**Table S1** All documented diagnostic (Class-IV or -V) variants in studies reporting on the incremental yield of exome sequencing over chromosomal microarray analysis or karyotyping in fetuses with prenatally detected non-immune hydrops fetalis

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ID | Ultrasound phenotype (in addition to NIHF) | Variant  | Zygosity | Monoallelic (M) or Biallelic (B) | Clinical syndrome | Ref |
| SR001 | Polyhydramnios | EPHB4 c.2354G >A dn | Het | M | Lymphatic malformation 7 | 19 |
| SR017 | Pulmonary artery stenosis, cardiomegaly | MYH7 c.2135G > A dn | Het | M | Cardiomyopathy, dilated, 1S | 19 |
| SR018 | Micrognathia, talipes, polyhydramnios | ACTA1 c.110 T > G dn | Het | M | Nemaline myopathy 3 | 19 |
| SR022 | Isolated NIHF | FOXC2 p.E111X  | Het | M | Lymphedema-distichiasis | 20 |
| SR037 | AVSD, lateral neck cyst, ductusvenosus agenesis, cleftpalate, micrognathia,posterior fossa anomaly | PTPN11 c.214G>A p.Ala72Thr dn | Het  | M | Noonan Syndrome 1 | 24 |
| SR040 | Flexion of elbows, talipes, stomach not seen | RYR1 c.C328T; p.H110Y and c.T14927C; p.L4976P  | Comp Het | B | Neuromuscular disease, congenital, with uniform type 1 fiber | 18 |
| SR041 | Multiple joint contractures, pulmonary hypoplasia | RYR1 c.9221C>T p.(Ser3074Phe) and c.14130-2A>G p.?  | Comp Het | B | Neuromuscular disease, congenital, with uniform type 1 fiber | 23 |
| SR042 | ACC, hypertrophic cardiomyopathy, pulmonary hypoplasia, VM | MRPS22 c.878+1G>T p.? and c.509G>A p.(Arg170His)  | Comp Het | B | Combined oxidative phosphorylation deficiency 5 | 23 |
| SR043 | Arthrogryposis  | RYR1 c.14344G>A p.(Gly4782Arg) and c.12013-2A>G p.?  | Comp Het | B | Neuromuscular disease, congenital, with uniform type 1 fiber | 23 |
| SR045 | Isolated NIHF | PIEZO1 c.307C>T p.Arg103Ter and c.7129+1G>C | Comp Het  | B | Lymphatic malformation 6 | 22 |
| SR047 | Isolated NIHF | PIEZO1 p.E679X  | Hom | B | Lymphatic malformation 6 | 25 |
| SR050 | Isolated NIHF | HRAS p.G13D | Het | M | Costello syndrome | 25 |
| SR051 | Contractures, echogenic kidney,placentomegaly | FOXP3 p.R337X | Hemi | M | Immunodysregulation, polyendocrinopathy, and enteropathy, X-linked | 25 |
| SR052 | CNS malformations, cardiomyopathy | MRPS22 c.768\_769del andp.R170H dn | Comp Het | B | Combined oxidative phosphorylation deficiency 5 | 25 |
| SR053 | Cardiomegaly | CYP11A1 p.R120X  | Hom | B | Adrenal insufficiency, congenital, with 46XY sex reversal, partial or complete | 25 |
| SR054 | CNS malformation, cardiac defect | RIT1 p.F82C dn | Het | M | Noonan syndrome 8 | 25 |
| SR078 | Isolated NIHF |  RIT1 c.[268A>G];[268A=] dn | Het | M | Noonan syndrome 8 | 26 |
| SR080 | CDH, polyhydramnios | ANKRD11 c.6504del p.Ala2170fs dn | Het | M | KBG syndrome | 27 |
| SR081 | Polyhydramnios, absent DV, macrosomia, brachycephaly. persisting right umbilical vein.  | SOS1 c.508A>G p.(Lys170Glu) dn | Het | M | Noonan syndrome 4 | 27 |
| SR086 | N/S | PIEZO1 c.3206G>A and c.6208A>C  | Comp Het | B | Lymphatic malformation 6 | 29 |
| SR094 | Skeletal anomalies, echogenic kidneysand VSD | WDR19 c.275T>G (p.L92X) and c.880G>A (p.G294R)  | Comp Het | B | Short-rib thoracicdysplasia type 5 with or without polydactyly | 30 |
| SR096\* | Arthrogryposis | CHRND c.459dup p.Val154SerfsTer24 and c.1010\_1011del p.His337LeufsTer60 | Comp Het | B | Multiple pterygium syndrome, lethal type | 8 |
| SR098\* | Micrognathia, kyphoscoliosis, COA | NRAS c.34G>C p.Gly12Arg dn | Het | m | Noonan syndrome 6 | 8 |
| SR106† | Isolated NIHF | KMT2D c.14341G>T p.Glu4781Ter dn | Het | M | Kabuki syndrome 1 | 8 |
| SR114† | Isolated NIHF | BRAF c.1782T>Gp.Asp594Glu dn | Het | M | Noonan syndrome 7 | 8 |
| SR118\* | Isolated NIHF | KMT2D c.3536del p.Gly1179AlafsTer33 dn | Het  | M | Kabuki syndrome 1 | 8 |
| SR120† | Joint contractures | RYR1 c.2045G>A p.Arg682Gln and c.8342\_8343del p.Ile2781ArgfsTer49 | Comp Het | B | Neuromuscular disease, congenital, with uniform type 1 fiber | 8 |
| SR121† | Stomach not visible, fixed flexion deformities | RYR1 420bp microdeletion | Hom | B | Neuromuscular disease, congenital, with uniform type 1 fiber | 8 |
| SR129 | Isolated NIHF | RASA p.Asp816Leufs mat | Het | M | Capillary malformation-arteriovenous malformation 1 | 16 |
| SR131 | Arthrogryposis, micrognathia, bilateral VM | SCN2A p.Ile874Phe (mosaic) dn | Het | M | Developmental and epileptic encephalopathy 11 | 16 |
| SR132 | Isolated NIHF | SOS1 p.Thr378Ala pat | Het | M | Noonan syndrome 4 | 16 |
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| SR134 |

 | Polyhydramnios | PTPN11 p.Glu139Asp mat | Het | M | Noonan syndrome 1 | 16 |
| SR135 | Polyhydramnios | PIEZO1 p.Arg1070Cys | Hom | B | Lymphatic malformation 6 | 16 |
| SR136 | Bilateral talipes & clenched hands | KLHL40 c.1608-1G>A | Hom | B | Nemaline myopathy 8, autosomal recessive | 16 |
| SR137 | VM, large echogenic lungs | FGFR2 p.Lys660Asn dn | Het | M | FGFR2 related craniosynostosis | 16 |
| SR141 | Abnormal facial profile, placentomegaly | PIEZO1 p.Arg189\*  | Hom | B | Lymphatic malformation 6 | 16 |
| SR144 | Polyhydramnios | PTPN11 p.Glu139Asp dn | Het | M | Noonan syndrome 1 | 16 |
| SR147 | N/S | ITGA9 p.G404S pat | Het | M |  | 31 |
| SR148 | N/S | ITGA9 p.G404S pat | Het | M |  | 31 |
| SR149 | N/S | ITGA9 p.G404S mat | Het | M |  | 31 |
| SR150 | N/S | ITGA9 p.G404S dn | Het | M |  | 31 |
| SR151 | N/S | ITGA9 p.G404S mat + dn | Hom | M |  | 31 |
| SR152 | N/S | PTPN11 p.D61A dn | Het | M | Noonan syndrome 1 | 31 |
| SR153 | N/S | PTPN11 p.Y62D dn | Het | M | Noonan syndrome 1 | 31 |
| SR154 | N/S | VEGFR3 p.L1044P dn | Het | M | Lymphatic malformation 1 | 31 |
| SR174 | Isolated NIHF | PTPN11 c.227A>T p.Glu76Val dn | Het | M | Noonan Syndrome 1 | 32  |
| SR180 | Bilateral echogenic kidneys | RAF1 c.770C4T p.Ser257Leu dn | Het | M | Noonan syndrome 5 | 33 |
| SR181 | Hydronephrosis, polyhydramnios, talipes | RAF1 c.775T4C p.Ser259Pro dn | Het | M | Noonan syndrome 5 | 33 |
| SR182 | Isolated NIHF | PTPN11 c.1381G4A p.Ala461Thr dn | Het | M | Noonan syndrome 1 | 33 |
| SR183 | Isolated NIHF | PTPN11 c.227A4T p.Glu76Val dn | Het | M | Noonan syndrome 1 | 33 |
| SR195 | Small stomach bubble | GBA c.1448T>C p.Leu483Pro  | Hom | B | Gaucher disease, type II | 17 |
| SR196 | Talipes | GUSB c.1192C>T Arg398Cys | Hom | B | Mucopolysaccharidosis VII | 17 |
| SR197 | Isolated NIHF | GUSB c.104C>A p.Ser35\* and c.1091C>T p.Pro364Leu.  | Comp het | B | Mucopolysaccharidosis VII | 17 |
| SR198 | Talipes | GUSB c.1610T>C p.Ile537Thr and c.323C>T p.Pro108Leu | Comp het | B | Mucopolysaccharidosis VII | 17 |
| SR199 | Talipes | GBE1 c.1229T>G p.Ile410Arg and c.773C>, p.Ala258Val | Comp het | B | Glycogen storage disease IV | 17  |
| SR200 | Narrow thorax, clenched hands | RAPSN c.1119\_1121del p.Lys373del | Hom | B | Myasthenic syndrome, congenital, 11, associated with acetylcholine receptor deficiency | 17  |
| SR201 | Polyhydramnios | PIEZO1 c.5366\_5367dupAG,p.Leu1790Serfs\*132 and c.7049+1G>C | Comp het | B | Lymphatic malformation 6 | 17 |
| SR202 | Isolated NIHF | FOXC2 c.361C>T p.Arg121Cys dn | Het | M | Lymphedema-distichiasis syndrome | 17 |
| SR203 | COA, ectopic kidney | LZTR1 c.1A>G p.Met1? and c.27dupG p.Gln10Alafs\*24  | Comp het | B | Noonan syndrome  | 17  |
| SR204 | Isolated NIHF | FOXP3 c.1120\_1122del p.Phe374del  | Hemi | M | Immunodysregulation, polyendocrinopathy, and enteropathy, X-linked | 17  |
| SR223 | COA | PTPN11 c.214G>C p.Ala72Pro dn | Het | M | Noonan syndrome 1 | 2 |
| SR224 | VM, polyhydramnios | PTPN11c.922A>Gp.Asn308Asp dn | Het | M | Noonan syndrome 1 | 2 |
| SR225 | VM, COA, biventricular hypertrophy, absent DV, hepatomegaly, pyelectasis, polyhydramnios | PTPN113 c.854T>Cp.Phe285Ser dn | Het | M | Noonan syndrome 1 | 2 |
| SR226 | Short long bones, placentomegaly | PTPN11 c.854T>Cp.Phe285Ser dn  | Het | M | Noonan syndrome 1 | 2 |
| SR227 | VSD, LSVC, SUA, polyhydramnios | KRAS c.220A>C p.Thr74Pro dn | Het | M | Noonan syndrome 3 | 2 |
| SR228 | Polyhydramnios | RIT1 c.246T>G p.Phe82Leu dn | Het | M | Noonan syndrome 8 | 2 |
| SR229 | Placentomegaly | SHOC2 c.519G>A p.Met173Ile dn | Het | M | Noonan syndrome-like with loose anagen hair 1 | 2 |
| SR230 | Intraabdominal calcification | HRAS c.37G>Cp.Gly13Arg dn | Het | M | Costello syndrome | 2 |
| SR231 | Absent CSP, cardiomegaly, hepatomegaly, clubbed feet, placentomegaly | BRAF c.1741A>C p.Asn581His dn | Het | M | Noonan syndrome 7 | 2 |
| SR232 | Biventricularhypertrophy,placentomegaly | NPC1 c.3182T>C p.Ile1061Thr and c.2072C>Ap.Pro691Gln  | Comp het | B | Niemann-Pick disease, type C1 | 2 |
| SR233 | VSD, pelvic kidney, SUA | GLB1 c.931G> p.Gly311Arg and c.75+1delG | Comp het | B | GM1-gangliosidosis | 2 |
| SR234 | Unilateral MCDK, oligohydramnios | GUSB c.35T>C p.Leu12Pro and C.210+1G>A | Comp het | B | Mucopolysaccharidosis VII | 2 |
| SR235 | Oligohydramnios | GUSB homozygous exon 9 deletions (chr7:65,435,095-65,435,534) | Hom | B | Mucopolysaccharidosis VII | 2 |
| SR236 | Absent CSP, absent stomach, short long bones, scoliosis, abnormal vertebrae, clubbed hands and feet | MYH3 c.2114T>C p.Ile705Thr dn | Het | M | Contractures, pterygia, and spondylocarpostarsal fusion syndrome 1A | 2 |
| SR237 | Clenched hands, polyhydramnios | KLHL40 c.1516A>Cp.Thr506Pro | Hom | B | Nemaline myopathy 8, autosomal recessive | 2 |
| SR238 | Isolated NIHF | FLT4 c.3121C>T p.Arg1041Trp pat | Het | M | Lymphatic malformation 1 | 2 |
| SR239 | Polyhydramnios | PIEZO1 c.5716\_5738del p.Pro1906Lysfs\*55 and c.6809T>C p.lle2270Thr | Comp het | B | Lymphatic malformation 6 | 2 |
| SR240 | Cardiomegaly, short long bones, small thorax | ACAD9 c.1109delC p.Pro370fs\*13and c.796C>T p.Arg266Trp | Comp het | B | Mitochondrial complex 1 deficiency, nuclear type 20 | 2 |
| SR241 | Cardiomegaly, ventricular hypertrophy and dilatation, dysplastic AV, FGR | NEXN c.646C>T p.Arg216\*and c.1606\_1607del p.Lys536fs | Comp het | B | Cardiomyopathy, dilated 1CC | 2 |
| SR242 | Small bilateral jugular sacs, small aorta, persistent LSVC, heterotaxy, umbilical cyst | MYRF c.789dupC p.Ser264fs dn | Het | M | Cardiac-urogenital syndrome | 2 |
| SR243 | SUA | RPL11 c.314\_315delTT p.Phe105fs\*15 dn | Het | M | Diamond-Blackfan anemia 7 | 2 |
| SR244 | Scalloping of frontal skull, polyhydramnios | PIEZO1 c.2610G>AA p.Met870lle mat (mosaic) | Het | M | Dehydrated hereditary stomatocytosis | 2 |
| SR245 | Hypoplastic nasalbone, brachycephaly,SUA, spherocytes in fetal blood | PIEZO1 c.1792G>A p.Val598Met dn | Het | M | Dehydrated hereditary stomatocytosis | 2 |
| SR246 | Isolated NIHF | STAT3 c.1022C>T p.Thr341IIe dn | Het | M | Hyper-IgE recurrent infection syndrome | 2 |
| SR247 | Absent stomach, clubbed feet, SUA | FOXP3 c.543-2A>G | Hemi | M | Immunodysregulation, polyendocrinopathy, and enteropathy , X-linked  | 2 |
| SR248 | Left atrial isomerism, ventricular noncompaction, arrhythmia, polyhydramnios | DNAH9 c.2984delG p.Arg995fs\*5 | Hom | B | Ciliary dyskinesia, primary, 40 | 2 |
| SR249 | Isolated NIHF | SUZ12 c.1451delG p.Gly484fs dn | Het | M | Imagawa-Matsumoto syndrome | 2 |
| SR302 | Isolated NIHF | RASA1 c.2603+2T>A mat | Het | M | Capillary malformation-arteriovenous malformation 1 | 34 |
| SR303 | Abnormal cerebellum | KIAA1109 c.3296T>A p.(Leu1099\*) and c.12406\_12409delTCAG p.(Ser4136Thrfs\*2)  | Comp het | B | Alkuraya-Kucinskas syndrome | 34 |

ACC, Absence of corpus callosum; AV, aortic valve; AVSD, atrial-ventricular septal defect; CDH, congenital diaphragmatic hernia; CNS, central nervous system; COA, coarctation of the aorta; comp het, compound heterozygous; CSP, cavum septum pellucidum; dn, de novo; DV, ductus venosus; FGR, fetal growth restriction; hemi, hemizygous; het, heterozygous; hom, homozygous; LSVC, left sided superior vena cava; mat, maternal; MCDK, multi-cystic dysplastic kidney; NIHF, nonimmune hydrops fetalis; N/S, not stated; pat, paternal; Ref, reference; SUA, single umbilical artery; VM, ventriculomegaly; VSD, ventricular-septal defect. Inheritance in heterozygous conditions only stated where documented. \*previously reported in PAGE study publication; † unreported in PAGE study publication

**Table S2** All documented variants of uncertain significance (Class III) in studies reporting on the incremental yield of exome sequencing over chromosomal microarray analysis or karyotyping in fetuses with prenatally detected non-immune hydrops fetalis

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ID | Ultrasound phenotype (in addition to NIHF) | Variant  | Class | Zygosity | Monoallelic (M) or Biallelic (B) | Clinical syndrome | Ref |
| SR002 | Polyhydramnios, Absence of stomach bubble | RFWD3 c.879G > T and c.835A > G | III and III | Hom | B | Fanconi anemia, complementation group W | 19 |
| SR019 | Isolated NIHF | LAMB2 c.4304C > Tand c.3339G > T | III and III | Comp Het | B | Pierson syndrome | 19 |
| SR023 | Isolated NIHF | EPHB4 p.V870G | III | Het | M | Capillary malformation-arteriovenous malformation 2 | 20 |
| SR024 | FGR | RYR1 p.P816L and p.2567S | III and III | Comp Het | B | Neuromuscular disease, congenital, with uniform Type 1 fiber | 20 |
| SR025 | Isolated NIHF | CRELD1 p.R329C mat/pat | III  | Het | M | Atrioventricular septal defect, partial, with heterotaxy syndrome | 20 |
| SR026 | SVT | ACTN2 p.T412M mat/pat | III | Het | M | Cardiomyopathy, hypertrophic, 23, with or without LVNC | 20 |
| SR045 | Isolated NIHF | PIEZO1 c.307C>T p.Arg103Ter and c.7129+1G>C | IV and III | Comp Het | B | Lymphatic malformation 6(Defined as causative – see Table S1) | 22 |
| SR077 | Isolated NIHF | FOXP3 c.[1072C>T];[0] | III | Hemi | M | Immunodysregulation, polyendocrinopathy, and enteropathy , X-linked | 26 |
| SR094 | Skeletal anomalies, echogenic kidneysand VSD | WDR19 c.275T>G (p.L92X) and c.880G>A (p.G294R)  | V and III | Comp Het | B | Short-rib thoracicdysplasia type 5 with or without polydactyly(Defined as causative – see Table S1) | 30 |
| SR095 | Isolated NIHF | AHI1 c.3368C>T and c.2798A>G | III and III | Comp Het | B | Joubert syndrome type 3 | 30 |
| SR105† | Hypoplastic aortic arch, abnormal head shape | LRBA c.369G>C p.Met123IIe and c.787C>G p.Leu263Val | III and III | Comp Het | B | Immunodeficiency, common variable, 8, with autoimmunity | 8 |
| SR109† | Isolated NIHF | ATR c.7578\_7585del p.Met2526IlefsTer9 and c.6023G>T p.Arg2008Leu | III and III | Comp Het | B | Seckel syndrome 1 | 8 |
| SR205 | Isolated NIHF | RAPSN comp het | IV and III | Comp Het | B | Myasthenic syndrome, congenital, 11, associated with acetylcholine receptor deficiency | 17 |
| SR206 | Additional anomalies not specified | RAPSN comp het | IV and III | Comp Het | B | Congenital myasthenic syndrome | 17 |
| SR207 | Isolated NIHF | PIEZO1 variant compound het | III and III | Comp Het | B | Lymphatic malformation 6 | 17 |
| SR208 | Isolated NIHF | PIEZO1 variant | III | Het | M | Dehydrated hereditary stomatocytosis with or without psuedohyperkalemia and/or perinatal oedema | 17 |
| SR209 | Isolated NIHF | PROC variant | III | Het | M | Thrombophilia due to protein C deficiency, autosomal dominant | 17 |
| SR292 | Unilateral VM, echogenic bowel, cardiomegaly, placentomegaly | GATA1 p.Pro385Leu | III | Hemi | M | Anemia, X-linked, with/without neutropenia and/or platelet abnormalities | 2 |
| SR294 | Hypertrophic cardiomyopathy, placentomegaly | POU3F3 p.His180Pro | III | Het | M | Developmental delay | 2 |
| SR295 | Echogenic bowel, small stomach bubble, VSD, right atrial dilation, bilateral cardiac ventricular hypertrophy, small bladder, oligohydramnios, placentomegaly, intraperitoneal calcification | KMT2D p.Ala4568Pro  | III | Het | M | Kabuki syndrome 1 | 2 |
| SR296 | Polyhydramnios | FBXW11 p.Arg98\*  | III | Het | M | Neurodevelopmental, jaw, eye, and digital syndrome | 2 |
| SR297 | Enlarged CM, bilateral cataracts, abnormal facial profile, mild VM, FGR | ERCC5 p.Asp730Gly  | III | Hom | B | Cerebrooculofacioskeletal syndrome 3 | 2 |
| SR298 | Short long bones, placentomegaly, thickened cardiac ventricular walls | PIEZO1 p.Arg2336Trp, p.Phe1247Cys | III and III | Comp het | B | Lymphatic malformation 6 | 2 |
| SR299 | Polyhydramnios | FLT4 p.Ser1275Gly | III | Het | M | Lymphatic malformation 1 | 2 |
| SR300 | Hepatomegaly, polyhydramnios, possible aortic coarctation | LZTR1 p.Arg412Cys  | III | Het | M | Noonan Syndrome 10 | 2 |

CM, cisterna magna; comp het, compound heterozygous; FGR, fetal growth restriction; hemi, hemizygous; het, heterozygous; hom, homozygous; NIHF, nonimmune hydrops fetalis; Ref, reference; SVT, supraventricular tachycardia; VM, ventriculomegaly; VSD, ventricular-septal defect †unreported in PAGE study publication