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Fetal Description of the Pancreatic Agenesis and Holoprosencephaly Syndrome associated to a specific *CNOT1* variant

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Abstract:

Holoprosencephaly (HPE) is a clinically and genetically heterogeneous disease, which can be associated with various prenatal comorbidities not always detectable on prenatal ultrasound. We report on the case of a foetus carrying a semi-lobar HPE diagnosed at ultrasound, for which a fetal autopsy and a whole exome sequencing were performed following a medical termination of pregnancy. Neuropathological examination confirmed the semi-lobar HPE and general autopsy disclosed a total pancreas agenesis. Whole exome sequencing found the *CNOT1* missense c.1603C>T, p.(Arg535Cys), occurring *de novo* in the foetus. The same variant was previously reported in five unrelated children. All individuals had HPE, and 4 out of 5 presented endo- and exocrine pancreatic insufficiency or total pancreas agenesis. *CNOT1* encodes a subunit of the CCRN4-NOT complex, expressed at the early stage of embryonic development. This report is the first fetal description of

the phenotype associating HPE and pancreatic agenesis linked to the recurrent *CNOT1* missense c.1603C>T, p.(Arg535Cys). This finding strengthens the hypothesis of a specific recurrent variant associated with a particular phenotype of HPE and pancreas agenesis. The fetal autopsy that revealed the pancreas agenesis was crucial in guiding the genetic diagnosis and enabling accurate genetic counselling.

Introduction:

HPE can be diagnosed with ultrasound from the first trimester of pregnancy^{1,2}. HPE is clinically and genetically heterogeneous. Both environmental and genetic causes have been identified such as chromosomal abnormalities, teratogenic exposure during pregnancy (poorly controlled diabetes, infections, alcohol and cigarette smoking, drugs), and single-gene disorders³⁻⁵. For molecular genetic anomalies, inheritance pattern is mostly autosomal with dominant transmission and concerns major HPE genes (*SHH*, *ZIC2*, *SIX3*, *FGFR1*). Other rare inheritance patterns described involves an autosomal recessive inheritance and concerns minor HPE genes (*STIL*, *DISP1*, *TGIF1*, *FGF8*), while oligogenic inheritance could explain the variable expressivity in several families⁶⁻⁸.

De novo variants in *CNOT1* (CCR4-NOT Transcription Complex Subunit 1, OMIM #604917) have been associated with neurodevelopmental delay and a wide spectrum of comorbidities, such as seizures, hypotonia, behavioral problems and organ dysfunction⁹. The specific missense c.1603C>T, (p.Arg535Cys) has been reported in five individuals with a singular phenotype associating partial/semi-lobar HPE, pancreas agenesis or neonatal pancreatic insufficiency with diabetes mellitus, and gallbladder agenesis in two cases^{10,11}. A sixth case of semi-lobar HPE, pancreas and gallbladder agenesis was reported in 2017 with no molecular diagnosis in the genes known to be associated with pancreatic aplasia or neonatal diabetes but *CNOT1* was not assessed. For these patients, the pancreatic anomaly was detected postnatally in the first days of life.

We report on a fetus with semi-lobar HPE prenatally diagnosed, pancreas agenesis identified at fetal autopsy and associated to the specific *CNOT1* variant c.1603C>T, (p.Arg535Cys).

Case report:

Pregnancy

A genetic consultation took place following observation of abnormalities in an ultrasound examination in the first trimester of pregnancy, for a 27-year-old, gravida 1 para 0 woman. The parents were unrelated and had no relevant personal or familial medical history.

At the routine first trimester ultrasound examination, the clinician suspected a malformation of the cephalic pole and referred the pregnant woman to the gynaecology department of the Rennes University Hospital. A high-accuracy ultrasound examination was performed at the estimated term of 15+2 weeks of gestation and evidenced a progressive intrauterine monofetal pregnancy with semi-lobar holoprosencephaly and a large median facial cleft. Nuchal translucency was at 1.40 mm (5th percentile), cranio-caudal length was 75.2 mm (75th percentile), and estimated fetal weight was 71 g (40th percentile)¹².

The parents requested an assisted abortion, which was accepted by the maternity centre of the ethics committee and took place at 17+1 weeks of gestation.

Post-mortem examination

Post-mortem examination of the placenta revealed a small placenta, with weight corresponding to that of a 13 weeks gestation (55g). Microscopic examination was normal.

Bone age corresponded to the estimated age of 16 weeks gestation. The post-mortem examination showed a female fetus with appropriate development for the gestational age. She had dysmorphic features with hypertelorism, low-set posteriorly rotated ears, a large unilateral cleft lip and palate, and an edematous neck. Her extremities were normal. The autopsy showed pancreatic agenesis with no other visceral malformations (gallbladder present). The neuropathological examination showed a semi-lobar holoprosencephaly (fusion of the left and right frontal and parietal lobes,

interhemispheric fissure only in the occipital portion) with arhinencephaly and absent corpus callosum. Photographs of the fetopathological and neuropathological examination are shown in Figure 1. X-rays revealed short tubular bones consistent with an estimated gestational age (EGA) of 16 weeks.

Genetic investigations

Genetic investigations were performed after delivery. Parental information and consent were assessed.

The fetal karyotype realised on blood from the umbilical cord was normal (46,XX).

An array-CGH analysis showed a 414 Kb heterozygous deletion in the 15q11.2 chromosomal region (arr[GRCh37] 15q11.2(22765628_23179948)x1). This deletion encompasses the OMIM genes *NIPA1* and *NIPA2*, and was solely considered as a locus for susceptibility to neurodevelopmental disorders with incomplete penetrance and variable phenotypes.

A MLPA (Multiplex Ligation-dependent Probe Amplification) analysis was performed for the genes most frequently implicated in holoprosencephaly (*SHH*, *ZIC2*, *SIX3*, *TGIF1*, *PTCH1*, *GLI2*, *TMEM1* and *FBXW11*) and found neither duplication nor deletion in these genes.

Whole exome sequencing found the missense heterozygous variant c.1603c>t, p.(Arg535Cys) in the *CNOT1* gene (NM_016284.5). Sanger analysis carried out on both parents revealed that the variant occurred *de novo* in the fetus. The variant c.1603c>t was classified as pathogenic (ACMG class⁵¹³) based on the following argument: reported in ClinVar with concordant phenotype, absent from GnomAD, affected a residue highly conserved across species (up to *C. elegans*), deleterious effect on protein function with in silico prediction tools used (Align GVGD, SIFT, Mutation Taster and PolyPhen accessed through AlamutVisual). No other significant variant was found.

Discussion:

We report here on the sixth proband with the *CNOT1* variant c.1603C>T, p.(Arg535Cys) and a similar phenotype to individuals previously described. All patients reported with the same variant in PubMed presented HPE, and pancreas anomalies (except one who had no pancreatic insufficiency nor diabetes mellitus at 6.5 years old). Intrauterine growth retardation was reported for 4/6 patients. The phenotypes are shown in Table 1.

CNOT1 encodes a subunit of the CCR4-NOT complex, implicated in the post-transcriptional mRNA degradation and expressed in early embryonic development^{10,11}. *CNOT1* contains two HEAT-like repeat domains in C-terminal, providing a rigid scaffold to the complex subunit formed by *CNOT1* and the heterodimer *CNOT2-CNOT3*¹⁴. The variant c.1603C>T, p.(Arg535Cys) is located in the HEAT domain of the protein. In a cohort study of 39 individuals with the *CNOT1* variant distributed throughout the protein, Visser et al.⁹ found one other variant in the HEAT domain (c.1924C>G, p.(Gln642Glu)). The individual also had *de novo* variants in *ATP6V0C*, *XPO7* and *ETNK1*, and presented with a severe intellectual deficiency, hypotonia, Lennox-Gastaut epilepsy and an Arnold-Chiari malformation requiring surgery at 14 months old, but neither pancreatic disorder nor HPE were reported. De Franco et al.¹⁰ suggested a potential effect of the c.1603C>T, p.(Arg535Cys) variant in the Sonic Hedgehog pathway at an early stage of embryonic stem cell differentiation. The variant could maintain an increased expression of SHH by maintaining its inhibition activity on Gata4 and Gata6 transcription factors and, therefore, blocking the differentiation of pluripotent cells into pancreatic cells.

Prenatal diagnosis of HPE should lead to specific genetic counselling, as HPE is a severe condition linked to multiple aetiological factors. A rigorous clinical evaluation of the family history, environmental factors, and associated features should be the first step of the consultation¹⁵. While HPE can be diagnosed by ultrasound at the first trimester of pregnancy, the pancreas is an organ that is difficult to examine by ultrasound and normal reference ranges allow a reliable assessment after 19 weeks of gestation¹⁶. In this case report, pancreas agenesis has not been evidenced at the 17th gestational week and was discovered at the post-mortem examination.

In conclusion, this sixth consistent description of an association between the *CNOT1* variant c.1603C>T, p.(Arg535Cys) and a particular phenotype of HPE and pancreas agenesis strengthens the hypothesis of a specific recurrent nonsense variant in *CNOT1*. As the pancreas is difficult to explore through prenatal ultrasound examination or in-utero MRI examination during the pregnancy, fetal autopsy remains a major element to guide genetic diagnosis and to allow accurate genetic counselling.

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Legends:

Figure 1: Photographs of the fetopathological and neuropathological evaluation. A and B: dysmorphic features with hypertelorism, low-set posteriorly rotated ears and large unilateral cleft lip and palate. C: anterior view of the brain with fusion of the frontal and parietal lobes and D: posterior view of the brain with interhemispheric fissure only in the occipital portion. E: coronal section of the brain showing a single anterior hemisphere and a unique ventricle.

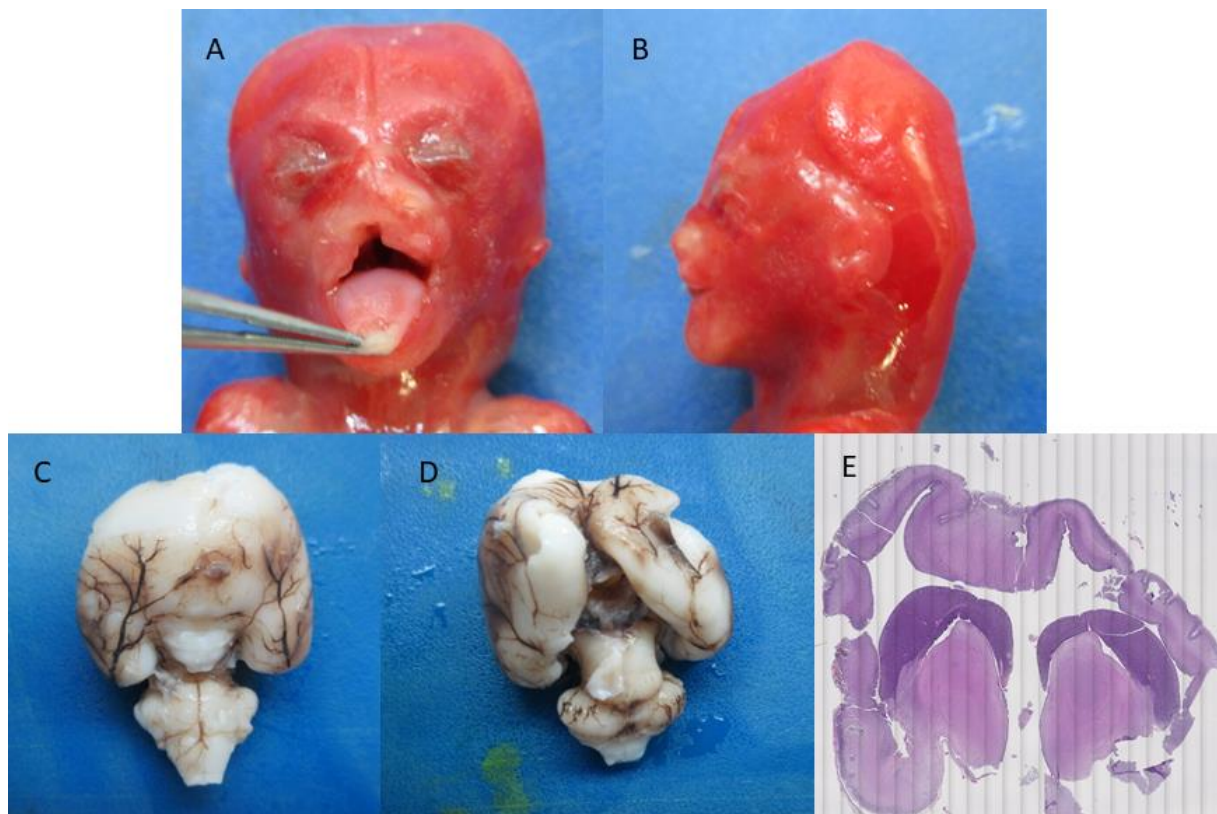


Table 1: Phenotypic description of the reported case and five other individuals reported with the *CNOT1* variant p.(Arg535Cys).

* Brain MRI showed dysplastic frontal horns of the lateral ventricles, missing septum pellucidum, broadly joined cella media of the lateral ventricles, and hypoplasia of the corpus callosum (absent frontal parts).

	Number of cases involved	P1 (Kruszka et al.2019)	P2 (Kruszka et al.2019)	P3 (De Franco et al. 2019)	P4 (De Franco et al. 2019)	P5 (De Franco et al. 2019)	P6 (Reported case)
Intrauterine growth retardation	4/6	X		X	X	X	
Holoprosencephaly	6/6	Semilobar HPE	Semilobar HPE	Lobular* HPE	Semilobar HPE	Consistent dysmorphic features	Semilobar HPE
Pancreas anomalies	5/6						
Only pancreatic insufficiency and diabetes mellitus		X					
Pancreas agenesis				X	X	X	X
Gallbladder agenesis	2/6			X	X		
Dysmorphic features		Epicanthal folds, depressed nasal bridge, hypotelorism and long philtrum	Microcephaly, epicanthal folds and long philtrum	Low-set ears	Receding forehead, cylindrical nose, mild hypotelorism, dysplastic left ear and hypoplastic zygomatic bone	Prominent occiput, low-set ears, high arched palate and prominent central incisors	Hypertelorism, low-set posteriorly rotated ears, a large unilateral cleft lip and palate, and an edematous neck
Prognosis		Developmental delay. Death at 16 months	Developmental delay, muscle spasticity	Mild learning difficulties. Complex focal seizures	Death at 3 months	No developmental delay	Medical termination of pregnancy