Appendix 1: Classification of the study collective – Details of prenatal ultrasound and laboratory findings

Group and details of anomalies	No	Group and details of anomalies	No
Isolated	**70**	**Lymphatic**	**11**
Chromosomal	**456**	Cystic hygroma colli	7
Trisomy 21	173	Chylothorax	3
Turner	127	Lymphatic dysplasia	1
Trisomy 18	106	**Thoracic**	**8**
Trisomy 13	21	Diaphragmatic hernia	3
Unbalanced structural anomaly	12	CCAML	3
Triploid	9	Ectopia cordis	1
Trisomy 14	2	CHAOS	1
Tetraploid	2	**Urogenital**	**7**
Trisomy 22	1	Potter	1
Tetrasomy 12p	1	Obstructive Uropathy	1
Trisomy 23	1	Infravesical obstruction	1
Trisomy 16	1	Renal agenesis	1
Cardiovascular	**64**	Renal vein thrombosis	1
Cardiac arrhythmia	12	Renal dysfunction	1
Other heart defect	8	LUTO	1
Valvular heart disease	7	**Feto-maternal unit**	**3**
Hypoplastic left heart	6	Placental hematoma	1
Cardiomyopathy	5	Villous maturation disorder	1
VSD	5	Umbilical hernia	1
Arteriovenous fistula	3	**Gastrointestinal**	**3**
AVSD	3	Intrahepatic venous anomaly	1
Fallot	2	Omphalocele	1
Ebstein-Anomalia	2	Lesions of liver parenchyma	1
Coarctation of the aorta	2	**Metabolic**	**2**
Univentricular heart defect	2	Thanatophoric dwarfism	1
Interrupted aortic arch	2	Mucopolysaccharidosis	1
Hypoplastic right heart	2	**Extrathoracic tumor**	**2**
Double outlet right ventricle	1	Teratoma	2
ASD	1	**Hematologic**	**2**
Agenesis of ductus venosus	1	Non-immune erythroblastosis	1
Infection	**32**	Unclear anemia	1
Parvovirus	25	**Miscellaneous**	**20**
CMV	5	Unspecific syndromic	8
Toxoplasmosis	1	Asphyxiated skeletal dysplasia	2
Coxsackie	1	Cerebral malformation	2
Syndromes	**20**	Anencephaly-Exencephaly	1
Pena shokeir	4	Exencephaly	1
Pterygium	3	Iniencephaly	1
Arthrogryposis congenita multiplex	2	Cleft lip and palate	1
Cantrell	2	Ventral cleavage	1
Dandy Walker	2	Pachygyria	1
Noonan	2	Gastroschisis	1
Right sided atrial isomerism	1	Rachischisis	1
			Total 700

ASD: atrial septal defect; AVSD: atrioventricular septal defect; CCAML: congenital cystic adenomatoid malformation; CHAOS: congenital high airway obstruction syndrome; CMV: Cytomegalovirus; LUTO: lower urinary tract obstruction; VSD: ventricular septal defect4

Appendix 2: Results from prenatal and postnatal molecular biology

Case	Group	Prenatal classification from ultrasound and laboratory findings	Karyotype	Techniques of molecular biology	Suspected disease	Genloci/a-cGH Results	Conspicuous findings
1[a]	**Isolated NIHF**	Isolated NIHF	46,XY	a-CGH (prenatal)	Chromosomal microdeletions and microduplications	No abnormal a-CGH Results	No
2	Non-isolated NIHF	Unbalanced structural anomaly	46,XXadd(4)(p15.2)	a-CGH (prenatal)	Chromosomal microdeletions and microduplications	1. Deletion 4p16.3 to 4p16.1 (size 8.8Mb, containing 94 genes). 2. Duplication 4p16.1 to 4p15.31 (size 10 Mb, containing 29 genes) 3. Duplication 4q25 to 28.3 (size 24.7 Mb, 90 genes) 4. Duplication 4q28.3 to q35.2 (size 57.6 Mb, 184 genes)	**Yes**
3	Non-isolated NIHF	Cardiac arrhythmia	46,XY	Direct DNA sequencing (prenatal)	Noonan-Syndrome. Genes: PtPn11, SOS1, RAF1 and KRAS	PTPN11: exons 1-15. SOS1 exons: 3,4,6,7,10,11,13,14,15. RAF1: exons 7, 14, 17. Kras: 2,3,4,5,6.	No
4	Non-isolated NIHF	Hygroma colli	46,XX	Direct DNA sequencing (prenatal)	Noonan-Syndrome. Genes: PtPn11, SOS1, RAF1 and KRAS	PTPN11: exons 1-15. SOS1 exons: 3,4,6,7,10,11,13,14,15. RAF1: exons 7, 14, 17. Kras: 2,3,4,5,6.	No
5	Non-isolated NIHF	CMV Infection	46, XY	DNA sequencing with multiplex PCR and oligo-ligation (prenatal)	Cystic fibrosis. Gen: CTFR.	31 mutations	No
6	Non-isolated NIHF	Ventricular septal defect	46, XY	DNA sequencing with multiplex PCR and oligo-ligation (prenatal)	Noonan-Syndrome. Gen: PtPn11	PTPN11: exons 3,8,13	No
7	Non-isolated NIHF	Ebstein-Anomalia	46,XX	MLPA (Multiplex ligation-dependent probe amplification) (prenatal)	1. Beckwith-Wiedemann-Syndrome 2. Noonan-Syndrome	Quantitative analysis: Exclusion of deletions or duplications on 11p15; Microsatellite analysis: Exclusion of uniparental disomy 11 (No CDKN1C sequencing); Methylation analysis: KvDMR1 und H19-DMR	No
8	Non-isolated NIHF	Hypoplastic left heart	46,XX	Molecular cytogenetic FisH (prenatal)	DiGeorge-Syndrome. Gen: 22q11.2	D22S553, D22S609, D22S942	No
9	Non-isolated NIHF	Tetralogy of Fallot	46,XX	Molecular cytogenetic FisH (prenatal)	DiGeorge-Syndrome. Gen: 22q11.2	D22S553, D22S609, D22S942	No
10	Non-isolated NIHF	Complex structural heart defect	46,XY	Molecular cytogenetic FisH (prenatal)	1. DiGeorge 22q11 2. Noonan-Syndrome 10p14	22q11: D22S553, D22S609, D22S942.	No
11[b]	**Isolated NIHF**	Isolated NIHF	46,XX	a-CGH **(postnatal)**	Unspecified syndrome	1. Duplication 4q31 2. Heterozygous deletion 6p12 3. Insertion 19q13 (size 350 kb)	**Yes**
12[c]	**Isolated NIHF**	Isolated NIHF	46,XY	a-CGH **(postnatal)**	1. Hellkamp-Syndrome 2. Noonan-Syndrome 3. Metabolic disorder	Duplication 4p15.33 (size 472-495 kb)	**Yes**

aCGH: array comparative genomic hybridization; NIHF: non-immune hydrops fetalis

[a] Case 12 in the individual case survey on anomalies in the long-term development of iNIHF (Table 3)

[b] Case 2 in the individual case survey on anomalies in the long-term development of iNIHF (Table 3)

[c] Case 8 in the individual case survey on anomalies in the long-term development of iNIHF (Table 3)