Lessons from exome sequencing in prenatally diagnosed heart defects: a basis for prenatal testing

Running head: Exome sequencing in prenatal diagnosed heart defects

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Conflicts of interest

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request**.**

Contributorship statement

Study design: DSW, TM, RO, MW

Drafting the manuscript: DSW, GSL, MW

Obtaining clinical data: DSW, EO, ERF, GW, RO

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Abstract

Congenital heart defects (CHDs) are the most common birth defect with 30-40% being explained by genetic aberrations. With next generation sequencing becoming widely available, we sought to evaluate the clinical utility of exome sequencing (ES) in prenatally diagnosed CHD.

We retrospectively analyzed the diagnostic yield as well as non-conclusive and incidental findings in 30 cases with prenatally diagnosed CHDs using ES, mostly as parent-child trios.

A genetic diagnosis was established in 20% (6/30). Non-conclusive results were found in 13% (4/30) and incidental findings in 10% (3/30). There was a phenotypic discrepancy between reported prenatal and postnatal extracardiac findings in 40% (8/20). However, none of these additional, postnatal findings altered the genetic diagnosis.

Herein, ES in prenatally diagnosed CHDs results in a comparably high diagnostic yield. There was a significant proportion of incidental findings and variants of unknown significance as well as potentially pathogenic variants in novel disease genes. Such findings can bedevil genetic counselling and decision making for pregnancy termination. Despite the small cohort size, our data serve as a first basis to evaluate the value of prenatal ES in CHD for further studies emerging in the near future.

Key words: congenital heart disease, genetics, clinical genetics, prenatal, exome sequencing

INTRODUCTION

Congenital heart defects (CHDs) are the most common birth defect with an observed birth prevalence of approximately 7-11 per 1,000 (1, 2) representing the leading cause of newborn mortality in developed countries. About 30% of CHDs (1% of all live born) are life threatening and require surgical interventions within the first year of life (3-5).

Genetic abnormalities are found in 30-40% of CHDs (6, 7), comprising chromosomal abnormalities, smaller copy number variations and single gene defects explaining about 10% of CHDs, each (6, 8). A small number of unsolved cases might be explained by mutations in unknown disease genes, which still have to be discovered. The remaining proportion of CHDs are likely explained by environmental factors and polygenic inheritance, reflected by a recurrence risk that depends not only on the affected family member, but also on the kind of heart defect. Tetralogy of Fallot (ToF) for example has a higher recurrence risk in first degree relatives than D-transposition of the great arteries (9). In line with this hypothesis, exome sequencing (ES) in patient-parents trios with CHDs and control families demonstrated an enrichment of deleterious variants in genes that are expressed in the developing heart (10).

In most industrialized countries ultrasound screening for CHDs is offered around the 20th week of gestation as a basic cardiac examination with a four-chamber view and an extended cardiac examination with a routine view of the outflow tract (11, 12). The overall prenatal detection rate of

CHDs by ultrasound is 45.1% but highly depends on the type of defect (13). In case of a fetal CHD, the parents are usually offered prenatal karyotyping and array based CNV analysis. These methods, however, cannot detect point mutations, which is the reason why there is a growing urge for next generation sequencing technologies in everyday practice.

Management of pregnancies, in which CHDs are identified, highly depends on the postnatal prognosis of affected children, which in turn often depends on the genetic diagnosis. In a Danish study with 14,688 cases, 57.8% of pregnancies with prenatally diagnosed fetal CHDs were terminated (14), highlighting the necessity in establishing an early genetic diagnosis in order to provide adequate counselling for affected families.

Therefore, the aim of this study was to evaluate the utility of ES in fetuses from interrupted pregnancies or live born patients that had been diagnosed with CHDs prenatally. First we evaluated the diagnostic yield. Second, we were interested in the rate of inconclusive genetic findings, such as novel candidate genes or variants of unknown significance, as well as incidental findings that can all bedevil genetic counselling. Third, we evaluated potential discrepancies between prenatal ultrasound findings and postnatal phenotypes, in order to assess the possible use of prenatal ES for the future.

MATERIAL AND METHODS

Study structure

The study was performed according to the declaration of Helsinki and the STROBE guidelines and was approved by the local ethic committee. We performed a retrospective analysis of cases where exome sequencing was performed between January 2016 and August 2018 with the indication of a prenatally diagnosed CHD at our institute. Patients were selected if the clinical information provided clearly stated that a CHD was diagnosed prenatally. Indication for ES was established by an interdisciplinary team consistent of pediatric cardiologists, gynecologists and geneticists if the expected diagnostic yield was above 10%. These cases include severe cardiac defects or syndromic CHDs. Cases were categorized as syndromic if there was at least one extracardiac sonographic finding not including soft markers such as a singular umbilical artery. The decision was made independently from previously performed genetic testing with negative results such as chromosome or array analysis. Written informed consent was obtained from both parents for genetic testing as well as the publication of the data. Participants were informed about the possibility of incidental findings. Incidental findings were reported if they were considered as "actionable", i.e. with medical consequences in regard to possible preventative measures following the "ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing" (15), but not exclusively restricted to this recommendation. Findings were reported to the patients or parents by medical geneticists or clinicians with genetic expertise in his or her field.

Exome sequencing and variant interpretation

DNA for ES was extracted from peripheral and umbilical blood, skin, chronic villous samples, cultivated amniocytes or umbilical tissue (Supplementary table 1) using the Chemagic DNA Blood Kit

on a chemagic 360 instrument (PerkinElmer, Baesweiler, Germany) according to the manufacturer's instructions. For DNA extraction from tissue, a tissue specific lysis buffer was used. ES was performed using a Sure Select Human All Exon 60Mb V6 Kit (Agilent, Santa Clara, USA) for enrichment and sequencing was done on a HiSeq 4000 engine (Illumina, San Diego, USA) as previously described (16). Reads were aligned to the University of California Santa Cruz (UCSC) human reference assembly (hg19) with BWA v.0.5.8. Single nucleotide variants and small insertions and deletions were detected with SAMtools v.0.1.7. Variant prioritization was performed based on an autosomal recessive pattern of inheritance (homozygous or putative compound heterozygous variants with a minor allele frequency <1%), and an autosomal dominant pattern of inheritance (heterozygous variants with a minor allele frequency <0.01%). For this search, a three-step approach was used (Supplementary figure 1). First (step I), a phenotype-based gene list was used to prioritize potential disease causing genes (Supplementary table 2). The gene list was created based on a full-text OMIM search for "congenital heart defect". Second, variants in all other OMIM genes were analyzed for potentially causative variants to account for the limited prenatally available phenotypic information (step II). Third (step III), all genes were searched for potential novel disease causing genes (list of all potentially deleterious variants in Supplementary table 3).

In case of trio ES, variant prioritization was also based on the *de novo* status of variants in the patient's DNA (minor allele frequency <0.02%). Variants were classified according to the ACMG standards and guidelines for the interpretation of sequence variants (17) with the limitation of having reduced phenotypic data. All pathogenic and likely pathogenic variants were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/submitters/500240/).

Study group

30 cases with prenatally diagnosed CHDs as by ultrasound were sequenced from January 2016 to August 2018 and included in this study. All statistical parameters can be found in Table 1. 83.3% of these cases were sequenced as parent–child trios. Three cases were sequenced as single exomes, whereby in one of these cases the affected father was sequenced instead of his daughter, because she showed pre- and postnatally the same symptoms as himself (individual 2). In one family, two affected brothers and their parents were sequenced as quattro exome, since both siblings were affected by CHDs (individuals 26-1 and 26-2). 29 cases were sequenced postnatally and one prenatally (individual 18). Median maternal age at pregnancy was 32 years and median gestational age at diagnosis of CHD was 20 weeks of gestational age. 43.3% (13/30) of the fetuses were born alive, while all of the remaining pregnancies were terminated (Supplementary table 1). Median gestational age at birth was 39 weeks, with only one premature birth (individual 4 in week 32). Except for one child (individual 4) that died shortly after birth, all other live born children underwent medical intervention (i.e. interventional heart catheter, surgery) within the first year of life (Table 2).

RESULTS

Most of the cases with prenatally diagnosed CHDs are sporadic cases

About 87% (26/30) of the prenatally diagnosed CHDs were sporadic cases and four were familial cases. In two families, one of the parents had the same congenital CHD as the child. Individual 2 and her father had an aortic isthmus stenosis accompanied by bilateral cleft palate. Individual 18 was prenatally diagnosed with a CHD composed of pulmonary atresia, ventricular septal defect and aorto-pulmonal collaterals while her mother had a TOF and aorto-pulmonal collaterals, as well. Individuals 26-1 and 26-2, were brothers. The CHD from 26-1 was consistent of azygos continuation, double outlet right ventricle, heterotaxy and suspected aortic isthmus stenosis. Individual 26-2 showed signs

of a double outlet right ventricle, malposition of the great arteries, ventricular septal defect and suspected pulmonary stenosis. Individual 6 was classified as a sporadic case, despite the unknown identity of the mother (due to egg donation); the father had no history of CHDs (Table 3).

Definitive molecular diagnosis was established in 20% of prenatally diagnosed CHDs

In 70% (21/30) of cases, genetic analysis such as karyotyping, array analysis and single gene sequencing had already been performed before ES (Supplementary table 4). All results were unsuspicious. ES established a definite genetic diagnosis in 20% (6/30) of cases (Table 3).

In two cases, *de novo* variants were identified as disease causing (2/30, 6.7%). In individual 8, "Noonan syndrome 1" (MIM: #163950) was diagnosed by identifying a *de novo* missense variant in *PTPN11* (step I finding). The variant has already been reported as pathogenic in the ClinVar database (Variation ID: 177754). Individual 24 was diagnosed with "Kabuki syndrome 1" (MIM: #147920). The variant in *KMT2D* (step I finding) has not been reported yet, but was classified as pathogenic due to its predicted loss-of-function effect and its *de novo* status (Table 3).

In four cases, parentally inherited disease causing variants were identified. In individual 10, compound-heterozygous missense variants, of which one is a novel mutation, were identified in *DNAI1* (step II finding), which is associated with "Ciliary dyskinesia, primary, 1, with or without situs inversus" (MIM: #244400) (18). Since the fetus showed signs of heterotaxy matching the associated phenotype, the variants were reported as causative. In individual 17, an approximately 165 kb spanning microdeletion 9q34.4 (ca. chr9:139,255,000-139,420,000) was identified. The deletion affected exon 3 to 34 of *NOTCH1* (step II finding). *NOTCH1* is known to be associated with CHDs and "Adams-Oliver syndrome 5" (MIM: #616028) and the deletion was therefore reported as pathogenic. The variant could also be identified in the unaffected mother who, however, objected cardiologic

examination. In the likewise affected individual 18 and his mother, a heterozygous missense variant in *TBX1* (step I finding) was identified. *TBX1* is, amongst others, associated with "Tetralogy of Fallot" (MIM: #187500) and the variant has already been reported as pathogenic in an individual (19). Genetic testing for individual 18 was the only that was done prenatally in this study. Subsequently, the mother of individual 18 decided to complete pregnancy, with the reason that she considered her the quality of life restrictions of her own disease not sufficiently severe. The child was born alive. In individual 19, who was affected by heterotaxy, compound-heterozygous causal variants in *MMP21* (step I finding) were identified (Table 3) leading to the diagnosis of "Heterotaxy, visceral, 7, autosomal" (MIM: #616749).

Off note, 16 out of 30 individuals of our study group had isolated CHDs and 14 had additional extracardiac findings on prenatal examination that defined these cases as syndromic. Two cases (Individuals 24 and 27) were regarded as non-syndromic in context of prenatal findings, but were reclassified based on postnatal examination. A causative variant could be identified in four cases with syndromic CHDs whereas a genetic diagnosis was established in two of the isolated cases. Consequently, the diagnostic yield reached 28.6% in the subgroup of syndromic and 12.5% in isolated CHDs. This difference, however is not significant (p=0.27, Chi-Square test). Lager study groups are needed to further analyze the diagnostic yield in CHD subgroups.

Inconclusive findings were reported in 13% of individuals with CHDs

Inconclusive findings were reported in 13.4% (4/30) of cases (Table 3). In two cases, variants of unknown significance (VUS) were reported, offering a possible explanation for the cardiac phenotype without a definitive diagnosis. In individual 9, two missense variants in *ZNF423* (step II finding) were identified. *ZNF423* is associated with "Joubert syndrome 19" with four reported families published to

date (MIM: #614844), but the variants in the fetal DNA had not been reported yet. A homozygous missense variant in *MYH6* (step II finding), identified in individual 23, was also classified as VUS. Individual 23 had a hypoplastic left heart syndrome (HLHS). Homozygous and compound-heterozygous variants in *MYH6* have been implicated with HLHS, but a causal association is yet to be established (20).

In two cases with inconclusive findings, novel candidate genes that might explain the cardiac phenotype were reported in context of research. In individual 6, a missense variant, reported in patients with hypertrophic cardiomyopathy (HCM) (21), was identified in compound-heterozygosity with a microdeletion 14q11.2 spanning both *MYH6* and *MYH7* (step II finding) partially (ca. chr14:23869377-23897879). Functional follow-up studies concerning that finding are still ongoing. In individual 21, a heterozygous loss of function variant in *PUM1* (step III) was identified. Since the variant was *de novo*, and *PUM1* has a probability of loss function intolerance of 1.00 in the Exome Aggregation Consortium dataset and might play a role in embryogenesis (22), *PUM1* was reported and explained to the parents as a potential novel CHD gene even though *PUM1* knockout mice show no signs of heart defects (23). After the genetic report was created missense variants in *PUM1* were associated with “Spinocerebellar ataxia 47” (MIM #617931) (24).

All pathogenic as well as inconclusive findings in known disease causing genes could have been identified independently from the used gene list (step I findings) by phenotype based variant prioritization in OMIM listed genes. However, it helped to prioritize and classify variants as the specificity of testing was considered higher if a variant was found within the candidate gene list.

Incidental findings were reported in 10% of individuals with CHDs

In 10% (3/30) of cases, incidental findings were reported (Table 3). In individual 4 who was included in the study with aortic valve stenosis and a hypoplastic aortic arch as well as the unaffected father, we identified a heterozygous splice variant in *PKP2*. The variant has already been associated with arrhythmogenic right ventricular cardiomyopathy (ARVC) (25) and was considered as an incidental finding and not directly associated with the CHD due to the phenotypic discrepancy. The already mentioned missense variant in *MYH7* identified in individual 6 was paternally inherited. The variant was described in the literature in patients with HCM (21). Both fathers, of individual 4 and 6, were referred to a cardiologic specialized center. Cardiac MRI in individual 4's father did not reveal any pathological findings. The results of the examinations of individual 6's father are unknown.

In individual 19, a maternally inherited missense variant in *COL4A5* was reported as incidental finding. *COL4A5* is associated with X-chromosomal inherited Alport syndrome and the variant has already been classified as pathogenic in the ClinVar database (Variation ID: 24455). The pregnancy was terminated because of the CHD. The mother, however, was referred to a nephrological specialized center, since female variant carriers have an increased risk of developing renal insufficiency with progressing age (26, 27) and possibly benefit from treatment with angiotensin converting enzyme (ACE) inhibitors (28). However, to date no hematuria or proteinuria were detected in the mother of individual 19.

Prenatal-postnatal phenotypic discrepancy did not influence the diagnostic outcome

Genetic diagnostics highly depend on the phenotypic description, especially in genome-wide analyses. We therefore analyzed the differences between the prenatal sonographic findings and postnatal phenotype as evaluated by imaging or post-mortem autopsy with focus on extracardiac

abnormalities which influence the interpretation of variants more than the cardiac phenotype. Extracardiac symptoms could be compared in 20 cases of which twelve (60%) had a correct prenatal diagnosis (Supplementary table 5). In two out of eight cases, where there was a discrepancy between the prenatal and postnatal findings, the findings could not be identified by prenatal ultrasound such as developmental delay or iris coloboma. In the remaining six cases, the differences were minimal and could have developed after ultrasound examination (e.g. minimal hydronephrosis) or are difficult to detect in prenatal ultrasound examination (e.g. esophageal atresia, polyspenia/asplenia, double left kidney). In all cases, we performed an analysis of the genetic data based on the prenatal and the postnatal phenotype and we observed no differences in the diagnoses as well as the classification of variants.

DISCUSSION

Since next generation sequencing (NGS) becomes more available these days and CHDs belong to the most frequent inborn anomalies, we aimed to illuminate the clinical utility of exome sequencing (ES) in cases with prenatally diagnosed CHDs.

The overall diagnostic yield was 20% in this study which is higher than previous published data and the expected rate of single gene defects of 12% in CHDs (6), even when considering the fact that in most of the cases (70%, 21/30) chromosomal abnormalities had previously been excluded. However, the comparably high diagnostic yield in prenatally diagnosed CHDs by ES will be biased by the inclusion criteria as we focused on severe and syndromic CHDs (46.7% of cases were prenatally considered syndromic CHDs and at least 55% postnatally) increasing the probability of a monogenetic cause. Even if the diagnostic yield did not differ significantly (p=0.27) between prenatally syndromic (28.6%) and isolated CHDs (12.5%) the tendency indicates that a large proportion of isolated CHDs cannot be explained by ES. Two cases were classified as non-syndromic based on the prenatal findings but turned out to be syndromic postnatally, indicating that not all syndromic CHDs will correctly be classified as such by prenatal examination. These discrepancies complicate the selection of cases for prenatal ES. All of the established diagnoses are associated with postnatal phenotypes that cannot be determined prenatally such as mental retardation or hematological complications in Noonan syndrome, or symptoms that might have been missed in the prenatal ultrasound before, like other organ involvement in heterotaxy. Therefore, the genetic diagnoses established by ES are of high significance when it comes to the decision process of terminating a pregnancy due to a detected CHD. But regardless of the decision to terminate a pregnancy, the diagnoses are also important for prenatal counseling since counseling regarding developmental outcomes and extra-cardiac findings is useful for families who are opposed to termination in terms of screening and planning. For example,

the family of individual 18 decided to pursue pregnancy after having noted that the mother was affected by the genetic diagnosis as her child.

In 13% (4/30) of cases, genetic findings were inconclusive, because variants were either classified as VUS or variants were identified in putative novel disease genes. These kind of findings represent a difficult situation in counselling. On the one hand, these findings can lead to an uncertainty due to an elevated recurrence risk without practical consequences for the parents at the moment of diagnosis. On the other hand, it is important to be aware of such VUS since a reanalysis of the exome data after a certain period can help to clarify their pathogenicity. The same applies to ES with no findings, especially in cases with a high *a priori* chance of a genetic caused CHD, like in familial or syndromic cases.

A critically discussed side effect of ES are incidental findings, i.e. the identification of pathogenic variants in genes not associated with the observed phenotype. Most laboratories follow the guidelines and recommendation for reporting incidental findings as suggested by the ACMG where those variants are reported back to the family, which are considered actionable (15). The rate of incidental findings in ES is estimated 1% to 4.5% (29-31). In our study, variants that were considered actionable were identified in 10% (3/30) of cases. This discrepancy might be due to the relative small study group. However, it might also represent an enrichment of risk variants in a polygenic context as two of three variants affect genes associated with heart conditions. Such an enrichment has already been shown in exome sequencing analyses of patients with CHDs e.g. for variants in *MYH6* (32). It should be noted that one of the reported genes, *COL4A5*, was reported due to its possible therapeutic consequences, although it is not listed in the recommendation by the ACMG.

We also aimed to evaluate if the prenatal phenotype was precise enough for the interpretation of genetic data. We limited this analysis to the extracardiac abnormalities as first, the cardiac phenotypic spectrum of the disorders that we identified is broad and therefore mostly unspecific for genetic testing. Second, some of the discrepancies are open to interpretation and third, some cannot be detected prenatally such as patent foramen ovale. An extracardiac phenotypic discrepancy between the reported prenatal and postnatal findings was found in overall 40% (8/20) of cases. None of these discrepancies led to differences in the evaluation of the genetic data in respect to genetic diagnoses or the interpretation of the pathogenicity of identified variants. Even though the prenatally determined phenotype was sufficient for the interpretation of genetic data in the study group, it is too small to derive a general rule. Additionally, the fact that ES was mostly performed postnatally, restricts the generalization of our findings.

In the present study, we aimed to perform exome sequencing as parent-child trios in all cases. A trio analysis helps to identify those variants, which are of *de novo* origin and therefore constitute variants with a higher probability of being pathogenic. It also serves to prove compound-heterozygosity for those cases where two variants are identified in one gene. In four out of six solved cases (individuals 8, 10, 18 and 19) parental testing was necessary to classify the variants as clinically relevant which indicates that prenatal exome sequencing needs to be performed as parent-child trios to make a fast and precise diagnosis. In the only case in our study that was sequenced prenatally, the time from DNA quality check to the finished exome report took 20 days. This time span is within the range of recently published data concerning prenatally performed ES (33) and should be sufficient for the prenatal use of ES.

In our study, trio ES in cases with prenatally diagnosed CHDs results in a high diagnostic yield that is consistent with or higher than the genetic diagnostic yield in CHDs in general, limited by the small

study group. There was a notable fraction of reported incidental and inconclusive findings. All patients or parents, respectively, should be counselled accordingly before giving consent to ES. The report of incidental finding should be discussed in each case individually and is difficult to standardize. Despite the limitations with only 30 cases included, our data serves as a first basis to evaluate the value of prenatal ES in case of CHD for further studies emerging in the near future.

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Table 1: Statistical summary of exome sequencing approaches and pregnancy demographics

Whole exome sequencing (WES)	
- Single	10% (3/30)[1]
- Trio	83.3% (25/30)
- Quattro	6.7% (2/30)[2]
Median maternal age at pregnancy in years	32 (range 17 to 44)
Median gestational week at CHD diagnosis	20 (range 12 to 36)
Terminated pregnancies	56.7% (17/30)

WES was performed from January 2016 to August 2018
[1] One of the single exome was performed in an also affected father of the patient
[2] The two patients were affected brothers

Table 2 Outcome and interventions in livebirths

Total number of livebirths	13
Median gestational week at birth	39 (range 32 to 42)
Median APGAR score	8/8/9 (each range 1 to 10)
Median birth length percentile	45th (range <1st to 84th)
Median birth weight percentile	19th (range 3rd to 97th)
Medical intervention within first year of live	12/12[1]

[1] one child died shortly after birth

Table 3: Overview of clinical and genetic findings in all 30 individuals included in the study

Individual	CHD	Prenatally detected extracardiac anomalies	Affected 1st-degree relative	Finding	Disease	Classification	Incidental Finding	Disease
1	AVS	-	-	-	-	-	-	-
2	ISTA, PLSVC	bilateral cleft palate	father with same phenotype	-	-	-	-	-
3†	HLHS	choroid plexus cyst, growth retardation, increased nuchal transparency	-	-	-	-	-	-
4‡	AVS, HAA	-	-	-	-	-	*PKP2* (NM_004572.3): c.2146-1G>C in father and index	Arrhythmogenic right ventricular dysplasia 9 MIM: #609040
5†	HA, susp. PLSVC	agenesis of the corpus callosum, diminished gyration, hypoplastic vermis, ventriculomegaly	-	-	-	-	-	-
6†	HPV, MGA, TA, VSD	singular umbilical artery	-	*MYH7* (NM_000257.2): c.1727A>G, p.His576Arg microdeletion 14q11.2 (approx. chr14:23869377-23897879, partially including *MYH6*/*MYH7*) compound-heterozygous	-	research	*MYH7* (NM_000257.2): c.1727A>G, p.His576Arg in father and index	Cardiomyopathy, hypertrophic, 1 MIM: #192600
7†	susp. EA, PA, TD	-	-	-	-	-	-	-
8†	susp. AVSD	hydrops, lateral neck cyst, ductus venosus agenesis, susp. cleft palate, susp. micrognathia, posterior fossa	-	*PTPN11* (NM_002834.3): c.214G>A, p.Ala72Thr heterozygous, *de novo*	Noonan syndrome 1 MIM: #163950	solved	-	-
9†	susp. DORV, PLSVC, VSD	agenesia of the right kidney, cerebellar hypoplasia, hydrocephalus, macrocephaly, partial rhombencephalosynapsis	-	ZNF423 (NM_015069.3): c.2852C>T, p.Thr951Met, c.2108C>T, p.Ser703Leu compound-heterozygous	Joubert syndrome 19 MIM: #614844	VUS	-	-
10†	DORV, PAPVC	susp. polysplenia	-	*DNAI1*	Ciliary dyskinesia,	solved	-	-

				(NM_012144.3): c.1003G>T, p.Val335Phe, c.1543G>A, p.Gly515Ser compound-heterozygous	primary, 1, with or without situs inversus MIM: #244400			
11†	DIRV, ISTA, TGA	-	-	-	-	-	-	-
12	HA, small LV, PLSVC, VSD	growth retardation, singular umbilical artery	-	-	-	-	-	-
13†	PA, VSD	growth retardation	-	-	-	-	-	-
14†	HLHS	-	-	-	-	-	-	-
15†	HLHS	hydrops	-	-	-	-	-	-
16‡	HLHS, possible NCCM	susp. aplasia of right lung	-	-	-	-	-	-
17	susp. SYPCA, left SVC, PA, VSD	cystic hygroma colli, one-sided club foot, retrognathia	-	microdeletion 9q34.3 (approx. chr9:139252466-139418430, including *NOTCH1*) heterozygous, inherited by unaffected mother	*NOTCH1*: Adams-Oliver syndrome 5 MIM: #616028	solved	-	-
18	PA, SYPCA, VSD	-	mother with TOF, SYPCA	*TBX1* (NM_005992.1): c.385G>A, p.Glu129Lys heterozygous, inherited by affected mother	Tetralogy of Fallot MIM: #187500	solved	-	-
19†	PA, UH, VSD	hygroma coli, right-sided stomach	-	*MMP21* (NM_147191.1) c.1372C>T, p.Arg458*, c.281G>C, p.Arg94Pro compound-heterozygous	Heterotaxy, visceral, 7, autosomal MIM: #616749	solved	*COL4A5* (NM_000495.4): c.1871G>A, p.Gly624Asp, in mother and index	Alport syndrome MIM: #301050
20†	EFE, HLHS	-	-	-	-	-	-	-
21†	AA, HRV, MGA	-	-	*PUM1* (NM_014676.2) c.1738C>T, p.Arg580* heterozygous, *de novo*	-	(research)	-	-
22†	HLHS	-	-	-	-	-	-	-
23	HLHS, susp. Shone's Complex	-	-	*MYH6* (NM_002471.) c.831G>T,	-	VUS	-	-

24‡	HLHS	-	-	p.Gln277His, homozygous *KMT2D* (NM_003482.3) c.11093dup, p.(Phe3699Leufs*14) heterozygous, *de novo*	Kabuki syndrome 1 MIM #147920	solved	-	-
25†	PA, SYPCA, VSD	agenesis of the corpus callosum, cerebellar hypoplasia	-	-	-	-	-	-
26-1	AC, DORV, heterotaxia, susp. ISTA, VSD	-	brother of 26-2	-	-	-	-	-
26-2	DORV, susp. PS, VSD	-	brother of 26-1	-	-	-	-	-
27	HAA, VSD	-	-	-	-	-	-	-
28†	AVS, EFE, HAA	-	-	-	-	-	-	-
29	HAA, ISTA, VSD	Polyhydramnios, low-set ears, singular umbilical artery	-	-	-	-	-	-

† Fetus in pregnancies that were terminated

‡ Children that died after birth

AA, aortic atresia; AC, azygos continuation; AVS, aortic valve stenosis; AVSD, atrioventricular septal defect; DIRV, double inlet right ventricle; DORV, double outlet right ventricle; EA, Ebstein's Anomaly; EFE, endocardial fibroelastosis; HA, hypoplastic aorta; HAA, hypoplastic aortic arch; HLHS, hypoplastic left heart syndrome; HPV, hypoplastic pulmonary valve; HRV, hypoplastic right ventricle; ISTA, aortic isthmus stenosis; LV, left ventricle; MGA, malposition of the great arteries; NCCM, non-compaction cardiomyopathy; PA, pulmonary atresia; PAPVC, Partial anomalous pulmonary venous connection, PLSVC, persistent left superior vena cava; PS, pulmonary stenosis; susp., suspected; SVC, superior vena cava; SYPCA, systemic-pulmonary collateral arteries; TA, tricuspid atresia; TD, tricuspid dysplasia; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; UH, univentricular heart; VSD, ventricular septal defect; VUS, variant of unknown significance