Paternal mosaicism for a novel PBX1 mutation associated with recurrent perinatal death: Phenotypic expansion of the PBX1-related syndrome

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Abstract
Autosomal dominant (de novo) mutations in PBX1 are known to cause congenital abnormalities of the kidney and urinary tract (CAKUT), with or without extra-renal abnormalities. Using trio exome sequencing, we identified a PBX1 p.(Arg107Trp) mutation in a deceased one-day-old neonate presenting with CAKUT, asplenia, and severe bilateral diaphragmatic thinning and eventration. Further investigation by droplet digital PCR revealed that the mutation had occurred post-zygotically in the father, with different variant allele frequencies of the mosaic PBX1 mutation in blood (10%) and sperm (20%). Interestingly, the father had subclinical hydronephrosis in childhood. With an expected recurrence risk of one in five, chorionic villus sampling and prenatal diagnosis for the PBX1 mutation identified recurrence in a subsequent pregnancy. The family opted to continue the pregnancy and the second affected sibling was stillborn at 35 weeks, presenting with similar severe bilateral diaphragmatic eventration, micro-splenia, and complete sex reversal (46, XY female). This study highlights the importance of follow-up studies for presumed de novo and low-level mosaic variants and broadens the phenotypic spectrum of developmental abnormalities caused by PBX1 mutations.

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1 | INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) refer to inborn morphogenic defects of the renal system and urinary tract and represent a broad spectrum of clinical manifestations. The large phenotypic heterogeneity of CAKUT ranges from subclinical abnormalities present in the general population, to severe malformations which are sometimes incompatible with life (Knoers & Renkema, 2019; Nicolaou, Renkema, Bongers, Giles, & Knoers, 2015).

De novo mutations in \textit{PBX1} were first identified as a cause of CAKUT in humans in 2017 (MIM: 617641) following earlier work demonstrating renal hypoplasia, renal ectopia, and renal agenesis in a \textit{Pbx1}−/− mouse model (Heidet et al., 2017; Le Tanno et al., 2017; Schnabel, Selleri, & Cleary, 2003; Slavotinek et al., 2017). The reported heterozygous disease causing mutations in \textit{PBX1} include partial and complete gene deletions, truncating, splice site and missense variants (Figure S1). These de novo variants have been described in individuals with renal hypoplasia/dysplasia and associated urinary tract abnormalities, with a broad range of extra-renal abnormalities. These include facial dysmorphism, and anatomic abnormalities of the ear, genitalia, heart, and lung. Developmental delay/intellectual disability is common, and growth restriction and hearing loss have also been reported (Table S1: Alankarage et al., 2019; Eozenou et al., 2019; Heidet et al., 2017; Kia, Sarafoglou, Mooganayakanakote Siddappa, & Roberts, 2019; Riedhammer et al., 2017; Slavotinek et al., 2017; Sun et al., 2019; Le Tanno et al., 2017). We present two cases of perinatal death, from independent pregnancies in one family, with a recurrent disease-causing mutation in \textit{PBX1} due to paternal mosaicism for the mutation.

2 | METHODS

2.1 | Patient consent and ethics approval

This study was performed in concordance with the declaration of Helsinki. The family were referred to the clinical genetics unit for consideration of an underlying genetic diagnosis following the first affected pregnancy. The family were counseled and provided consent for enrolment into the Genomic Autopsy Study (HREC/15/WCHN/35, family study ID: PED043), a National Health and Medical Research Council (NHMRC) funded study.

2.2 | Genomic analysis

Parents-proband trio whole exome sequencing (WES) was performed on DNA isolated from whole blood. Exonic sequences were enriched from genomic DNA using an illumina exome capture (38 Mb target) and sequenced at the Genomics Platform of the Broad Institute (Boston, MA). Sequencing reads were aligned to the human reference genome GRCh37/hg19 (BWA 0.7.12) and single nucleotide variants (SNVs) and small insertions/deletions were called using GATK HaplotypeCaller (package version 3.8.0). Copy number variants (CNVs) were detected using an in-house (unpublished) algorithm. Data from 97 unrelated samples sequenced in the same batch were used to normalize read depth signals before partitioning into bin sizes optimal for the exome capture. Variant filtering was performed to select for rare protein altering variants (SNVs: gnomAD and in-house frequencies <1% for autosomal recessive [AR] and <0.01% for autosomal dominant [AD], CNVs: no reciprocal overlap with known benign CNVs ≥70%; Lek et al., 2016).

2.3 | Droplet digital PCR

Given the father’s low-level mosaicism in blood, droplet digital PCR (ddPCR) was performed on a sperm sample to define the recurrence risk. The ddPCR assay for the \textit{PBX1} c.319C > T variant was ordered from Thermo Fisher Scientific (Waltham, MA). The ddPCR assay was performed in triplicate on DNA from four samples (fetal, maternal and paternal blood, and paternal sperm) using a BioRad QX200 instrument.

3 | RESULTS

3.1 | Family presentation

The proband (II.3) is a one-day old deceased female infant with syndromic CAKUT (Figure 1a). Her nonconsanguineous healthy parents have no family history of CAKUT. However, the father had an ultrasound in childhood which demonstrated subclinical dilation of one renal pelvis in an otherwise normal urinary tract. He has had no renal-related illnesses during his adult life.

The couple’s firstborn, a 5-year-old healthy female, was antenatally diagnosed with an ovarian cyst but did not show any renal anomalies on abdominal ultrasound. Their second pregnancy resulted in a miscarriage before 12 weeks’ gestation for which no genetic testing was performed.

The proband was born following their third pregnancy in which a mildly increased nuchal translucency (3.0 mm) and polyhydramnios were noted at 12 weeks’ gestation. Early morphology ultrasound (16 weeks) identified hypoplastic lungs, dilated bilateral renal pelves, a horseshoe kidney, small abdominal and chest circumferences, an apparent left-sided diaphragmatic hernia, and confirmed polyhydramnios. The couple was counseled regarding aneuploidy risk but opted to forego invasive testing and continued the pregnancy. The female infant was born via elective cesarean section at 38 weeks’ gestation (birthweight 2,920 g). After delivery, the infant required
ventilatory support for severe persistent pulmonary hypertension secondary to pulmonary hypoplasia but deteriorated over 24 hours and died.

Karyotype of the female proband was normal (46, XX) and microarray analysis (Illumina CytoSNP-850 k) did not identify any CNVs. At autopsy, external examination revealed mildly dysmorphic facial features including hypertelorism, prominent forehead, and downturn corners of the mouth. (Figure 1b). Extremely thin, transparent diaphragm (white arrow) was noted. Internal examination showed severe pulmonary hypoplasia associated with a markedly thinned (transparent) diaphragm leading to eventration without true diaphragmatic hernia (Figure 1d). Other findings included asplenia, a horseshoe kidney with bilateral pelvic dilatation, abnormal ureter insertion into the bladder, and a bicornuate uterus (†). Histology of diaphragm demonstrating complete absence of muscle cells (white arrow). (j) Normal histology of fallopian tube (white arrow) and ovary (white arrowhead).

FIGURE 1 (a) Pedigree, (b–d) Autopsy photographs of the proband (II-3). (b) Dysmorphic features include hypertelorism, prominent forehead, and downturn corners of the mouth. (c) Horseshoe kidney. (d) Extremely thin, transparent diaphragm (white arrow). (e–j) Autopsy photographs and histology images of the affected sibling (II-5). (e) Dysmorphic features include deep-set eyes and prominent forehead. Note normal female genitalia despite 46, XY karyotype. (f) Hypoplastic spleen (white arrow) and tiny accessory spleen (white arrowhead). (g) Paper-thin diaphragm with forceps visible through diaphragm (white arrow). (h) Macroscopically normal ovary (white arrow), fallopian tube (white arrowhead), and uterus (†). (i) Histology of diaphragm showing complete absence of muscle cells (white arrow). (j) Normal histology of fallopian tube (white arrow) and ovary (white arrowhead).
early morphology ultrasound showed a dilated renal pelvis and possible small diaphragmatic hernia. A follow-up (20-week) ultrasound revealed a ventricular septal defect, an overriding aorta, possible pulmonary atresia, bilateral renal pelvis dilatation, and ambiguous genitalia. The diaphragmatic hernia was not confirmed. Decreased fetal movements were noted at 35 weeks and the mother presented with a stillbirth shortly after. Autopsy demonstrated marked thinning/aplasia of the diaphragm leading to evagination, normal female appearing external genitalia with a normally formed vagina, uterus, fallopian tubes and ovaries, a small spleen and smaller accessory spleen, bilaterally small kidneys and bilateral ureteric dilatation, a bell-shaped chest, and pulmonary hypoplasia (Figure 1e–j). A precise cause of death was not identified at autopsy; however, multiple congenital anomalies and congenital heart disease are both separately associated with an increased risk of stillbirth (Liu et al., 2019; Mcclure et al., 2015). Autopsy identified some subtle placental abnormalities including subchorionitis, early choriodeciduitis, and a small localized infarct but these findings were not considered causative of stillbirth as they are relatively nonspecific common findings. Given the discordant chromosomal (46, XY) and phenotypic sex, the karyotype was checked on a separate sample, and was again confirmed as 46, XY.

3.2 | Genomic analysis

Filtering for rare, nonsynonymous variants in the proband (II.3) yielded high quality variants affecting one AD and three AR genes (Table S2). Further prioritization for genes associated with renal disorders highlighted one AD de novo variant; a missense variant in PBX1 (Chr1(hg19):g.164761784C > T; NM_002585.3:c.319C > T; NP_002576.1:p.(Arg107Trp)), that was absent from population databases and not called in either parent. However, manual inspection of sequencing reads showed the variant at low level (7%, 10/136 variant reads; Figure S2) in the paternal blood sample. The PBX1 p.Arg107Trp variant was predicted to be deleterious by multiple computational pathogenicity predictors, such as CADD, SIFT, PolyPhen, and Mutation Taster (Table S3). Follow-up by ddPCR revealed that the variant was present in 10.4% of paternal blood cells and 20.1% of sperm cells (Figure S3).

4 | DISCUSSION

PBX1 encodes the Pre-B Cell Leukemia Transcription Factor, which regulates the expression of genes involved in tissue development, including morphologic patterning and hematopoiesis (Ficara, Murphy, Lin, & Cleary, 2008; Heidet et al., 2017; Slavotinek et al., 2017; Le Tanno et al., 2017). Highlighting the importance of PBX1 in organogenesis, Pbx1−/− mice displaying asplenia, hyposplenia, and diaphragmatic muscularization and tissue patterning defects (Brendolan et al., 2005; Koss et al., 2012; Russell et al., 2012).

Here, we present paternatal mosaicism for a PBX1 mutation leading to perinatal death in two siblings, with the identified variant, p.Arg107Trp, underlying a severe presentation of the multisystem disorder caused by PBX1 mutations. The congenital abnormalities observed in our siblings expand the phenotypic spectrum of PBX1-associated disease with unique features including severe diaphragmatic evagination, and asplenia/microsplenia. While unilateral diaphragmatic evagination has been reported in two patients (Slavotinek et al., 2017), our patients’ diaphragms were entirely membranous and devoid of muscle tissue.

Genital abnormalities are commonly reported in male patients with PBX1 mutations (10/14), with the phenotypic spectrum ranging from cryptorchidism to partial development of female internal and/or external genitalia (Alankaraage et al., 2019; Ezenou et al., 2019; Kia et al., 2019; Riedhammer et al., 2017; Slavotinek et al., 2017; Le Tanno et al., 2017). Interestingly, the second affected sibling (II-5; 46, XY) presented here showed complete sex reversal of both internal and external organs, without any signs of male gonad development (no streak gonad). In line with earlier reports (Ezenou et al., 2019; Slavotinek et al., 2017), the phenotypic heterogeneity among patients carrying the same mutation indicates a limited genotype-phenotype correlation, possibly influenced by factors such as gender, genetic modifiers, or skewed gene expression.

In recent years, the contribution of parental mosaicism to de novo mutations has been reported for several (neuro-)developmental disorders (Acuna-Hidalgo et al., 2015; Wright et al., 2019). However, potential recurrence of presumed de novo mutations is still not routinely considered in research and diagnostics. Using ddPCR, the low-level paternal mosaic PBX1 mutation was detected in 20.1% of sperm cells, indicating an estimated recurrence risk of one in five. For this family, unfortunately, their subsequent affected pregnancy was conceived before these results were available and assisted reproductive options could be considered.

Altogether, we identified recurrence for a novel PBX1 mutation in two siblings, leading to severe congenital abnormalities and perinatal lethality. The severe diaphragmatic hypoplasia, asplenia/microsplenia and complete sex reversal observed in our patients further expand the clinical phenotypes associated with the PBX1-related complex multisystem abnormality syndrome. In addition, this report also represents the first mosaic PBX1 mutation in a parent leading to recurrence, highlighting the relevance of follow-up screening.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

P.A., J.G., A.B.B., C.B. and H.S.S. drafted the manuscript. P.A., M.B. and S.L.K.-S. coordinated the study. J.F., T.H., P.W. and A.W.S. processed WES data and performed preliminary analyses, and P.A. performed tertiary data analysis. P.A., J.G., A.B.B., T.S.E.H., T.H., S.L.K.-S.,
REFERENCES

Acuna-Hidalgo, R., Bo, T., Kwint, M. P., Van De Vorst, M., Pinelli, M., Veltsman, J. A., ... Slavotinek, A., Selleri, L., Alankarage, D., Szot, J. O., Pachter, N., Slavotinek, A., Selleri, L. (2005). A Pbx1-dependent genetic and transcriptional network regulates spleen ontogeny. Development, 132(13), 3113–3126. https://doi.org/10.1242/dev.01884

Eozenou, C., Bashamboo, A., Bignon-Topalovic, J., Merel, T., Zwermann, O., Lorenco, D., ... Brauner, R. (2019). The TALE homeodomain of PBX1 is involved in human primary testis-determination. Human Mutation, 40(8), 1071–1076. https://doi.org/10.1002/humu.23780

Ficara, F., Murphy, M. J., Lin, M., & Cleary, M. L. (2008). Pbx1 regulates self-renewal of long-term hematopoietic stem cells by maintaining their quiescence. Cell Stem Cell, 2(5), 484–496. https://doi.org/10.1016/j.stem.2008.03.004

Heidet, L., Morinière, V., Henry, C., De Tomasi, L., Reilly, M. L., Humbert, C., ... Jeannier, C. (2017). Targeted exome sequencing identifies PBX1 as involved in monogenic congenital anomalies of the kidney and urinary tract (CAKUT) in humans. Journal of Medical Genetics, 54(7), 502–510. https://doi.org/10.1136/jmedgenet-2016-104435

Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., ... MacArthur, D. G. (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616), 285–291. https://doi.org/10.1038/nature19057

Liu, C., Lodge, J., Flatley, C., Gooi, A., Ward, C., Eagleson, K., & Kumar, S. (2019). Obstetric and perinatal outcomes in pregnancies with isolated foetal congenital heart abnormalities. Journal of Maternal-Fetal and Neonatal Medicine, 32(18), 2985–2992. https://doi.org/10.1080/14767058.2018.1453799

Mcclure, E. M., Saleem, S., Goudar, S. S., Moore, J. L., Garces, A., Esamai, F., ... Wallace, D. D. (2015). Stillbirth rates in low-middle income countries study from the global network. Reproductive Health, 12(Suppl 2), S7. https://doi.org/10.1186/s12978-015-0843-2

Nicolao, N., Renkema, K. Y., Bongers, E. M. H. F., Giles, R. H., & Knoers, N. V. A. M. (2015). Genetic, environmental, and epigenetic factors involved in CAKUT. Nature Reviews Nephrology, 11(12), 720–731. https://doi.org/10.1038/nrneph.2015.140

Riedhammer, K. M., Siegel, C., Alhaddad, B., Montoya, C., Kovacs-Nagy, R., Wagner, M., ... Hoefele, J. (2017). Identification of a novel heterozygous de novo 7-bp frameshift deletion in PBX1 by whole-exome sequencing causing a multi-organ syndrome including bilateral dysplastic kidneys and hypoplastic clavicles. Frontiers in Pediatrics, 5 (November), 1–7. https://doi.org/10.3389/fped.2017.00251

Russell, M. K., Longoni, M., Wells, J., Maalouf, F. I., Tracy, A. A., Loscertales, M., ... Donahoe, P. K. (2012). Congenital diaphragmatic hernia candidate genes derived from embryonic transcriptomics. Proceedings of the National Academy of Sciences of the United States of America, 109(28), 10798–10803. https://doi.org/10.1073/pnas.1121621109

Schnabel, C. A., Selleri, L., & Cleary, M. L. (2003). Pbx1 is essential for adrenal development and urogenital differentiation. Genesis, 37(3), 123–130. https://doi.org/10.1002/gene.10235

Slavotinek, A., Risolino, M., Losa, M., Cho, M. T., Monaghan, K. G., Schneidman-Duhovny, D., ... Shieh, J. (2017). De novo, deleterious sequence variants that alter the transcriptional activity of the homeodomain of PBX1 are associated with intellectual disability and pleiotropic developmental defects. Human Molecular Genetics, 26(24), 4949–4960. https://doi.org/10.1093/hmg/ddx363

Sun, M., Lou, J., Li, Q., Chen, J., Li, Y., Li, D., ... Liu, Y. (2019). Prenatal findings and molecular cytogenetic analyses of a de novo interstitial deletion of 1q23.3 encompassing PBX1 gene. Taiwanese Journal of Obstetrics and Gynecology, 58(2), 292–295. https://doi.org/10.1016/j.tjog.2019.01.022

Wright, C. F., Prigmore, E., Rajan, D., Handsaker, J., McRae, J., Kaplanis, J., ... Hurles, M. E. (2019). Clinically-relevant postzygotic mosaicism in parents and children with developmental disorders in trio exome sequencing data. Nature Communications, 10(1), 1–11. https://doi.org/10.1038/s41467-019-11059-2

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