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RESEARCH ARTICLE

Prenatal profile of Pallister-Killian syndrome: Retrospective analysis of 114 pregnancies, literature review and approach to prenatal diagnosis

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Pallister-Killian syndrome (PKS) is a tissue limited mosaic disorder, characterized by variable degrees of neurodevelopmental delay and intellectual disability, typical craniofacial findings, skin pigmentation anomalies and multiple congenital malformations. The wide phenotypic spectrum of PKS in conjunction with the mosaic distribution of the i(12p) makes PKS an underdiagnosed disorder. Recognition of prenatal findings that should raise a suspicion of PKS is complicated by the fragmentation of data currently available in the literature and challenges in diagnosing a mosaic diagnosis on prenatal testing. Ultrasound anomalies, especially congenital diaphragmatic hernia, congenital heart defects, and rhizomelic limb shortening, have been related to PKS, but they are singularly not specific and are not present in all affected fetuses. We have combined prenatal data from 86 previously published reports and from our cohort of 114 PKS probands (retrospectively reviewed). Summarizing this data we have defined a prenatal growth profile and identified markers of perinatal outcome which collectively provide guidelines for early recognition of the distinctive prenatal profile and consideration of a diagnosis of PKS as well as for management and genetic counseling.

KEYWORDS
isochromosome 12p, macrosomia, mosaicism, Pallister-Killian Syndrome, polyhydramnios, prenatal diagnosis

1 | INTRODUCTION

Pallister-Killian syndrome (PKS, MIM 601803) is a multisystemic genetic disorder characterized by typical craniofacial findings, skin pigmentary anomalies, neurodevelopmental delay, intellectual disability, congenital diaphragmatic hernia, congenital heart defects (CHD), gastrointestinal malformations, genitourinary malformations, rhizomelic limb shortening, ophthalmologic involvement, hearing loss, and other systemic anomalies. A hallmark of this syndrome is the tissue limited mosaicism for isochromosome 12p. The percentage of mosaicism is

higher in skin fibroblasts, amniocytes or chorionic villi cells than in rapidly growing lymphocytes. Overtime the tetrasomic cells are rapidly replaced by the euploid cells in the blood generally requiring skin fibroblasts or buccal smear analysis to make a diagnosis in individuals beyond the neonatal period. Prenatal diagnosis of PKS is still a challenge due to difficulties in detecting the extra iso (12p) chromosome and the variable level of mosaicism, the rapid decrease of the supernumerary marker isochromosome during amniocyte sub-culturing, in conjunction with the variability in ultrasound manifestations.

Most often the consideration of a prenatal diagnosis of PKS usually arises in the presence of congenital diaphragmatic hernia (CDH) or incidentally as a result of chorionic villus sampling (CVS) or amniocentesis performed for example, advanced maternal age, nonspecific ultrasound abnormalities or more rarely in cases of prenatal screening test anomalies.

In a review of the literature, prenatal findings, including CDH, ventriculomegaly, congenital heart disease, polyhydramnios, and rhizomelic shortening have been described in over 80 probands with PKS. However, the majority have been reports of isolated cases (Gilgenkrantz et al. 1985; Hunter et al.1985; Steinbach and Rehder 1987; Shivashankar et al. 1988; Soukup and Neidich 1990; Bresson et al. 1991; McLeod et al. 1991; Sharland et al. 1991; Priest et al. 1992; Tejada et al. 1992; Blancato et al. 1992; McLean et al. 1992; Bernert et al. 1992; Valerio et al. 1992; Boyle et al. 1993; Horn et al. 1995; Los et al. 1995; Brøndum-Nielsen and Mikkelsen 1995;Takakuwa et al. 1997; Chiesa et al. 1998; Zollino et al. 1999; Langford et al. 2000; Paladini et al. 2000; Choo et al. 2002; Velagaleti et al. 2003; Chiurazzi et al. 2004; de Ravel et al. 2004; Polityko et al. 2005; O Bartsch et al. 2005; Delahaye et al. 2006; Gerdes et al. 2006; Abad et al. 2006; Ramírez et al. 2007; Liberati et al. 2008; Kim et al. 2008; Kolarski et al. 2009; Kunz et al. 2009; Mourali et al. 2010; Chaouachi et al. 2010; Park et al. 2009; Chen et al. 2010; Sananes et al. 2010; Murakami et al. 2011; Johnstone and Jones 2012; Aydin et al. 2013; Özlü et al. 2014; Srinivasan and Wright 2014; Santamaria et al. 2016; Xi et al. 2015) or a small cohort of cases (Warburton et al. 1987; Wilson et al. 1994; Mowery-Rushton 1997; Schubert et al. 1997; Mathieu et al. 1997; Doray et al. 2002; Min-Hyoung Kim et al. 2008; de Athayde Costa et al. 2015; Desseauve et al. 2016; Libotte et al. 2016; Kucińska-Chahwan et al. 2017) often from an obstetrical or fetal pathology perspective. This has led to data fragmentation and probably to an overestimation of the prevalence of CDH or other major anatomic defects.

In this report, we retrospectively review prenatal findings in 114 probands enrolled in our PKS studies here at The Children's Hospital of Philadelphia (CHOP) and compare them with the data from the literature in order to characterize the prenatal profile in PKS and to delineate guidelines for a prenatal diagnostic approach.

2 | MATERIAL AND METHODS

This study has been conducted under an Institutional Review Board-approved protocol of informed consent held at The Children's Hospital of Philadelphia.

Our PKS patient database was retrospectively reviewed for individuals with a confirmed diagnosis of PKS and on whom we had prenatal clinical information available for the study. Additionally, a dedicated PKS prenatal survey was released in close collaboration with the PKS family support groups, to directly collect prenatal data from caregivers and from obstetrician's medical records when available. For cases diagnosed outside of our institution, clinical details, photographs, and in some cases, blood or other tissue samples were obtained to confirm the diagnosis of PKS.

Findings identified through ultrasound imaging were reviewed. When possible prenatal markers including serum markers (free beta–human chorionic gonadotropin [bhCG], pregnancy-associated plasma protein A [PAPP-A], and alpha-fetoprotein [AFP]), nuchal translucency (NT), growth parameters for gestational age and prenatal facial profile were evaluated. Percentiles for fetal biometric measurement were assigned for gestational age (GA) based on World Health Organization (WHO) fetal growth charts (Kiserud et al. 2017). Percentiles for biometric parameters at birth were assigned based on neonatal anthropometric charts (Bertino et al. 2010) and new intrauterine growth curves based on United States populations (Olsen et al. 2010).

At the same time, an extensive literature review of previously reported prenatal findings was conducted. Reference cases were ascertained through searches of all language articles in PubMed and Medline using the following keywords: PKS prenatal findings, PKS prenatal facial profile, PKS prenatal diagnosis, small supernumerary markers chromosomes. All available relevant references from these articles were obtained. A total of 142 cases were found in the literature, mainly represented by fetuses or newborns reported from 1985 up to November 2017 (Liehr et al. 2017). The prenatal findings of our cohort of 114 probands (Supporting Information Table S1) were then systematically compared to 86/142 cases previously reported in the literature with more detailed prenatal clinical information available (Supporting Information Table S2).

Data analysis was performed using the Prism software package, version 6, GraphPad Software Inc., San Diego, California, USA.

3 | RESULTS

3.1 | Demographic information

The average maternal age was similar in our cohort and in literature (respectively 32.1 vs. 35.3 years old). Two twin pregnancies (only one fetus affected in each case) were reported in our cohort and one (with both fetuses affected) in the prenatal published studies. Four pregnancies from our cohort resulted from assisted reproductive technologies (two of those were twin pregnancies), while the remaining 110 pregnancies occurred spontaneously. Among the 86 previously published cases, two singleton pregnancies have been reported to have resulted from assisted conceptions. Only three of the 114 cases from our dataset resulted in neonatal demise (respectively due to extreme prematurity subsequently to polyhydramnios and congenital diaphragmatic hernia in the remaining two cases) and one case of elective termination was reported. Conversely, more than half of the cases described in the literature resulted in voluntary termination (46/86 ~ 53%), 2%

(2/86) of cases in intrauterine demise and 24% (21/86) in perinatal death: both intrauterine fetal deaths occurred in fetuses with major malformations (CDH and left ventricular hypoplasia); while perinatal death was associated with CDH in 12 patients, prematurity in two patients, major cardiac anomalies in three cases (one with Ebstein anomaly and two with aortic stenosis/coarctation), isolated pulmonary dysplasia in one case, esophageal atresia in one case and omphaloceles in the two remaining cases. A vaginal delivery was reported in 54 on 101 (53%) cases with available information in our PKS population, while a cesarean section was carried out in 47/101 patients (47%) of which 18 were in emergency. Among live born cases reported in the literature 15 (18%) of cases were born by vaginal delivery and six (6%) cases by cesarean section. Emergency c-sections were usually performed due to premature membrane rupture, polyhydramnios, macrosomia, or to maternal complications (hypertension, eclampsia, etc).

3.2 | General pregnancy information

Fetal movements were reported as decreased in 25/114 (22%) of our database patients, while only in 2/19 (11%) of patients from the literature for which this information was specifically recorded. Preterm labor was reported respectively in 17% of patients from the literature and in 25% cases from our cohort. Maternal complications incidence range from 7% in the literature data to 25% in our population, mainly including gestational hypertension, gestational diabetes, nonspecific vaginal bleeding during the first trimester and eclampsia/HEELP (Hemolysis (H), Elevated Liver enzymes (EL) and Low Platelet count (LP)) syndrome.

3.3 | Noninvasive prenatal screening

3.3.1 | Ultrasound screening

Although specific prenatal growth measurements were available for only a small number of our patients, general prenatal ultrasound data was available for 104/114 (91%) of our subjects. The most common ultrasound finding was polyhydramnios that affected 58/104 (56%) screened pregnancies in our dataset and 32/86 (37%) pregnancies from the literature generally presenting after the 17, 18th week of gestation. The percentage with fetal macrosomia (20/104; 20%) was higher in our population as compared to previously published reports (7%). An intrauterine growth restriction profile (IUGR) was reported in five of our 104 (5%) patients, and most often occurred in the presence of gestational hypertension, multiple gestation and maternal exposure to tobacco. A prenatally identified shortening of the long bones was described in 20% of our cases and in 34% of cases from the literature (a femur length (FL) less-than fifth percentile was detectable as early as the 17th week of gestation).

Table 1 and Figure 1 document prenatal growth parameters and centiles of three PKS patients with at least two detailed sonographic measurements available. Bi-parietal diameter (BP) and head circumference (HC) measurements tended to be above the mean in all three patients, while fetal femur growth showed an absolute or relative limb shortening.

To further characterize the fetal growth profile, a total of 15 cases, (five from our study population and 10 from the literature (Gilgenkrantz et al. 1985, Tejada et al. 1992, Chiesa et al. 1998, Ramírez et al. 2007, Liberati et al. 2008, Kolarski et al. 2009, Mourali et al. 2010, Chen et al. 2010, Aydin et al. 2013, Srinivasan and Wright 2014) with at least one available measurement for each fetal parameter) were included to create an estimate of PKS prenatal growth curves representing a total of 14 BP measurements, 15 HC measurements, 17 abdominal circumference (AC) measurements and 19 FL measurements at different gestational ages (Figure 2). The majority of reported HC measurements (9/15) plotted above the 90th percentile, while almost all available FL observations (17/19) were under the 10th percentile (up to −6.6SD).

In terms of ultrasound abnormalities (Table 2, Figure 3), a prenatal cerebral anomaly was found in 13/104 (13%) of our cases and in 17/86 (20%) of the published cases, most often characterized by ventricular enlargement/ventriculomegaly (respectively 100% vs. 80%) or less frequently by Dandy Walker Malformation and/or cerebellar anomalies.

Ultrasonographic cardiac differences were reported less frequently in our population [10/104 (10%)] than in case from the literature [17/86 (16%)]. Gastrointestinal findings were reported more often in previously published cases (10%) including three with esophageal atresia, six reports of small stomach (associated with esophageal atresia in two cases), one malrotation and one nonspecific finding of bowl hyperechogenity. Only three minor nonspecific gastrointestinal differences were reported in our population, characterized by bowel hyperechogenity and small stomach. In both of our patients, the prenatal finding of bowel hyperechogenicity was postnatally related to anteriorly placed anus and in one case also to malrotation.

TABLE 1 Prenatal ultrasound growth measurements and centiles of three Pallister-Killian syndrome (PKS) cases with at least two detailed sonographic measurements available

	GA (weeks)	BP (mm)	HC (mm)	AC (mm)	FL (mm)
Fetus 1	21	51 (63th)	194 (68th)	149(15th)	32 (25th)
	30	77 (63th)	284 (58th)	270(15th)	40 (<1st)
Fetus 2	19	47 (91th)	176 (81th)	141(56th)	26 (21th)
	21	–	193 (65th)	161 (58th)	32 (25th)
	30	83 (99th)	289 (70th)	255 (56th)	51 (2nd)
Fetus 3	20	50.4(93th)	190.5 (85th)	151 (50th)	29.8 (14th)
	33	90.6 (99th)	341 (99th)	315 (91th)	66.6 (55th)

GA = gestational age; BP = biparietal diameter; HC = head circumference; AC = abdominal circumference; FL = femur length; Centile of measurement is in parentheses, – indicates absence of evaluation.

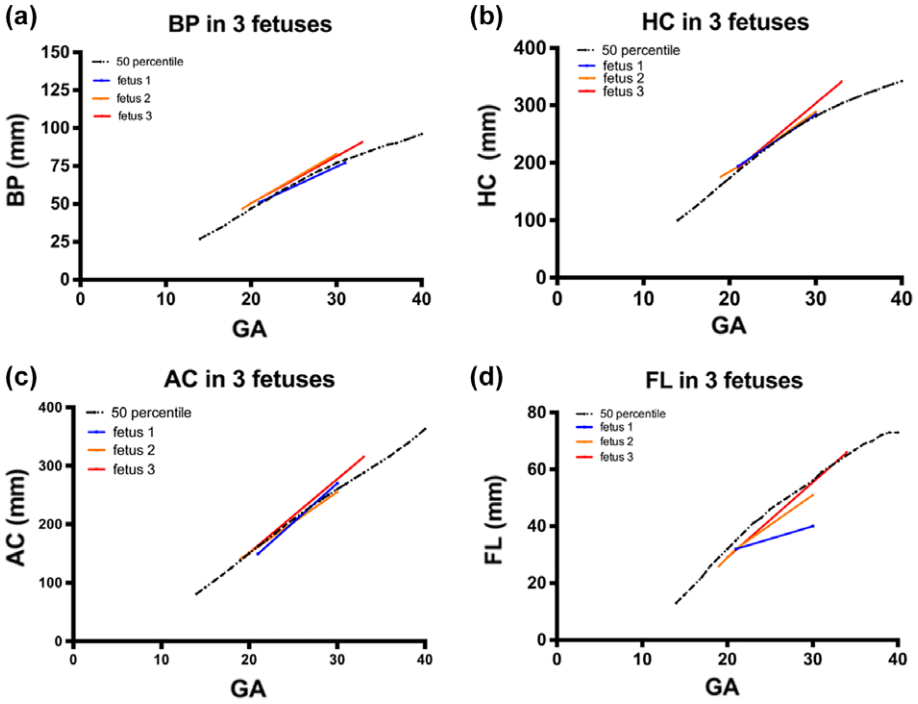

FIGURE 1 Plot of fetal growth measurements of three Pallister-Killian syndrome (PKS) cases with at least two detailed sonographic measurements available. The normal growth profile was drafted according to WHO Fetal Growth Chart (in black). GA = gestational age; BP = biparietal diameter; HC = head circumference; AC = abdominal circumference; FL = femur length [Color figure can be viewed at wileyonlinelibrary.com]

The percentage of genitourinary anomalies in our cases (13%), including hydronephrosis and calico-pelvic dilatation and/or kidney cysts, was similar to that in the literature data (10%). Among those, the most commonly seen finding of calico-pelvic dilation spontaneously resolved postnatally in three of four cases from literature and in eight of nine cases from our cohort.

Polydactyly (usually post-axial polydactyly) and foot anomalies were more often reported in the literature than in our population (19% vs. 5%).

The frequency of a prenatal CDH (27%) was significantly higher in the literature than in our population (5%) (likely representing an ascertainment bias as outlined below).

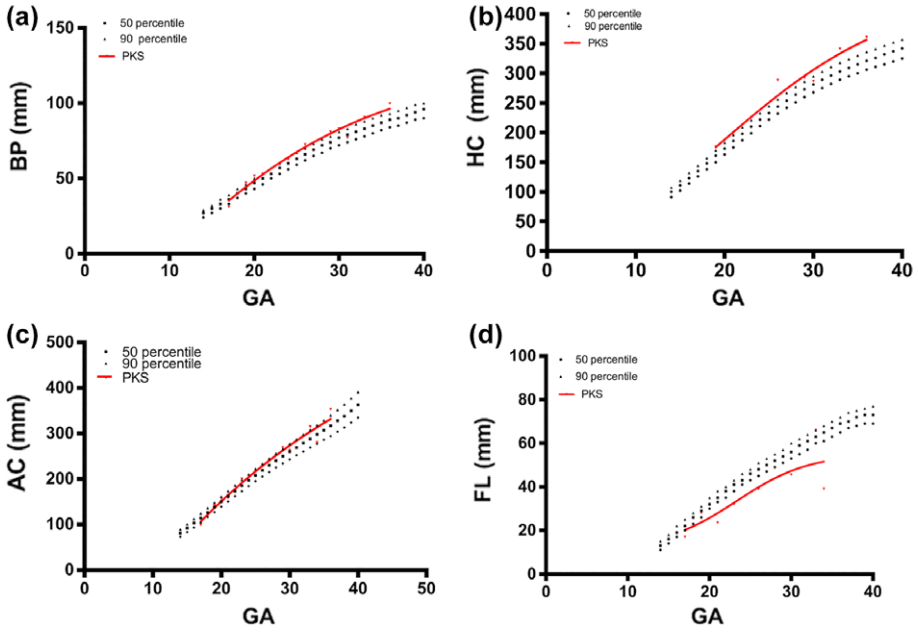

FIGURE 2 Prenatal Pallister-Killian syndrome (PKS) Growth Profile. Plot of fetal growth measurements from 15 patients (5 from our study and 10 from the literature). Percentiles for fetal biometric measurement were assigned for GA based on WHO Fetal Growth Chart (in black). PKS Growth curves (in red) were interpolated by using Prism software package, version 6. GA = gestational age; BP = biparietal diameter; HC = head circumference; AC = abdominal circumference; FL = femur length [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Pallister-Killian syndrome (PKS) prenatal findings

Ultrasound prenatal findings	This study	Literature
Cardiac involvement	10% (10/104)	16% (14/86)
ASD	50% (5/10)	7% (1/14)
VSD	10% (1/10)	14% (2/14)
Aortic valve anomalies (bicuspid aortic valve, aortic insufficiency, aortic stenosis)	30% (3/10)	14% (2/14)
Pulmonary valve anomalies	0	14% (2/14)
Ebstein anomaly	10% (1/10)	7% (1/14)
Double outlet right ventricle	0	7% (1/14)
Tetralogy of fallot	0	7% (1/14)
Left ventricular hypoplasia	0	29% (4/14)
CDH	5% (5/104)	27% (23/86)
Brain findings	13% (14/104)	20% (17/86)
Dandy Walker malformation	7% (1/14)	6% (1/17)
Cerebellar anomalies	7% (1/14)	12% (2/17)
Ventriculomegaly	86% (12/14)	71% (12/17)
Other (periventricular cyst, thinning of corpus callosum, mega cisterna-magna)	0	12% (2/17)
Gastrointestinal findings	3% (3/104)	10% (9/86)
Esophageal atresia	0	33% (3/9)
Malrotation	0	11% (1/9)
Bowel hyperechogenicity	67% (2/3)	11% (1/9)
Small stomach	33% (1/3)	67% (6/9) (1 associated with esophageal atresia, 1 with malrotation)
Genitourinary findings	13% (13/104)	9,3% (8/86)
Calico-pelvic dilatation	69% (9/13)	50% (4/8)
Kidney difference	38,5% (4/13)	13% (1/8)
Mullerian duct anomalies	0	38% (3/8)
Skeletal findings	48% (5/104)	19% (16/86)
Polydactyly	80% (4/5)	38% (6/16)
Inferior limb hypoplasia	20% (1/5)	50% (8/16)
Club feet	0	13% (2/16)(associated with inferior limb hypoplasia)
Club hands	0	1/16
Long bone shortening	20% (21/104)	34% (29/86)
Large for gestational age	19% (20/104)	7% (6/86)
IUGR	8% (8/104)	5% (4/86)
Polyhydramnios	56% (58/104)	37% (32/86)
Cystic hygroma	2% (2/104)	7% (6/86)
Nuchal fold	9% (9/104)	9% (8/86)
Pre-nasal edema	2% (2/104)	5% (4/86)

ASD = atrial septal defect; VSD = ventricular septal defect; IUGR = intrauterine growth restriction; CDH = congenital diaphragmatic hernia.

A prenatally identified typical facial profile was also described in 17/86 (20%) cases reviewed from the literature (including hypertelorism and flattered profile in nine cases, of which seven presented also a long philtrum and a small nose in six more patients) and in 4/104 (4%) of our patients more recently diagnosed (Figure 4). Additionally, a palate defect was prenatally identified in 22/104 (22%) of our cases.

3.3.2 | First trimester screening test

First trimester prenatal screening information was available on 19 cases in our cohort.

Similar to the literature data, no variation of the first trimester serum markers including bhCG and PAPP-A was observed.

NT was reported increased (> 3 mm) in 16% (3/19) of the screened fetuses in our cohort, while it was observed in 88% (14/16) of the literature cases who had first trimester clinical information available, with median NT of 4.2 mm. Cystic hygroma was more rarely detected in our study cohort (2/104–2%) than in PKS cases from the literature (6/86–7%).

Fetal nasal bones hypoplasia was only reported in two patients from our cohort (Table 3).

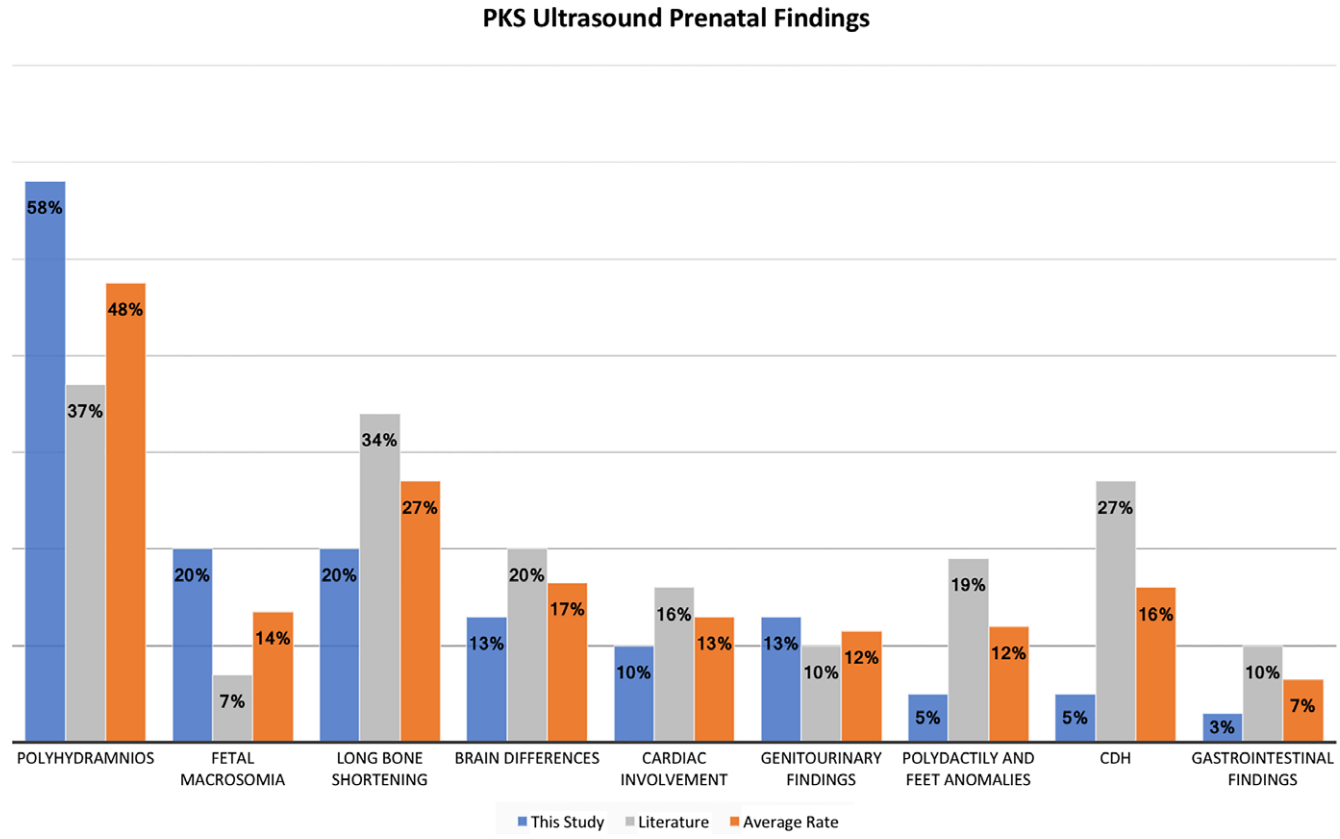

FIGURE 3 Chart of Prenatal Ultrasound data in Pallister-Killian syndrome (PKS) [Color figure can be viewed at wileyonlinelibrary.com]

3.3.3 | Second trimester screening test

Variation in level of the AFP was reported in 2/29 of our cases who underwent a second trimester screen and in five cases from the literature (increased in three and reduced in two PKS fetuses). An increased nuchal fold (>6 mm) reported after the 20th week gestation was identified in around 9% (8/86 vs. 9/104) in both cohorts. Prenasal edema was described in 5% (4/86) of previously published cases (Aydin et al. 2013; Desseauve et al. 2016; Kucińska-Chahwan et al. 2017) but only in 2% of our patients (Table 2).

3.4 | Invasive diagnostic tests

Among our cases amniocentesis were performed on 18/104 cases and CVS in 5/104 cases, and a prenatal diagnosis was established in 14/18 (78%) cases and in 2/5 (40%) cases respectively. Among the prenatal cases from the literature the detection rate of the amniocentesis was around 75% as well as in our cohort (the cytogenetic diagnosis was missed only in 3/63 investigated fetuses); while CVS was successfully carried out in 9/12 studied cases (one of the missed diagnoses were identified on a subsequent amniocentesis) (Table 4). All

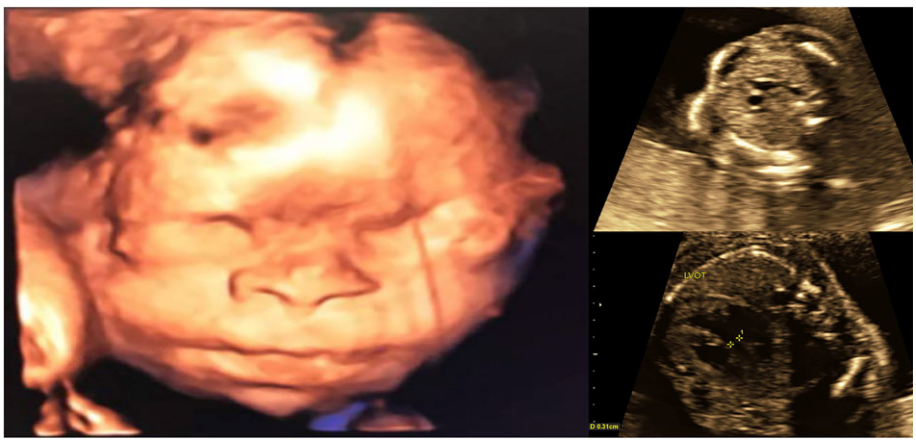

FIGURE 4 Ultrasound findings in a fetus with Pallister-Killian syndrome (PKS). Ultrasound findings at 29 weeks GA- From left to right: 3D facial appearance (high forehead, hypertelorism, flat facial profile with broad nasal bridge, small nose, long philtrum, thin upper lip, and everted lower lip), narrow aorta (at bottom: aorta diameter at level of valve less-than fifth centile and Peak Systolic Velocity in LVOT—95 cm/sec) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Screening markers in Pallister-Killian syndrome (PKS) pregnancies

Screening Markers First trimester screening test	This study N. screened patients: 19	Literature N. screened patients: 16
hCG above/ below median	0	0
PAPP-A above/ below median	0	0
NT	3/19	14/16
Fetal nasal bones	2/19	0
Tri/Quad screening test	**N. screened patients: 29**	**N. screened patients: 5**
Increased a-FP	2/29	3/5
Under median a-FP	0	2/5

hCG = human chorionic gonadotropin; PAPP-A = pregnancy associated plasma-protein A; a-FP = alpha-feto protein; NT = nuchal translucency.

other cases were cytogenetically diagnosed after birth by karyotype, Fluorescent in situ hybridization (FISH) or array-comparative genomic hybridization (a-CGH) analysis on peripheral blood cells and/or skin biopsy and/or buccal smear.

4 | DISCUSSION

Prenatal identification of PKS still remains a challenge due to the fragmentation of the data currently available in literature. This report provides the largest qualitative and quantitative summary of PKS prenatal findings including retrospective data analysis on 114 cases form our cohort here at the CHOP and the most extensive literature review of 86 cases published from 1985 through 2017.

Based on our analysis, PKS fetuses show a very typical growth pattern usually characterized by increased BP and HC often above the 90th percentile, associated with a significant femoral growth delay, which is usually markedly under the 10th percentile for gestational age. Interestingly fetal macrosomia is also described in 20% of cases, of which 70% also had polyhydramnios.

Overall, looking at the combined data from our study and literature (Figure 3), polyhydramnios is the most common prenatal finding (~ 50% of cases on average) followed by long bone shortening (~30% of cases on average) both detectable as early as the 17th week of gestation.

TABLE 4 Estimated detection rate of invasive diagnostic procedures for PKS

Invasive diagnostic testing	This study	Literature
Chorionic villus sampling	40% (2/5)	75% (9/12)
Amniocentesis	78% (14/18)	95% (60/63)

A percentile birth weight above the mean (>60th percentile based on gender and gestational age) was recorded in 73 (70%) of cases from our cohort of which 45 (43%) were large for gestational age (LGA > 90th percentile). Also, the presence of polyhydramnios correlates with an increased risk for LGA at birth (p value <.001: OR 3,181; 95 CI 1.4–7.1, Fisher's exact test) (Figure 5). In addition, polyhydramnios in PKS pregnancies represents a major risk factor for preterm labor (p value <.0001: OR 4.96; 95 CI 2.26–10.8, Fischer exact test).

The rate of major anatomic defects reported in the antenatal period, such as CDH, complex cardiac malformations and gastrointestinal malformations, appears lower among our retrospectively reviewed cases as compared to those from the literature (Figure 3). This bias likely reflects the variable clinical expression of PKS, but also demonstrates an ascertainment bias as our cohort have been primarily identified among children with PKS that survived the neonatal period (often PKS infants with major structural anomalies will not survive long postnatally). On the contrary, most of the previously published PKS prenatal information cases derive from spontaneous or voluntary terminated pregnancies following the prenatal discovery of life-threatening malformations. This likely is the cause of their comparative overrepresentation in the cases from the literature as compared to our cases. Therefore, the prevalence of CDH and other major PKS structural malformations associated with morbidity and mortality are likely to be represented as an intermediate frequency between the two cohorts compared in this study—not as common as depicted in the cases from the literature, but potentially more common than seen in our cohort.

Ventriculomegaly, cardiac septal defects, and calico-pelvic dilatation are frequently recognized PKS prenatal features in the second trimester (respectively 17%, 14%, and 11% of cases on average of the two analyzed cohorts), while hand and foot abnormalities are less likely to be identified prenatally. Although the presence of a caudal tag/appendage has been proposed as distinctive prenatal feature of PKS due to its rarity (Kucińska-Chahwan et al. 2017; McLeod et al. 1991), it was not detected during the fetal period in either of our

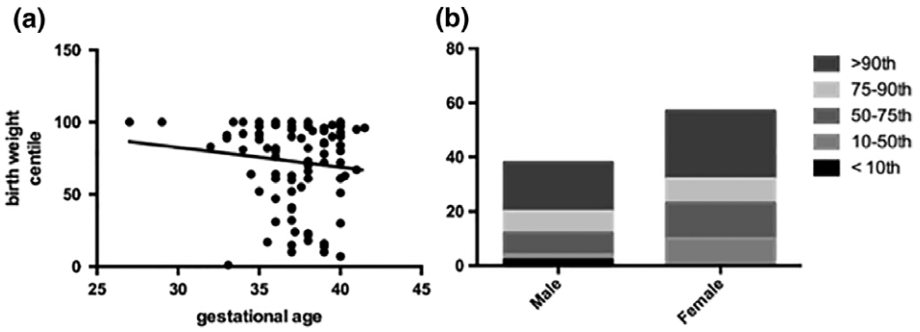

FIGURE 5 Percentile distributions of birth weights in Pallister-Killian syndrome (PKS) cases from our cohort. (a) Plot of birth weight percentiles according to gestational age from 96 patients. (b) Birth weight percentiles (plotted for gestational age) for male and female probands with PKS

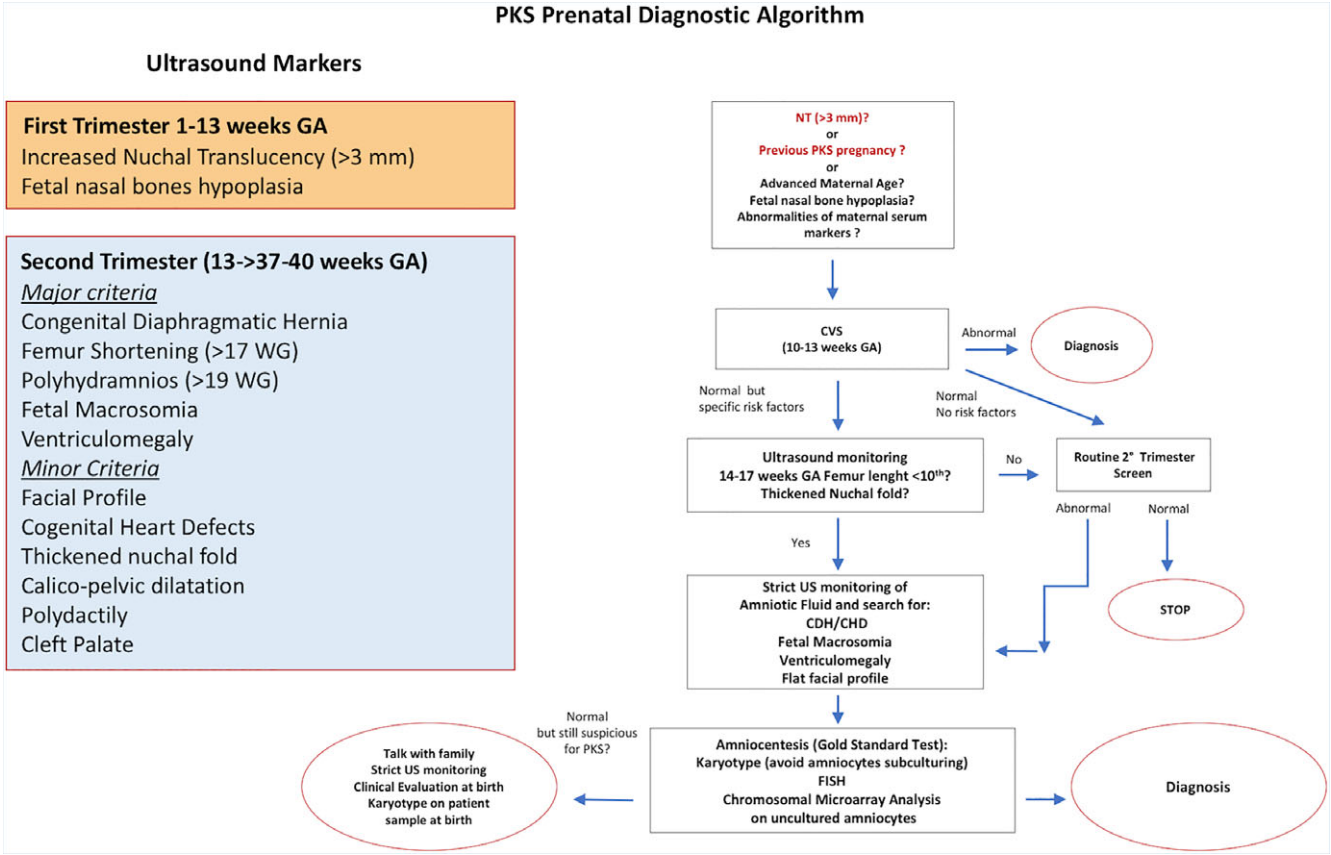

FIGURE 6 Flow-chart of recommended approach to prenatal diagnosis of Pallister-Killian syndrome. GA = gestational age; US = ultrasound; CVS = chorionic villus sampling; NT = nuchal translucency; CDH = congenital diaphragmatic hernia; CHD = congenital heart defect [Color figure can be viewed at wileyonlinelibrary.com]

cases with sacral appendage (2/114), suggesting that it is likely a fairly difficult feature to detect by ultrasonography.

The PKS facial profile is still poorly recognized prenatally and did not contribute to prenatal diagnosis for most of the CHOP collected cohort and cases reviewed from the literature. However, typical PKS features have been reported in the most recently reviewed cases, including hypertelorism/telecanthus, flattened facial profile, small nose, and long philtrum, sporadically associated with a prenasal edema. Although facial signs may be subtle, 3D ultrasound examination provides a useful tool for experienced clinicians and can aid in the diagnosis (Desseauve et al. 2016; Libotte et al. 2016; Sananes et al. 2010).

There have been a number of studies evaluating the predictive value of maternal serum markers on aneuploidies and adverse pregnancy outcome.

Particularly both increased and decreased concentrations of AFP have been considered markers for higher risk of neural tube defects and fetal aneuploidies. Also, specific changes in values of plasmatic PAPP-A and free bhCG have been associated with risk of low or high birth weight since the first trimester of pregnancy (Cignini et al. 2016; Morris et al. 2017). While the data from prenatal biomarker screening tests in PKS are poorly documented, none of the serum markers usually tested for appears to suggest any specificity for a PKS prenatal diagnosis or PKS fetal growth profile. Rarely changes in AFP levels have been reported during the second trimester.

Among the first trimester screening parameters, NT might likely have a potential predictive value on perinatal outcome of PKS fetuses. NT represents the normal fluid filled subcutaneous space between the back of the fetal skin and the overlying skin. Enlarged NT leads to lymphatic obstruction which in its most severe form results in cystic hygroma (Guraya 2013). Overall, adverse outcomes have been more commonly related in cases with an NT that exceed 3.5 mm that represents 99th percentile or greater throughout the gestational age window for first trimester screening (Roozbeh et al. 2017). Even though NT was increased in only 17% of tested PKS cases from our cohort, it was always associated with other major PKS findings such as CDH, heart defect, ventriculomegaly, long bone shortening, and polyhydramnios. Combining data from our cohort and the literature cohort of a total of 35 screened pregnancies, 17 fetuses (49%) showed a NT measurement of ≥3 mm, and in 12 of these 17 (71%) cases a life-threatening malformation was present, including CHDs and CDHs. In addition, all PKS fetuses with a prenatal finding of cystic hygroma were also diagnosed with CDH or major heart defects (including left ventricular hypoplasia, right ventricular hypoplasia associated with pulmonary stenosis atresia, and coarctation of the aorta with bicuspid aortic valve, and hypoplastic aorta) that were postnatally identified in half cases.

Analyzing all the data, an increased NT significantly correlates with an increased risk of CDH and CHD ($p < .005$, OR 10.40: 95 CI 2.0–53, Fisher's exact test) and, the presence of CDH, in turn, significantly increases the risk of perinatal death ($p < .0001$; OR 11.5: 95 CI

4.4–29, Fisher's exact test). Therefore, the prenatal identification of a CDH should always raise the suspicion for PKS and conversely, CDH should be carefully searched for in the presence of other suggestive PKS findings, as it is a severe life-threatening potentially treatable malformation, that may benefit from prenatal ascertainment and appropriate perinatal management.

In terms of invasive diagnostic tests for PKS, the analysis of prenatally diagnosed cases among both our cohort and previously reported cases, demonstrated that the presence of the isochromosome 12p was not identified on CVS in almost half investigated cases, while the detection rate of amniocentesis was closer to 90%.

5 | APPROACH TO GENETIC COUNSELING AND CONCLUSIONS

The establishment of a prenatal diagnostic algorithm for PKS is essential for appropriate and timely genetic counseling and management. As all reported cases of PKS have been sporadic, empirically the recurrence risk is close to that of the general population and based on our data, the risk increases with maternal age as already suggested (Doray et al. 2002). Theoretically, a recurrence risk could be possible if a parent presented with i(12)p germline mosaicism or a balanced chromosomal rearrangement on chromosome 12p (Izumi et al., 2010). For a complete evaluation of recurrence risk, cytogenetic discrimination between mosaic i(12)p and 12p duplication (often associated with a PKS phenotype), is also important (Izumi and Krantz 2014) as the 12p duplication may arise from a paternal balanced translocation. Overall, a high-resolution karyotype of both parents should always be performed to look for other small chromosomal rearrangements such as cryptic pericentric inversions which might play a causal role in duplications or isochromosome formation (Doray et al. 2002; Warburton et al. 1987).

Although no specific markers of PKS are identifiable during the first trimester of pregnancy, mosaic tetrasomy 12p may be potentially detected by the end of the first trimester by CVS avoiding long term culturing because of the progressive decrease of the isochromosome with culture aging (Doray et al. 2002; Kunz et al. 2009). Due to the low detection rate of the isochromosome by CVS (as documented in this study), if ultrasound findings remain suggestive for PKS, it is suggested to perform an amniocentesis by both direct and culture analysis on as many colonies as possible from different culture flasks (Doray et al. 2002; Kunz et al. 2009). FISH analysis using alpha-satellite or painting probes on metaphases or chromosome 12 fluorescent probes on interphase nuclei of amniotic cells usually increases the i(12p) detection rate (Doray et al. 2002). Array CGH methods on genomic DNA extracted from uncultured amniocytes may be efficient in detecting low levels of mosaicism and complex rearrangements (Kunz et al. 2009; Libotte et al. 2016).

In addition, digital droplet polymerase chain reaction (ddPCR) technology might represent a new potential adjunct in the early prenatal diagnosis of PKS. It has recently been proposed as a new, cheaper and faster postnatal diagnostic tool for PKS, able to quantify low percentages of mosaicism (around 5–10%) which is comparable to that of single nucleotide polymorphism (SNP) array (Conlin et al. 2012; Fujiki et al. 2016). As a prenatal diagnostic tool, microfluid digital PCR has been reported for rapid diagnosis (less-than 6 hr) of the most common fetal aneuploidies including trisomy 21, 13, and 18 on uncultured amniocytes and an octoplex droplet digital PCR has been demonstrated to meet the requirements for noninvasive prenatal testing of trisomy 21 on maternal plasma samples even when trisomic DNA content was as low as 5% (El Khattabi et al. 2016; Fan et al. 2009). Therefore, due to its relevant potential implications, further studies will be needed to assess the applicability of ddPCR and other technologies in PKS prenatal diagnosis and/or noninvasive first trimester prenatal testing.

The challenges in cytogenetic identification of the mosaic i(12p) makes an accurate ultrasound examination pivotal to guide the diagnostic recognition by the end of the first trimester or early second trimester. NT is a routine measurement during the first trimester screen test and although more data could be useful to define the relationship with PKS, an increased NT should be considered as a suggestive sign for this condition (after exclusion of more common associated syndromes) that requires more frequent ultrasound evaluations and more vigorous attention be paid in the identification of other anomalies, especially CDH, and CHD. Early ultrasounds can also identify the presence of a cystic hygroma as well as the presence of limb anomalies or long bone shortening that can also be suggestive of a diagnosis of PKS on the differential. A strict monitoring of amniotic fluid index and fetal growth profile should be performed in the presence of other suggestive findings such as rhizomelic shortening or increased NT.

Even if each of these previously described ultrasound findings is singularly nonspecific, when femur shortening, polyhydramnios and macrosomia are observed together, they are highly indicative of PKS even in the absence of other major congenital malformations. The analysis of the growth profile may also help to differentiate PKS from Fryns syndrome, which shares some common features such as CDH, as IUGR and microcephaly are more often seen in Fryns syndrome and uncommon in PKS (Ayme et al. 1989; Doray et al. 2002; Fryns et al. 1979). 3D ultrasound allows a more specific look at facial dysmorphic features that can support a suggested diagnosis of PKS (Fig. 6).

With the realization of a wide phenotypic spectrum of PKS, predictive features of prognosis in prenatally diagnosed patients are complicated and would benefit from more data. As the percentage of mosaicism does not correlate with the severity of congenital anomalies in PKS fetuses (Libotte et al. 2016) other markers of severity and poor outcomes would be helpful. Recently, abnormal extracellular microRNAs (miRNAs) in maternal plasma have been showed early in Down syndrome pregnancies and in those pregnancies complicated by gestational diabetes, preeclampsia, fetal growth restriction (Chiofalo et al. 2017; Kamhieh-Milz et al. 2014; Lagana' et al. 2018; Sebastiani et al. 2017). As five miRNAs located on 12p have already been reported to be overexpressed in skin fibroblasts from PKS patients, further studies on maternal plasma miRNAs profile in PKS pregnancies might open a window to identify new potential diagnostic and prognostic biomarkers (Izumi et al. 2014).

However, counseling based on a prenatal detection of PKS and the decision process should take into consideration the presence or absence of major malformations as well as other markers of poor perinatal outcome such as polyhydramnios (e.g., the high risk of perinatal death

related to CDH or complex cardiac anomalies and the greater percentage of preterm deliveries associated with polyhydramnios). Fetal macrosomia may also more frequently result in birth complications secondary to dystocic deliveries. Conversely prenatal detection of calico-pelvic dilation is rarely associated with subtle urinary tract malformation and often tends to spontaneously resolve after birth. Although fetal bowel hyperechogenicity seems to be rarely detected while the postnatal rate of gastrointestinal malformations in PKS is relatively high, when increased echogenicity persists over the third trimester and is associated with other anomalies the potential for gastrointestinal involvement should be considered and carefully monitored postnatally.

There are a few potential limitations related to this study. A major challenge is reflected in the ascertainment bias between the two cohorts as mentioned above. We attempted to overcome this by merging the data to arrive at an average analysis between the data from the literature (which generally represents the most severe cases that do not survive to the neonatal period) and the CHOP cohort (which represents a postnatally ascertained group who are generally milder as they have survived the prenatal and perinatal periods). In addition, other limitations may lie in the general poor knowledge itself of the PKS prenatal manifestations and accurate abilities to establish a cytogenetic diagnosis that may have affected a prompt recognition of PKS features resulting in underestimation of some data.

In spite of these limitations, through this extensive and systematic analysis of all PKS prenatal data currently available, we are able to present a preliminary descriptive set of relevant prenatal findings that suggest consideration of the diagnosis of PKS and approaches to optimally cytogenetically detect the presence of the mosaic i(12p). In addition, several prenatally ascertained findings may help in predicting perinatal outcome. An early diagnosis is essential to allow for effective counseling, decision making and anticipatory guidance and prenatal or newborn management especially of major congenital malformations leading to improved outcomes. In addition, the establishment of a prenatal profile consistent with a diagnosis of PKS is important for families with a prior PKS affected pregnancy in order to be reassuring in future pregnancies.

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SUPPORTING INFORMATION

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