Dysmorphism and immunodeficiency - one of the differential diagnoses is *PAX1* related Otofaciocervical Syndrome Type 2

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Abstract

Otofaciocervical syndrome (OTFCS) is a rare condition associated with short stature, abnormal facial features and conductive hearing loss. OTFCS type 2 (OTFCS) is an autosomal recessive form of this condition with associated T cell deficiency due to biallelic variants in *PAX1.* We report a consanguineous family with an affected female diagnosed with T cell immunodeficiency in the newborn period, who underwent haematopoietic stem cell transplant with cord blood at the age of 5 months. She had additional features of dysmorphism, ear abnormalities and spinal deformity. We present longitudinal follow-up of the proband who has responded well to transplant to add to the otherwise limited description of this rare condition. This case report expands on the limited literature available on this condition, with only five families reported to date and it further highlights the clinical utility of a gene-agnostic trio exome analysis in identifying a genetic diagnosis in patients who previously underwent genomic testing by gene panel analysis.

Keywords: Otofaciocervical syndrome, severe combined immunodeficiency, haematopoietic stem cell transplantation, PAX1.

Introduction

Otofaciocervical syndrome (OTFCS) was first described in 1967 in a family with an affected father and four children (Fara et al). The original features described included short stature, a long face with a broad forehead, narrow nose and mandible, sunken nasal roots, high arched palate, prominent ears with large conchae and preauricular fistulas. Some family members also had lateral cervical fistulas, long neck with downward sloping shoulders and clavicles as well as winged scapulae. They had bilateral conductive hearing loss and mild intellectual disability. Indeed the original description had overlapping clinical features with branchiootorenal syndrome and the autosomal dominant form similar to the original description (OTFCS1, OMIM 166780) has also been found to be due to *EYA1* mutations (Rickard et al). There has been a second autosomal recessive form of OTFCS with associated T cell deficiency (OTFCS2, OMIM 615560) associated with *PAX1* mutations (Pohl et al).

Descriptions of *PAX1* associated otofaciocervical syndrome (OTFCS2) remain limited with only thirteen cases documented to date from five consanguineous families and the majority with poor outcomes due to associated immunodeficiency (Pohl et al; Paganini et al; Patil et al; Yamazaki et al). There is therefore little description of the ongoing clinical course. Here we report the case of a female proband with longitudinal follow up diagnosed through Whole Exome Sequencing (WES) where outcomes have been more favourable.

Clinical Data

The proband was the second child of consanguineous Iraqi parents (see figure 1). Their first child was born two years earlier and presented in the neonatal period following an uneventful pregnancy with primary immunodeficiency, most consistent with haemophagocytic lymphohistiocytosis (HLH), although severe combined immunodeficiency (SCID) could not be excluded. The child was also noted to have an external right ear abnormality and facial dysmorphism. Genetic investigations were unable to confirm a molecular diagnosis at the time and the child died aged 2 months. There was no other family history of relevance and the couple have subsequently had a third child, who is healthy.

The proband was born at term following a normal pregnancy. Her birth weight was 2.9kg (11th centile) and she was well at birth. In view of family history, immunology testing was performed on day 2 of life and identified immunodeficiency. In the early neonatal period she suffered with recurrent infections requiring antibiotics. She underwent cord blood transplant at the age of 5 months. Her feeding was normal in the neonatal period but she subsequently required a gastrostomy post-transplant to support feeding due to poor weight gain. This was removed at the age of 6 years.

Her development was within normal limits, she had a social smile from 6 weeks, sat independently at 6 months and walked at 12 months. She required glasses but there were no other concerns with vision. She was diagnosed in the neonatal period with moderate conductive hearing loss. She was fitted with bone anchored hearing aids.

On examination at the age of 5 years (see figure 2), her height and weight were on the 2nd centile and her OFC was on the 0.4th centile (105cm, 16.2kg and 49cm, respectively). She was noted to have hypertelorism with depressed nasal bridge and a beak shaped nose. She had bilateral microtia with

no external ear canal on the left and narrow external ear canal on the right. She had thin upper lip. Her hands and feet were normal.

Subsequent review at 11 years of age when mother presented in pregnancy demonstrated no evidence of intellectual disability. She was noted to have narrow forehead with hypertelorism and small eyes. She had a narrow, high arched palate. She had a hypoplastic alae nasi. By this stage she required a back brace due to deterioration in her spine.

Results

Lymphocyte subsets on day 8 of life were consistent with T cell lymphopenia with complete absence of CD3+ T cells (0.1% 0.00,) but presence of B (55% 0.77,)and NK cells (31% 0.45). Lymphocyte subsets at age 11 demonstrated presence of near normal CD3+ T cells(36.8%, 0.95 with CD3/4 28.9% 0.74, CD3/8 7.8% 0.2) in addition to B (34.8% 0.89) and NK cells (27.1% 0.70). She has a normal proliferative response to PHA (unstimulated 284 cpm, 4 ug/ml PHa 33,802 cpm) and makes normal vaccine response to tetanus (>0.4 iu/ml) and has protective pneumococcal responses to 7/13 serotypes tested.

All genetic testing was performed on a DNA sample stored prior to allogenic haematopoietic stem cell transplantation (HSCT). Genome wide array-CGH analysis performed using Nimblegen 135K v3.1 at a resolution of 0.2Mb indicated no clinically significant copy number changes. Next generation sequencing (Agilent SureSelect + MiSeq) for a panel of 82 primary immunodeficiency genes performed when the proband was 6 years old identified two heterozygous variants of uncertain significance *PTRPC* c.128T>C p.(Val43Ala) and *DOCK8* c.663C>A p.(Asp221Glu).

Trio exome sequence analysis of the proband and both unaffected parents targeted the coding region and conserved splice sites of 23,244 genes by next generation sequencing (Twist Core Human Exome/Illumina NextSeq/NovaSeq). This gene-hyphothesis free approach was undertaken as previously described (Le Fevre et al) and it identified a homozygous frameshift variant in *PAX1:* NM_006192.5:c.501del p.(Ser168Alafs*4). Both parents were heterozygous carriers for this variant. The variant was classified as pathogenic based on the ACMG guidelines for variant classification using the following criteria: PVS1_Very Strong and PM2_Moderate.

Discussion

Severe combined immunodeficiencies (SCID) are rare disorders which are characterised by defective T-cell development, with B- and NK-cell deficiency sometimes associated. The majority of cases are due to variants in genes expressed in haematopoietic precursors. These can be successfully treated with allogenic HSCT. More rarely, SCID may be due to impaired thymic development. This has been described in several conditions classically DiGeorge syndrome due to *TBX1* haploinsufficiency, CHARGE syndrome due to heterozygous*CHD7* variants, Nude SCID due to biallelic *FOXN1* variants and subsequently biallelic *PAX1* variants (Giardino et al). There were no features suggestive of immunodeficiency in the original family diagnosed with PAX1 deficiency but all subsequent reports appear to have varying degrees of thymic dysfunction which is in keeping with the role of this gene. The PAX1 protein is a member of the paired box (PAX) family of transcription factors and plays a role in patterning of the pharyngeal endoderm during embryogenesis, this gives rise to the thymus, tonsils, parathyroid glands, thyroid and middle ear (Farley et al). Mouse models of *Pax1* deficiency

have vertebral column anomalies, thymic hypoplasia to variable degrees and abnormalities of thymocyte number and maturation (Adham et al; Wilm et al). Stem cells from previous patients with PAX1 deficiency have an altered transcriptional profile impacting thymus development and thymus shadows have been absent on X-ray suggesting thymus aplasia.

Variants reported to date have all been seen in consanguineous families in the homozygous state in affected individuals. It is also worth noting that all variants reported to date have been localised around the highly conserved paired box domain itself (Treisman et al). There does not appear to be a specific genotype associated with missense, nonsense, in frame deletions and insertions all reported, see table 1. The original missense variant described by Pohl et al (2013) demonstrated Pax1 transcription but this was reduced compared to wild type. This family had no reported features of immunodeficiency and it is possible that as a similar amino acid was substituted while there was a reduction in protein the residual functional protein may have allowed the development of some functional thymus tissue. Although extremely low TRECs have been detected in this patient (227/10^6 T cells) it cannot be determined if these are from her cord blood transplant or a result of some residual thymus function. Yamazaki et al (2020) demonstrated that p.(Val147Leu), p.(Asn155del) and p.(Cys368*) variants also all alter PAX1 transcriptional activity but as yet no clear genotype phenotype correlations have emerged.

The preceding four patients with PAX1 deficiency where HSCT had been attempted had failed to correct T cell deficiency despite engraftment in three of them. This is not the case in our proband who has responded well to cord HSCT. This may be because all the T cells coming across with the graft would have been naïve T cells and these are likely to have engrafted, expanded and persisted as there was an empty niche which may explain the proband's lasting T cells compared to previous cases. Early recognition of the underlying cause of immunological problems may assist in planning intervention as considering the underlying pathology thymus transplant may be a more appropriate management for future patients.

In addition to the immunological features, the other clinical features in the proband described here are comparable to those reported previously. There appears to be characteristic dysmorphic facies with affected individuals having features including hypertelorism, microretrognathia and malformed pinna with microtia and similarities of skeletal malformation which include kyphosis and winging of the scapulae.

The majority of cases of OTFCS2 have been in early childhood. Pohl et al reported a pedigree with four affected family members outside and in addition to physical features reported mild intellectual disability. This is not the case in our proband who has normal neurodevelopment with no evidence of intellectual disability suggesting that this aspect of the phenotype may be variable.

While panel testing was performed on this patient prior to whole exome sequencing, this testing was performed before *PAX1* had been associated with immunodeficiency and therefore this gene had not been included in the panel. Rapid trio whole exome sequencing during her mother's pregnancy resulted in a diagnosis with clinical utility for the family. Scans could be performed to assess for dysmorphism and testing performed on the baby from cord blood at delivery with a medical plan in place for management of the immunological phenotype based on the specific underlying cause if the baby was found to be affected.

In summary, this report further confirms that immunodeficiency is a feature of PAX1-related disorder. The power of a gene-agnostic analysis where the analysis includes all known disease-genes rather than a selected gene panel is also highlighted in this case report.

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Figure 1: Pedigree of the family. Filled circle indicates the affected proband with features of otofaciocervical syndrome. III:1 is displayed as a grey circle where the diagnosis is suspected.

Figure 2: (a-b) Images of the proband at age 5 years. (c-e) Images of proband at age 11 years. Images demonstrate hypertelorism, depressed nasal bridge, beak shaped nose, thin upper lip and abnormally shaped ears.

Table 1: Features of PAX1-related otofaciocervical syndrome in the proband compared to previously reported cases.