, alignment of the spine was maintained in most patients, however, evaluation of the longer-term outcome is beyond the scope of this study. We observed some differences in the radiological findings in our patients compared to those in previous reports. In particular, none of our patients had broad metacarpals and we did not identify broad ilia, as previously reported (Lida et al., 2013).

Comparison with patients with *TRPV4*-related brachyolmia shows considerable overlap of radiological features. The findings which are most helpful in distinguishing this latter form are more severe kyphoscoliosis and the configuration of the cervical vertebrae which may be more flattened and irregular (Rock et al., 2008). Bone age or carpal development might be expected to be delayed in some cases of TRPV4-related brachyolmia (Nemec et al; 2012), in contrast to PAPSS2-brachyolmia, where we have shown normal range bone age in all cases and sometimes advanced carpal development. Furthermore, we are not aware of prenatal presentation of TRPV4-related brachyolmia.

The patients we describe are from diverse ethnic backgrounds and include the first from Northern Europe and Africa. Previously published patients with *PAPSS2*-brachyolmia have been from Turkey, Syria, Lebanon, Kurdistan, Japan and Korea (Handa et al., 2016; Miyake et al., 2012; Lida et al., 2013). Genetic investigations revealed that 12 of our patients were either homozygous or compound heterozygous for *PAPSS2* c.809G>A. Our patients with this variant are all of White Northern European origin (Table 5).This variant has previously been reported once before, in the heterozygous state together with a frameshift variant (Oostdij et al., 2015). Their patient was described as having spondylodysplasia and androgen excess and we consider that the phenotype is consistent with *PAPSS2*-brachyolmia. The c.809G>A variant is recorded in the gnomAD database, occurring in the heterozygous state in 91 out of 276920 individuals and notably 83 of these are from European populations. We have not established any clear genotype-phenotype relationships with respect to the severity of spinal or other skeletal involvement. The propensity for variants in *PAPSS2* to result in a skeletal disorder is not surprizing as in a murine model *PAPSS2* is reported to be expressed in proliferating and differentiating chondrocytes (Stelzer et al., 2007). It is not clear though why the spine is predominantly affected and possibly vertebral chondrocytes are more vulnerable to sulphate deficiency during growth.

In conclusion, this study confirms that the principal features of *PAPSS2*-brachyolmia are disproportionate short stature and platyspondyly, in keeping with previous reports. The clinical symptoms and complications arise mainly from spinal shortening and deformity and we suggest that affected children should be monitored in an expert spine clinic, particularly through the pubertal growth phase when longitudinal spinal growth is relatively rapid. Few of our patients have required surgical intervention, but the evolution of spinal deformity is unpredictable. Likewise, lower limb alignment surgery is unlikely to be required, but may be necessary for a few individuals. We highlight that in our experience, prenatal presentation with short long bones in the fetal skeleton in the second trimester occurs in approximately 50% of patients. In contrast to some other skeletal dysplasias which present at this early stage, the long-term outcome of this condition seems to be favourable and compatible with most normal activities of living, although we acknowledge that this study does not address the experience of patients into their adult life. Distinguishing between the *PAPSS2* and *TRPV4*-related forms of brachyolmia may be possible on the basis of the facial features seen in the latter group, but expert radiological analysis and genetic testing are more definitive. Features favouring *PAPSS2*-brachyolmia include parental consanguinity (or a recessive pedigree), prenatal detection of short limbs, advanced carpal development and features of androgen excess (these latter 2 features are rare). This is important so that patients may be given accurate genetic advice and counselling. Finally, we show that autosomal recessive brachyolmia occurs across continents and speculate that it may be an under-recognised cause of short stature in children.

Acknowledgements / Declarations

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Data availability

All genotypic data is freely available in the LOVD database under the accession numbers 0000454922, 0000454924, 0000454950, 0000454951, 0000454952, 0000454953, 0000454954, 0000454955, 0000455087, 0000455088 and in DECIPHER database, under patient numbers 286552, 304822, 304827.

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DECIPHER https://decipher.sanger.ac.uk/

gnomAD https://gnomad.broadinstitute.org/

LOVD http://www.lovd.nl/3.0/home

Growth Charts www.rcpch.ac.uk/growthcharts

Figure and Table legends

FIG. 1. Phenotype of brachyolmia patients: above, illustrating the changing pattern of skeletal proportions from short limb to short spine in patient 2, at ages 2 years 4months (a) and 19 years (b), patient 3 at ages 1 year 8 months (c) and 12 years (d) and patient 8 at ages 18 months (e), 7 years (f) and 8 years (g) and below, showing skeletal proportions of patients 1 (h, aged 17 years), 4 (i, aged 16 years), 5 (j, aged 8 years), 9 (k, aged 7 years) and 10 (l, aged 9 years).

FIG.2. Radiological findings in brachyolmia: above, showing the spine of Patient 3 aged 10 years (a-d) and Patients 9 aged 10 years (e), 1 aged 15 years (f), and 4 at 16 years (g). Lateral spine radiographs and MRI (c) show platyspondyly, narrow disc spaces, vertebral end plate irregularity and short pedicles. The AP spine radiographs show over-faced pedicles. Some patients have scoliosis (d, e, and f), kyphosis (g) and/or lumbar lordosis (c). Below, mild radiological anomalies in the limbs including genu valgum in Patients 8 and 9 at 12 and 10 years (h and i), showing medial hemi-epiphysiodesis using 8-plates (h), relatively short tibiae in Patients 8 and 10 at 12 and 9 years (h and j), striations of distal radius and ulnar and short 4th and 5th metacarpals in Patient 2 at 14 years (k) Metaphyseal striations and relatively flat capital femoral epiphyses in Patient 9 at 8 years (l). Metaphyseal striations and flattened epiphyses are also seen around the hips and knees of Patient 9 (i) and knee of Patient 10 (j).

Table 1. Recent height, weight and Body Mass Index (BMI) measurements with z scores of patient with *PAPSS2*-related brachyolmia, in ascending age order. Centile values for heightof patients and predicted mid-parental centile heights for parents are also shown (derived from UK Royal College of Paediatrics www.rcpch.ac.uk/growthcharts). Z scores calculated using British 1990 reference data. †Estimate of mother's height

Table 2. Endocrine investigations in patients with *PAPSS2*-related brachyolmia.

Table 3. Summary of clinical findings in patients with *PAPSS2*-related brachyolmia. (+) feature present, (-) feature absent, (NK) Not known.

Table 4. Summary of radiological findings in patients with *PAPSS2*-related brachyolmia. Including data for previous published patients where available. †Only included those which were of the long bones other than proximal femur. ‡ Other than short femoral neck and fingers. ¶Bone age was calculated by Greulich-Pyle method and was less than 2 years advanced.

Table 5. *PAPSS2* variants: homozygous c.809G>A was common in the European brachyolmia patients. Variant nomenclature uses the accession number NM_001015880.1.

Supplementary material

FIG.3. Image displays growth chart longitudinal height data for selected male patients. Growth chart image is copyright 2009 Royal College of Paediatrics and Child Health. Original growth chart has been cropped for illustration.

FIG.4. Image displays growth chart longitudinal height data for selected female patient. Growth chart image is copyright 2009 Royal College of Paediatrics and Child Health. Original growth chart has been cropped for illustration.

Table 6. Longitudinal growth data for individual patients.