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Phenotypic spectrum of fetal Smith-Lemli-Opitz syndrome

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Abstract

The Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive multiple congenital malformation syndrome caused by dehydrocholesterol reductase deficiency. The diagnosis is confirmed by high 7- and secondarily 8-dehydrocholesterol levels in plasma and tissues and/or by detection of biallelic mutations in the *DHCR7* gene. The phenotypic spectrum of SLOS is broad, ranging from a mild phenotype combining subtle physical anomalies with behavioral and learning problems, to a perinatally lethal multiple malformations syndrome. The fetal phenotype of SLOS has been poorly described in the literature. We report a series of 10 fetuses with molecularly proven SLOS. Even in young fetuses, the facial dysmorphism appears characteristic. Genital abnormalities are rare in 46, XX subjects. Gonadal differentiation appears histologically normal and in agreement with the chromosomal sex, contrary to what has been previously stated. We observed some previously unreported anomalies: ulnar hypoplasia, vertebral segmentation anomalies, congenital pulmonary adenomatoid malformation, fused lungs, laparoschisis, holomyelia and hypothalamic hamartoma. This latter malformation proves that SLOS phenotypically overlaps with Pallister-Hall syndrome which remains clinically a major differential diagnosis of SLOS.

Introduction

Smith-Lemli-Opitz syndrome (SLOS, MIM270400) is an autosomal recessive multiple congenital malformation syndrome with growth failure and mental retardation, first delineated in 1964. SLOS results from reduced activity of 7-dehydrocholesterol reductase, the final enzyme of the cholesterol biosynthesis pathway {Porter, 2011 #28} that converts 7-dehydrocholesterol to cholesterol. SLOS diagnosis can be confirmed by demonstrating, by gas chromatography-mass spectrometry (GC-MS), elevated precursor sterols, mainly 7- and 8-dehydrocholesterol in plasma and tissues, sometimes with low plasma cholesterol levels. In 1998, mutations in the *DHCR7* gene were shown to cause SLOS . Over 130 different mutations have now been identified in SLOS patients (detection rate 96%), among which the common, recurrent mutation IVS8-1G>C and two frequent mutations in Poland (W151X and V326L) . The incidence of SLOS has been estimated to be 1/20 000-1/70 000 but is strikingly different among various ethnic groups. The frequency of heterozygote is estimated as 3 to 4% in the Caucasian populations, a value that seems incompatible with the low reported incidence .

The phenotypic spectrum of SLOS is very broad, ranging from mild minor physical anomalies with behavioral and learning problems to a prenatally or perinatally lethal multiple congenital anomaly (MCA) syndrome. Post-natal clinical findings in molecularly proven SLOS and their frequency have been reported by Kelley *et al.* and by Goldenberg *et al.* , but the fetal phenotype has rarely be addressed. Goldenberg *et al.* briefly described 12 fetuses among a series of 45 SLOS . The same authors reviewed ultrasound findings in a series of 30 biochemically confirmed SLO cases : nuchal edema (during the first trimester of pregnancy), renal, cardiac or cerebral anomalies, ambiguous genitalia or a 46,XY karyotype in a phenotypically female fetus and polydactyly were the most common findings. Only two other biochemically confirmed fetal cases have been reported .

Prenatal diagnosis of SLOS can be essentially done either by sterols profiling in amniotic fluid or chorionic villi, or by *DHCR7* molecular analysis . Moreover maternal urine or serum steroid measurements by GC-MS (pregnanetriol, 7-dehydropregnanetriol, 8-dehydropregnanetriol, oestriol and 8-dehydroesteriol) should be a reliable non-invasive technique between 14 and 22 weeks' gestation . Antenatal diagnosis of SLOS is often made following familial history or ultrasound findings (mainly growth retardation with genital abnormalities). A better knowledge of the fetal phenotype of SLOS could help the practitioners of fetal medicine units to diagnose more accurately SLOS during pregnancy.

We report here the phenotype of 10 fetal SLOS confirmed by biochemical assays and molecular studies.

Materials and Methods

The series was build retrospectively through a conjoint effort of the members of the French fetal pathology society (SOFFOET). We collected data on 10 fetuses with Smith-Lemli-Opitz syndrome (born between 1999 and 2010). Termination of pregnancy (TOP) was performed in accordance with the French legislation and was followed by fetopathologic examination. The gestational age at which TOP was performed varied from 16 weeks of gestation (GW) to 34 GW. There was 5 46,XX and 5 46,XY fetuses. None of them was born to consanguineous parents and there was no positive family history of SLOS.

In accordance with the French bioethic laws, written signed consent was obtained from (at least) the mother for pathological examination and for genetic investigations. Standard fetal examination included photographs, evaluation of the fetal biometrics, X ray survey, external examination, autopsy and examination of placenta. All viscera, including the brain, were dissected, weighed and stained with hematoxylin-eosin (H&E) for histologic analysis. Fetal and/or placental tissue samples were frozen for biochemical analysis and DNA extraction.

Diagnosis of SLOS were suspected on fetal phenotype at prenatal ultrasound scan (USS) for cases 2 and 7 or at fetopathologic examination, and no other diagnostics tests has been performed, except *GLI3* sequencing in case 6 (see below). Elevated 7-dehydrocholesterol and 8-dehydrocholesterol were assayed in liver tissue, lung tissue or fetal blood, using GC-MS. Molecular analysis of the *DHCR7* gene was performed using standard molecular procedures. Segregation of the variants was confirmed by studying parental DNA.

Results

Prenatally detected anomalies (Table 1)

At prenatal USS, four fetuses (on eight) showed increased nuchal translucency. The main USS anomalies were intrauterine growth retardation (IUGR) with oligo- or anhydramnios in seven cases, congenital heart defects, limb defects (mainly postaxial polydactyly), cerebral and renal anomalies. One fetus had only IUGR associated with pulmonary valvular dysplasia.

Fetal pathology (Tables 1 and 2)

Seven fetuses showed IUGR and two were between the 10th and the 25th centile. Most showed micromelia with short long bones. Facial dysmorphism was noted in all fetuses, mainly short nose with anteverted nares (Fig. 1). Cleft palate or bifid uvula was seen in 4 fetuses. All fetuses had some limb defects: syndactyly of toes II-III, postaxial polydactyly of hands and feet, short and proximally placed thumbs and deep distal palmar crease between the second and third fingers, giving sometimes a "split hand like" appearance (Fig. 2). All female fetuses had normal external genitalia, and all male fetuses had ambiguous genitalia (Fig. 3). One fetus (case 5) had bicornuate uterus bicollis (Fig. 4A). Microscopic examination of the gonads disclosed normal ovaries in all 46,XX fetuses and testes in all 46,XY fetuses, without histologic evidence of gonadal dysgenesis. Most fetuses (8/10) had abnormal lung segmentation and renal hypoplasia or agenesis (8/10). One fetus only had a normal heart. One fetus had anomalous pulmonary venous return. 3 fetuses had common mesentery. Two fetuses had polysplenia and two had enlarged pancreatic tail. Radiologic anomalies included shortened long bones and brachymesophanlangy of fingers II (Fig.5). Four fetuses had cerebral biometries on the 5th centile, and five had true microcephaly. Holoprosencephaly was present in one case. Anomalies not reported before in SLOS included congenital pulmonary adenomatoid malformation, fused lungs, laparoschisis (Fig 4A), hypothalamic hamartoma and holomyelia (Fig. 4B), vertebral segmentation defects and ulnar hypoplasia (Fig. 5).

Metabolic and genetic screening

The clinical diagnosis of SLO syndrome was confirmed by high levels of 7-dehydrocholesterol and 8-dehydrocholesterol in liver, lung or fetal blood in all cases but case 8 (Table 3). For case 7, the diagnosis was confirmed by prenatal sterol measurement performed on amniotic fluid (cholesterol 0.03 g/l, 7-DHC 0.023g/l and 8-DHC 0.017 g/l). Molecular analysis of the *DHCR7* gene showed compound heterozygote mutations in 7 cases and homozygote mutations in 3 cases (Table 3). In fetus 5, 3 mutations were identified in the *DHCR7* gene, two of which in exon 4 inherited from the father (c.208G>T and c.149C>T) and the third mutation inherited from the mother. Because of the existence of a hypothalamic hamartoma in case 6, a coincident mutation of *GLI3* was excluded by direct sequencing.

Discussion

Several differences exist between our prenatal series and the postnatal ones : renal anomalies (mainly hypoplasia) are reported in all cases of this series, versus 40%; pulmonary segmentation defects are present in 9/10 fetuses of our study, versus 17%-45%; incidence of intestinal malrotation was 6% in a previous series , but affected 60% of the fetuses of this study ; and finally brain

malformations are also more frequent in our study. Obviously, fetopathologists can describe anomalies which are less accessible to pediatric examination. To the contrary, some typical findings of SLOS, as cataracts or colonic aganglionosis, are difficult to assess histologically and can be missed antenatally. Incidence of others malformations, such as limb anomalies, is in agreement with the literature, except for absent or hypoplastic middle phalanx of the second finger that seems more frequent than previously suspected. Lin *et al.* reported five major types of heart defects: atrioventricular canal, atrial septal defect ostium secundum type, patent ductus arteriosus, abnormal pulmonary venous return (APVR) and ventricular septal defects. In this series, nine fetuses have cardiac defects, mostly atrioventricular canal. Facial dysmorphism is present even in midtrimester fetuses: bitemporal narrowing, hypertelorism, short nose with anteverted nares, microretrognathia, large mouth, low set and posteriorly rotated ears and short neck. In case 5, these anomalies are blurred out by holoprosencephaly, a malformation which has been reported previously in nine SLOS patients . All male fetuses have external genital anomalies ranging from bifid scrotum to apparently female appearance. To the contrary, the female fetuses of this series have no anomalies of the external genitalia. Case 5 has bicornuate uterus, a rare but already described malformation. We observed normal gonadal differentiation in all our XY fetuses, irrespective of the level of external ambiguity. Referring to an article published in 1974, when biochimical diagnosis of SLOS was not possible, Kelley *et al.* stated that gonads varies from normal testes to ovotestis and to normal ovaries, or may be missing. No publication has addressed this issue since the discovery of the metabolic anomaly of SLOS, and gonadal differentiation was not described in two recent fetal cases . We suspect that the 1974 paper was based on a syndromologically heterogeneous collection. Indeed, our observation is not surprising, considering the fact that gonadal differentiation is not influenced by gonadal steroids. Our series confirms the retrospective data of Goldenberg on prenatal USS signs . All fetuses but one have IUGR of variable severity. Microcephaly is the most common sign during the 2^{nd} and 3^{rd} trimester of pregnancy. Case 9 has normal growth parameters at 16 GW, but growth retardation may not be present as early. For case 1, IUGR was the major antenatal sign. Non-vascular IUGR should prompt the sonographer to consider SLOS, especially when karyotype is 46,XX. Two fetuses in our series were diagnosed early in the second trimester (fetus 9 and 10). Our study shows for the first time that early fetal phenotype of SLOS is recognizable and very similar to the phenotype of elder children. Indeed facial dysmorphism, already present, was felt to be a good handle to elicit the diagnosis in a MCA fetus. These two cases illustrate the high interest of pathological fetal examination even for young MCA fetuses who should therefore not be terminated by surgical aspiration to allow a complete fetal examination.

We observed atypical manifestations of SLOS. Many skeletal malformations have been reported in SLOS, as polydactyly, metacarpal/tarsal hypoplasia, syndactyly, disproportionate short limbs, chondrodysplasia punctata, ribs anomalies, scoliosis, kyphosis or high ovoid lumbar vertebral bodies . A single patient suspect of SLOS was reported with ectrodactyly, radial aplasia and monodactyly . We now definitively add ulnar hypoplasia (patient 3) and vertebral segmentation anomalies (patient 3) to this list. Five fetuses also have a deep distal palmar crease with widely separate the second and third fingers, giving a "split hand like" appearance (Fig. 2). Abnormal pulmonary development has already been reported in SLOS. We can add congenital pulmonary adenomatoid malformation and fused lungs (Fig. 4A) to the list. The laparoschisis observed in case 4 may be fortuitous, but could mislead the clinician. Fetus 8 (previously been reported by Marcorelles and Laquerriere) has holomyelia, which is a partial or complete fusion of both anterior horns of the spinal cord. Distal arthrogryposis may be the result of this developmental anomaly. The presence of a hypothalamic hamartoma in fetus 6 makes SLOS overlap with Pallister-Hall syndrome (PHS, MIM146510),ascribed to *GLI3* mutations. Hypothalamic hamartoma, short limbs, postaxial or central polydactyly (with Y shaped central metacarpal or tarsal) and dysplastic nails, bifid epiglottis or laryngotracheal cleft, imperforate anus and other visceral malformations (renal abnormalities, genitourinary anomalies or abnormal lung segmentation) are the hallmarks of PHS . Similarities between both syndromes have been stressed , but hypothalamic hamartoblastoma has never been formally reported with SLOS. When both diseases where still unexplained, Donnai *et al.* even suggested that the Pallister-Hall syndrome and severe Smith-Lemli-Opitz syndrome may represent phenotypic variations of one condition . Indeed, some ancient cases may have been misdiagnosed on clinical ground in the past. Verloes *et al*. suggested that patient 6 from the seminal report of PHS , who had an hamartoma, flat nose, low-set and posteriorly rotated ears, micrognathia, left postaxial polydactyly, bilateral 2/3 toes syndactyly, bilateral cataracts and Hirschprung disease, had SLOS and not PHS. Interestingly, this phenotypic overlap with PHS and the possible holoprosencephaly/midline defects observed in SLOS suggest a defect in SHH pathway in SLOS mutated patients. Indeed, SHH protein precursor undergoes autocatalytic internal cleavage mediated by covalently attached cholesterol to the N-terminal signaling domain, and the potential effects of perturbed cholesterol biosynthesis on embryonic signaling proteins has already been discussed in SLOS . Distinction between these two syndromes is of obvious significance for genetic counseling, as Pallister-Hall syndrome is an autosomal dominant disorder (mainly sporadic cases resulting from *de novo GLI3* mutations), whereas SLOS is recessively inherited. We report for the first time systematic histologic examination of SLOS placenta. This organ has grossly normal architecture, but three fetuses show

placental hypotrophy. It will be of interest to better study placentas of SLOS in order to confirm and precise these anomalies.

There is an apparent discordance between the low incidence of SLOS as compared to the estimate carrier frequency . Jezela-Stanek *et al.* established the prospective incidence of SLOS in Poland in order to compare it to a previous carrier frequency estimation, but doesn't resolve this discrepancy between the predicted and observed incidence . No segregation of SLOS has been published. The "missing SLOS" may correspond to the two tails of the clinical spectrum: SLOS may remain undiagnosed among mildly affected individuals who do not show the key features that would trigger cholesterol analysis, and severe cases may stay hidden among early miscarriages. In accordance with this hypothesis, 4 couples suffered multiple early spontaneous abortions (2 for case 6, 5 for case 8, 5 for case 9 and 1 for case 10). For case 1, pregnancy obtained by *in vitro* fertilization was initially gemellar bichorionic biamniotic and reduced spontaneously. For case 6, the fourth pregnancy ended at 6 GW and the sterols analysis by GC-MS in miscarriage product (case 6') showed high levels of 7- and 8-dehydrocholesterol, confirming recurrence (Table 3). Moreover, three fetuses had increased nuchal translucency in the first trimester of pregnancy. As Maymon *et al.*, we could speculate, that some of fetal loss associated with SLOS may be related to nuchal oedema and subsequent fetal hydrops, reflecting the severity of the phenotype in these families . SLOS could be an unsuspected cause of recurrent early spontaneous abortions without identified cause, but systematic screening of *DHCR7* In couples with recurrent miscarriages has never been carried on.

All the fetuses, who can be considered as severe (prenatally detected), were genotyped and found to be either homozygous or compound heterozygous, with at least one null allele. Our observation confirms that severe phenotypes are mainly associated with nonsense and splice-site mutations (null alleles) , although strict genotype-phenotype correlations are difficult and significant variation is seen in severity even among individuals with similar mutations, suggesting influences of other factors on the phenotype. Fetus 5 with holoprosencephaly has 3 deleterious mutations, which could partly explain the severity of the phenotype. The efficiency of cholesterol transport from the mother to the fetus early in pregnancy may be one of these factors. For example, Apolipoprotein E protein is a major component of the cholesterol transport system in humans and studies suggest that the maternal apolipoprotein E genotype (ApoE) was implicated in phenotype heterogeneity, the maternal apo epsilon2 genotype (APOE2 protein) being associated with a severe Smith-Lemli-Opitz syndrome phenotype . Another hypothesis might be modifier alleles of genes belonging to the SHH signaling pathway.

In summary, we report 10 fetuses with SLOS, illustrating the broad clinical variability in the severe end of spectrum of this disorder and question the potential role of *DHCR7* deficiency in early abortion. We illustrate the usefulness of systematic feto-placental examination in syndromes which are not universally lethal: several anomalies that we describe would probably never have been reported in pediatric series, as morphologically-oriented autopsies are rarely performed in elder children, and as feto-placental examination allows exhaustive description of anomalies which are not easily accessible by clinical inspection or ex vivo imaging.

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Tables

Table 1: Prenatally detected anomalies and external phenotypic description of the 10 fetuses with Smith-Lemli-Opitz syndrome

(AVC Atrioventricular Canal ; B Bilateral ; CCA Corpus Callosum Agenesis ; CLP Cleft Lip and Palate ; CH Crown-Heel lenght ; CIV Interventricular communication ; CRL: Crown-Rump Length ; GW: gestational weeks ; HLH Hypoplastic Left Heart ; L Left ; ND Not determined ; R Right ; SA Spontaneous Abortion ; VH Vertex-Heel length)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Previous pregnancy	G1P1	G2P2	G3P2	G2P2	G2P2	G3P1 (2SA)	G2P2	G6P1 (5SA)	G6P1 (5SA)	G4P2
Gestational Age (GW)	32	34	21	34	24	26	26	23	16	19
Karyotype	46,XX	46,XY	46,XY	46,XX	46,XX	46,XY	46,XY	46,XX	46,XY	46,XX
Fetal echocardiography										
increased nuchal translucency	-	-	+ (value ND)	-	-	+ (3,2mm for CRL of 49mm)	ND	ND	+ (5,2mm for CRL of 57mm)	+ (3mm for CRL of 58mm)
IUGR	+	short limbs	+	+	+	+	+	ND	-	+
oligo/anamnios	-	-	+	+	-	-	ND	+	+	-
extremities anomalies	-	postaxial polydactyly left hand	postaxial polydactyly	-	-	postaxial polydactyly of hands/irregular toes	polysyndactyly of feet	-	-	hexadactyly?
cardiopathy	pulmonary valvular dysplasia	AVC	CIV?	-	AVC	partial AVC	AVC	AVC	AVC, HLH, mitral hypoplasia	AVC
microcephaly / brain anomalies	microcephaly	CCA	partial CCA?	microcephaly	CC anomalies	-	microcephaly/ gyration retardation	CCA	-	-
others anomalies		renal/lung anomalies, sexual ambiguity		laparoschisis/ small kidneys	bilateral cleft lip and palate and renal anomalies		retrognatism	bilateral renal agenesis	kidneys and bladder not seen	stomach and kidneys not seen
Weight(g) /CH(cm) / foot length(cm)	1128/37,5/5,6	625/31,5/4,3	250/21/3	1896/43/6,8	390/27,5/3,7	677/31/4,6	545/29,5/4,1	337/23/3	89/15/1,7	130/18,5/2
IUGR	+	10-25th	+	10-25th	+	+	+	+	-	+
Microcephaly (centiles) / brain weight	+	5th / 87g	5th / 40g	+ / 212g	5th / 86,5g	+ / 91g	5th / ?	+ / 54g	- / 26g	+ / 19,5g

Facial dysmorphism										
bitemporal narrowing	+	-	+	+	-	-	+	+	-	-
hypertelorism	+	+	+	+	-	+	+	-	+	-
anteverted nares/upturned	+	+	+	+	-	+	+	+	+	+
microretrognathia	+	+	+	+	-	+	+	+	+	+
smooth/convex philtrum	-	+	-	+		+	+	+	+	+
short philtrum	+	-	+	+			+	+	-	-
low-set/posteriorly rotated ears	+	+	+	+	+	+	+	-	+	+
short neck	+	+	+	+	+	+	+	+	+	+
large mouth	-	-	+	-	+	+	+	+		+
midline cleft palate/bifid uvula	-	cleft palate	-	bifid uvula	bilateral CLP	-	-	-	-	cleft palate
narrow palate	+	ND	-	-		+	-	-	+	
Micromelia	+	+	+	+	+	+	+	+	+	+
Feet anomalies										
Syndactyly of toes II-III	+	+	+	+	+	+	+	+	+	+
short hallux	+	+	-	-	+	-	-	+	-	-
post-axial polydactyly (L,R or B)	-	-	+ L	-	-	+ B	+ R	+ R	+ B	-
Hands anomalies										
post-axial polydactyly (L,R or B)	-	+ L	+ B	-	-	+ B	-	+ B	+ B	-
short/proximally placed thumbs	+	+	+	+	+	-	-	-	+	+
extended distal palmar crease	+	+	+	+	ND	-	+	-	ND	ND
External genitalia anomalies	-	open urogenital groove and unfused genital swelling	bifid scrotum	-	-	external female genitalia	open urogenital groove and unfused genital swelling	-	+	-

Table 2: Visceral and radiological anomalies of the 10 fetuses with Smith-Lemli-Opitz syndrome

(AVCD Atrioventricular ; APVR Anomalous Pulmonary Venous Return ; CCA Agenesis of the corpus callosum ; HLH Hypoplastic Left Heart ; HPE Holoprosencephaly ; N Normal ; NA: not available ; VSD Ventricular Septal Defect)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Gonads	ovaries	testes	testes	ovaries	ovaries	testes	testes	ovaries	testis	ovaries
Kidney	sigmoid kidney / hypoplasia	Left pelvicaliceal dilatation	hypo/aplasia + cysts	hypoplasia	hypoplasia	hypoplasia	hypoplasia	Bilat agenesis	severe hypoplasia	left pelvic kidney
Cardiovascular	pulmonary valvular dysplasia, short ductus arteriosus.	AVC with riht ventricular hypoplasia	HLH, arch aortic tubular hypoplasia and subaortic VSD.	-	Partial AVCD	Left cavities hypoplasia, partial AVCD and APVR	AVC	AVC	AVCD and mitral atresia	AVC
Lung	Lobation anomalies	lobation anomalies /cysts	hypoplasia	lobation anomalies	N	absent lobation / fused lungs	lobation anomalies / hypoplasia	absent lobation	absent lobation	lobation anomalies
CNS	?	posterior CCA	hypothalamic and pallidum dysgenesis and cranial nerves ganglia lesions	microcephaly	lobar HPE	cerebellar and brainstem abnormalities / Hypothalamic hamartoma	NK	CCA / rare neuronal heterotopia / holomyelia	-	ACC
Gastrointestinal	-		intestinal malrotation	laparoschisis / common mesentery	-	Incomplete common mesentery	-	-	-	-
Others		enlarged caudal pancreas			bicornuate uterus bicolli	enlarged caudal pancreas	polysplenia		polysplenia /single umbilical artery	single umbilical artery/ bicornuate uterus
Skeletal										
Short long bones	+ (F= 49 mm)	+ (F= 35 mm)	+ Right ulnar hypoplasia (F= NK)	- (F=62 mm)	+ (F=36 mm)	+ (F= 35 mm)	+ (F= 36 mm)	+ (F= 29 mm)	+ (F= NK)	+ (F= NK)
Brachymesophalangy of 2d finger(s)	+	+	+	+	+	NK	NK	+	NK	+
Placenta (weight)	hypotrophy (229g ; 5-10th ; 325g=M 32)	N (235g ; 75-90th)	N (94g ; <10th)	N (417g ; 50-75th)	hypotrophy (128g ; 10th ; 190g=M 24)	N (240g ; 50-75th)	hypotrophy (120g ; 5-10th ; 226g=M 26)	155g	N (69g ; 25-50th)	N (97g ; 10-50th)

Table 3: Biochemical and genetic data of the 10 fetuses with SLOS

Cases	Tissus	Cholesterol (mg/g)	7-DHC (mg/g)	8-DHC (mg/g)	7+8DHC/Chol	Mutations (1 line per allele)
1	Liver	0,76	0,34	0,53	1,14	c.356del13nt (p.His119ProFsX23) c.906C>G (p.Phe302Leu)
2	Fetal blood	113 mg/l	77mg/l	299mg/l	3,33	c.1228G>A (p.Gly410Ser) c.964-1 G>C (IVS8-1G>C)
3	Lung	0,22	1,57	1,91	15,82	c.438C>G (p.Asn146Lys) IVS8-1G>C
4	Liver	1,6	0,05	0,08	0,08	c.506C>T (p.Ser169Leu) IVS8-1G>C
5	Lung	0,41	1	0,99	4,85	c.149C>T (p.Ala50Val) c.208G>T (p.Gly70Cys) c.628A>T (p.Lys210X)
6	Liver	0,21	0,6	1,14	8,29	IVS8-1G>C IVS8-1G>C
6' (miscarriage)	Trophoblast	2,22	0,372	0,088	0,21	-
7	Liver	0,26	2,2	3,33	21,27	IVS8-1G>C IVS8-1G>C
8		NA	NA	NA	NA	IVS8-1G>C c.452G>A (p.Trp151X)
9	Liver	0,18	0,55	1,69	12,44	IVS8-1G>C c.682C>T (p.Arg228Trp)
10	Liver	0,104	0,21	0,224	4,17	IVS8-1G>C IVS8-1G>C
Normal (n=28)	Liver	3.98±1.75	0.003±0.003	0.006±0.004	0.002±0.002	

Table 4: Phenotypic overlap of SLO and Pallister-Hall syndromes

	PHS	**SLOS**
Gene	*GLI3*	*DHCR7*
Inheritance pattern	Autosomal Dominant	Autosomal Recessive
IUGR	+++	+
Renal	Hypoplasia/agenesis	hypoplasia
Lund segmentation defects	+	+
Cardiovascular defects	rare	+
Imperforate anus	+	rare
Hypothalamic hamartoma	+	rare
Epiglottic/laryngeal abnormalities	+	-
Extremities	Post-axial and central polydactyly, dysplastic nails, brachytelephalangism	Post-axial polydactyly and 2/3 toe syndactyly
Genital	Rare (hydrometrocolpos, vaginal atresia, urogenital sinus, cloaca)	+ (male)
Facial dysmorphism	Downward slanting palpebral fissures, short nose with anteverted nares	Bitemporal narrowing, anteverted nares

Legend to figures

Figure 1: Facial dysmorphism of fetuses 1 to 10

(A) Profile pictures of fetuses 1 to 10 – (B) Frontal view of fetuses 1 to 8. Note bitemporal narrowing, hypertelorism, short nose with anteverted nares, microretrognathia (severe for case 3), full cheeks, large mouth, low set and posteriorly rotated ears and short neck (figure numbering refers to the case numbers).

Figure 2: Acral anomalies in fetal SLOS

Note deep distal palmar crease between second and third fingers which have sometimes widely spaced ("split hand like") in cases 1, 2, 3, 4 and 7. Cases 2, 3, 6, 8 and 9 have postaxial hexadactyly. Case 8 has no palmar crease. Cases 8 and 10 have bilateral ulnar club hands. Thumbs are often short and proximally placed. Note some fetuses with camptodactyly and fifth finger clinodactyly. All fetuses except case 9 have bilateral syndactyly of toes II-III. Case 3 has syndactyly of toes II-III and IV-V. Cases 3, 6, 7, 8 and 9 have unilateral or bilateral post-axial hexadactyly. Case 8 has fibular deviation of toes. Hallux are sometimes short and broad.

Figure 3: Abnormal external genitalia in SLOS

Cases 1 and 5 are female with normal external genitalia. Cases 2 and 7 have ambiguous genitalia with small genital tubercle, open urogenital groove and unfused genital swelling. Case 3 has bifid or incomplete fused scrotum. Case 6 is 46,XY with phenotypically female appearance.

Figure 4A: Unusual malformations in SLOS

(a): congenital pulmonary adenomatoid malformation within the left upper lobe in fetus 2 - (b): absent pulmonary lobulation with posterior fused lungs in fetus 6 - (c,d): bicornuate uterus bicollis - (e): laparoschisis .

Figure 4B: CNS unusual malformations in SLOS

(a,b): hypothalamic hamartoma (arrow) in fetus 6 - (c): anterior spinal cord fusion (holomyelia) in fetus 8 (arrow).

Figure 5: Radiographic highlights in SLOS

(A): brachymesophanlangy of second finger in seven fetus - (B): associated right ulnar hypoplasia in case 3 - (C): vertebral segmentation defects (hemivertebra, agenesis of last sacral vertebra) in case 3.

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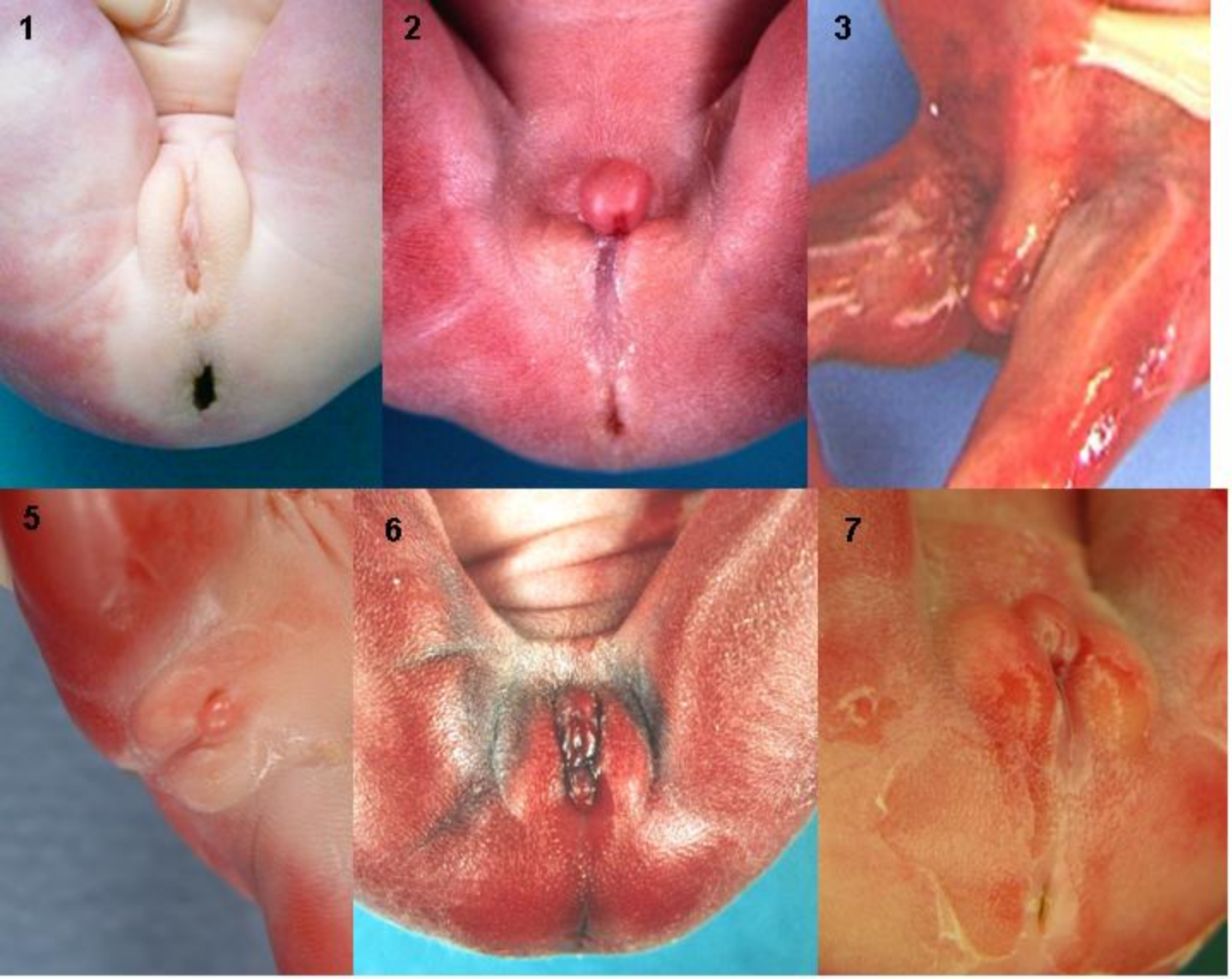
Figure 1

Figure 2



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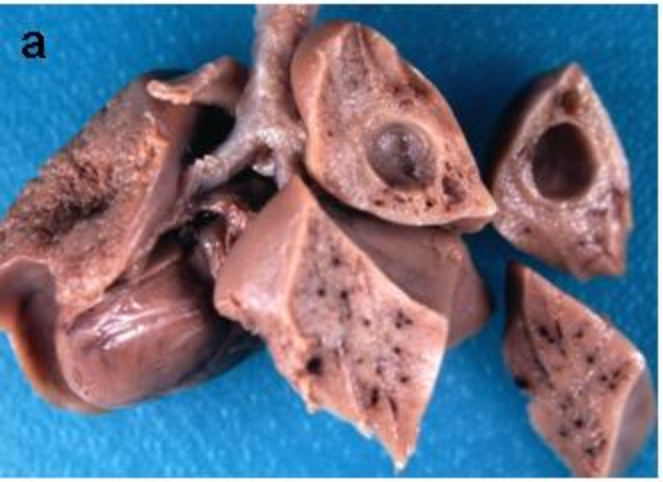
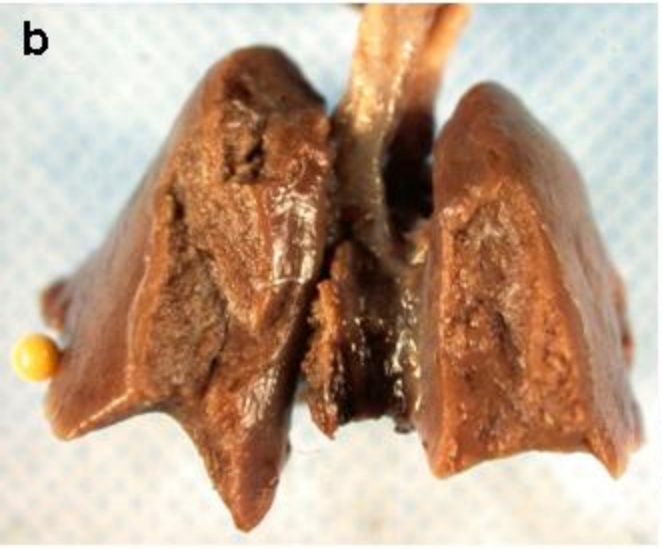




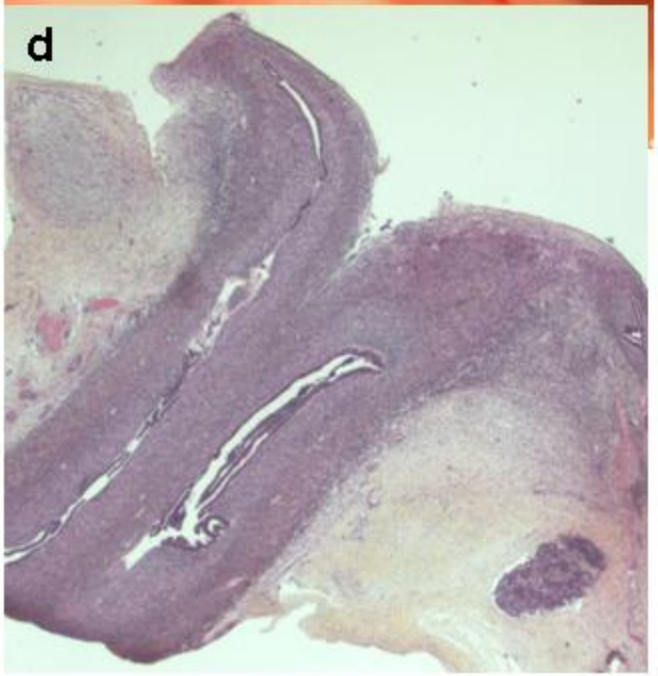
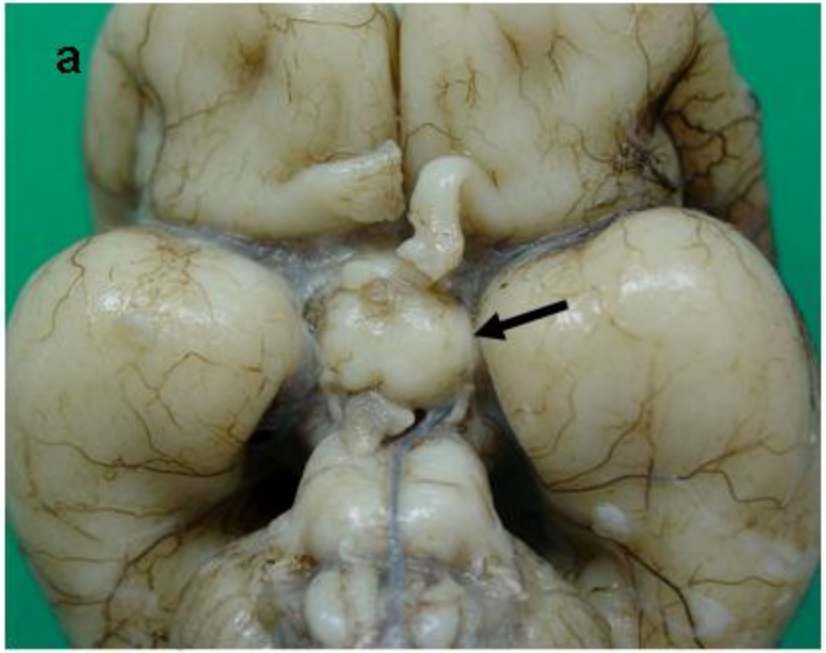
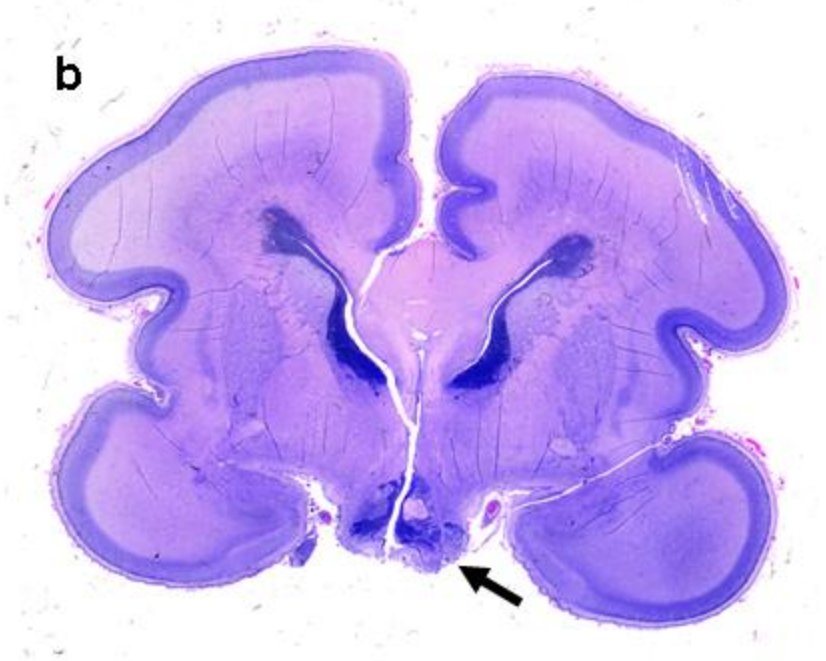


Figure 4A





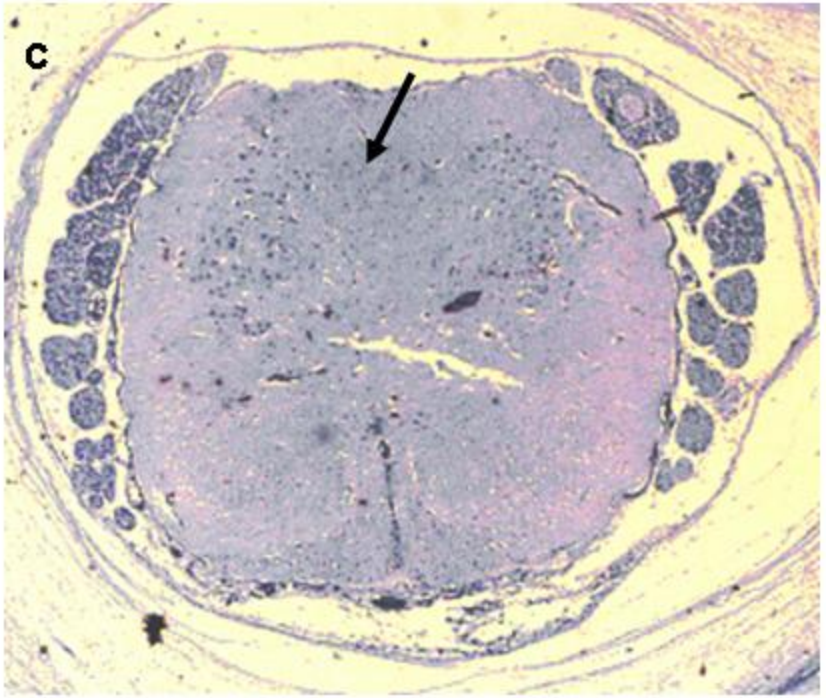


Figure 4B

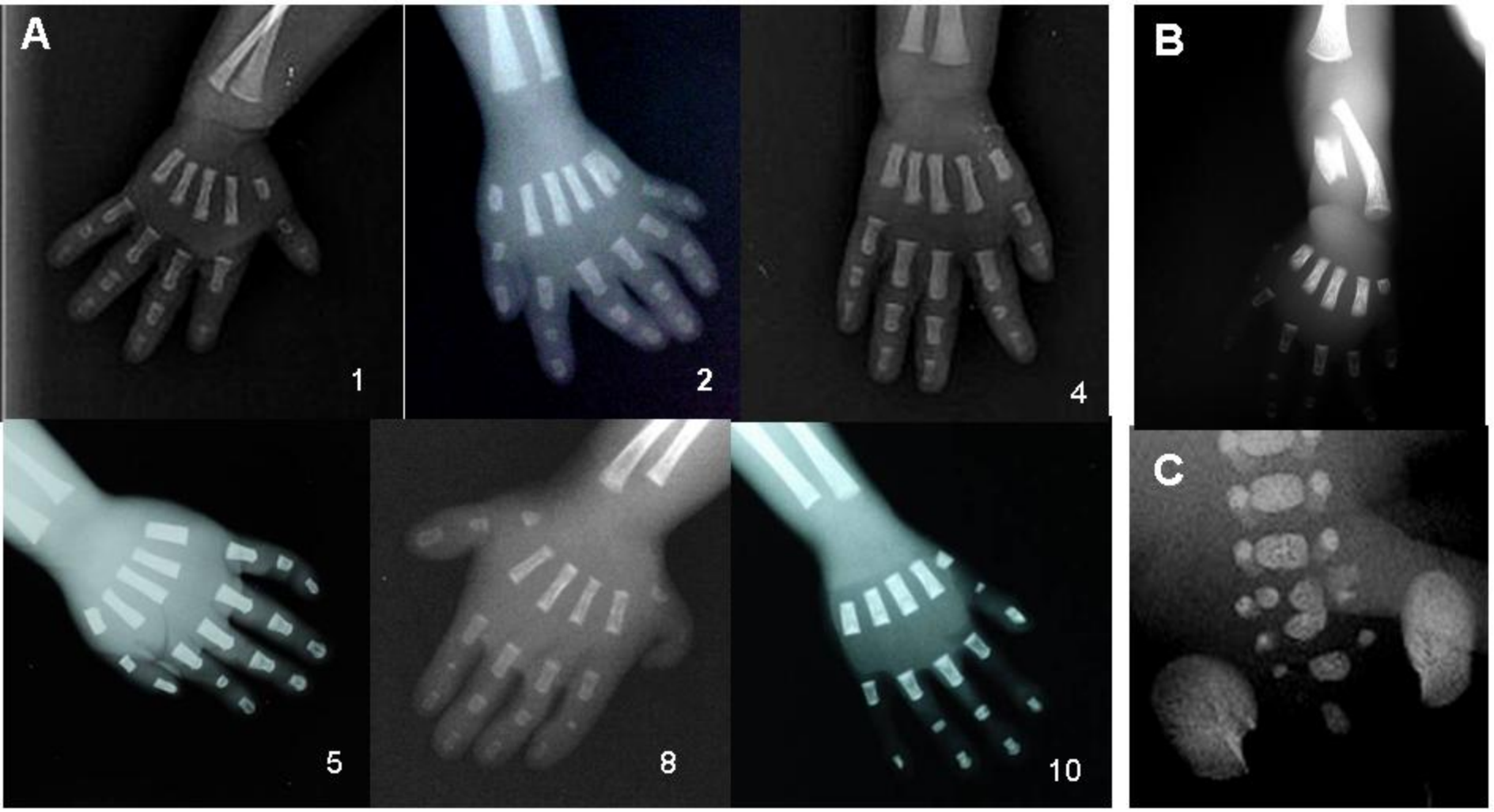


Figure 5