Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Table S1. Demographics, phenotypic features, and pregnancy outcomes by ES results.

Demographic	Cases with diagnostic variants (N=37)	Cases without diagnostic variants (N=90)
Median maternal age (IQR) - years	34 (30-36)	31 (28-35)
Nulliparous – no. (%)	19 (51.4%)	38 (42.2%)
Assisted reproductive technology – no. (%) *	1 (2.7%)	11 (12.2%)
Median GA at diagnosis of NIHF (IQR) - weeks	18.7 (13.3-24.5)	20.6 (14.0-24.6)
Prior pregnancy with NIHF – no. (%)	3 (8.1%)	7 (7.8%)
Biological parents consanguineous – no. (%)	0 (0.0%)	4 (4.4%)
Prenatal phenotype at enrollment †		
Increased NT or cystic hygroma – no. (%)	9 (24.3%)	20 (22.2%)
Isolated increased NT or cystic hygroma – no. (%)	1 (2.7%)	14 (15.6%)
Concurrent structural anomaly – no. (%)	6 (16.2%)	3 (3.3%)
>1 additional abnormal fluid collection – no. (%)	2 (5.4%)	3 (3.3%)
Single abnormal fetal fluid collection – no. (%)	2 (5.4%)	19 (21.1%)
Isolated single abnormal fetal fluid collection – no. (%)	0 (0.0%)	4 (4.4%)
Concurrent structural anomaly – no. (%)	2 (5.4%)	15 (16.7%)
≥2 abnormal fetal fluid collections – no. (%)	26 (70.3%)	51 (56.7%)

Isolated abnormal fetal fluid collections – no. (%)	13 (35.1%)	26 (28.9%)
Concurrent structural anomaly – no. (%)	13 (35.1%)	25 (27.8%)
Fetal sex		
Female – no. (%)	20 (54.1%)	45 (50.0%)
Male – no. (%)	17 (46.0%)	45 (50.0%)
Maternal race		
White – no. (%)	22 (59.5%)	52 (57.8%)
Asian – no. (%)	6 (16.2%)	13 (14.4%)
Mixed – no. (%)	4 (10.8%)	14 (15.6%)
Latina – no. (%)	4 (10.8%)	8 (8.9%)
Black – no. (%)	1 (2.7%)	2 (2.2%)
Unknown – no. (%)	0 (0.0%)	1 (1.1%)
Region of United States		
West – no. (%)	24 (64.9%)	62 (68.9%)
Midwest – no. (%)	4 (10.8%)	9 (10.0%)
South – no. (%)	3 (8.1%)	5 (5.6%)
Northeast – no. (%)	6 (16.2%)	14 (15.6%)
Pregnancy outcome		
Ongoing pregnancy – no. (%)	3 (8.1%)	20 (22.2%)
Live infant – no. (%)	5 (13.5%)	13 (14.4%)

Postnatal death – no. (%)	7 (18.9%)	19 (21.1%)
Stillbirth – no. (%) ‡	4 (10.8%)	11 (12.2%)
Pregnancy termination – no. (%)	15 (40.5%)	20 (22.2%)
Selective reduction of affected twin – no. (%)	0 (0.0%)	3 (3.3%)
Spontaneous loss – no. (%) §	3 (8.1%)	4 (4.4%)
Median GA at delivery (IQR) - weeks ¶	30.9 (29.7-32.0)	32.1 (27.0-34.3)

* 11 pregnancies resulted from in vitro fertilization and 1 from intrauterine insemination.

† Prenatal phenotype at enrollment in study and completion of ES.

‡ Intrauterine fetal demise at ≥20 weeks.

§ Spontaneous pregnancy loss prior to 20 weeks.

¶ Among ongoing pregnancies that did not result in spontaneous loss, termination, or stillbirth.

ES, exome sequencing. GA, gestational age. IQR, interquartile range. NIHF, non-immune hydrops fetalis. NT, nuchal translucency.

Table S2. Full details of diagnostic variants, prenatal phenotypes, and pregnancy outcomes in NIHF cases.

Case	Gene	Genetic disorder	Genomic coordinates	Reference/ Alternate *	Transcript ID	Coding alteration	Protein alteration	Variant type	Zygosity	Novel or reported †	Full prenatal phenotype	Pregnancy outcome
RASopathies												
H025	PTPN11	Noonan syndrome	12:112,888,198	G/C	NM_002834.3	c.214G>C	p.Ala72Pro	Missense	Heterozygous	Reported	CH, pleural effusions, skin edema, suspected aortic coarctation	Stillbirth
H089	PTPN11	Noonan syndrome	12:112,915,523	A/G	NM_002834.3	c.922A>G	p.Asn308Asp	Missense	Heterozygous	Reported	Pleural effusions, ascites, skin edema, mild ventriculomegaly, polyhydramnios	Neonatal death
H086	PTPN11	Noonan syndrome	12:112,915,455	T/C	NM_002834.3	c.854T>C	p.Phe285Ser	Missense	Heterozygous	Reported	Pleural effusions, skin edema, mild ventriculomegaly, suspected aortic coarctation, biventricular hypertrophy, absent DV, hepatomegaly, pelviectasis, polyhydramnios	Neonatal death

H095	PTPN11	Noonan syndrome	12:112,915,455	T/C	NM_002834.3	c.854T>C	p.Phe285Ser	Missense	Heterozygous	Reported	Pleural effusions, ascites, skin edema, short long bones, placentomegaly	Stillbirth
H036	KRAS	Noonan syndrome	12:25,380,238	T/G	NM_033360.2	c.220A>C	p.Thr74Pro	Missense	Heterozygous	Novel	Pleural effusions, ascites, skin edema, VSD, left SVC draining into coronary sinus, SUA, polyhydramnios	Stillbirth
H042	RIT1	Noonan syndrome	1:155,874,285	A/C	NM_006912.5	c.246T>G	p.Phe82Leu	Missense	Heterozygous	Reported	Pleural effusions, ascites, skin edema, polyhydramnios	Neonatal death
H073	SHOC2	Noonan syndrome-like, loose anagen hair	10:112,724,635	G/A	NM_007373.3	c.519G>A	p.Met173Ile	Missense	Heterozygous	Reported	Pleural effusions, ascites, skin edema, placentomegaly	Stillbirth
H008	HRAS	Costello syndrome	11:534,289	C/T	NM_176795.3	c.34G>A	p.Gly12Ser	Missense	Heterozygous	Reported	Diffuse scalp edema, clenched hands, arrhythmia, very large abdomen, absent stomach, severe polyhydramnios	Living infant
H016	HRAS	Costello syndrome	11:534,286	C/G	NM_176795.3	c.37G>C	p.Gly13Arg	Missense	Heterozygous	Novel	CH, pleural effusion, pericardial effusion,	Stillbirth

											ascites, skin edema, intraabdominal calcification	
H119	HRAS	Costello syndrome	11:534,288	C/T	NM_176795.3	c.35G>A	p.Gly12Asp	Missense	Heterozygous	Reported	CH, levorotated heart, small stomach, abnormal posturing of extremities, large abdomen with hepatomegaly, possible demineralization in some bones, polyhydramnios	Pregnancy termination
H020	BRAF	Cardiofacio-cutaneous syndrome	7:140,453,987	T/G	NM_004333.4	c.1741A>C	p.Asn581His	Missense	Heterozygous	Reported	CH, pleural effusion, pericardial effusion, skin edema, absent CSP, cardiomegaly, hepatomegaly, clubbed feet, placentomegaly	Stillbirth
Inborn errors of metabolism												
H005	NPC1	Niemann Pick C disease	18:21,116,700 18:21,124,366	A/G G/T	NM_000271.4 NM_000271.4	c.3182T>C c.2072C>A	p.Ile1061Thr p.Pro691Gln	Missense Missense	Compound heterozygous	Reported Novel	Pericardial effusion, ascites, scalp edema, biventricular	Neonatal death

											hypertrophy, placentomegaly	
H019	GLB1	GM1 gangliosidosis	3:33,093,274 3:33,138,501	C/T C/-	NM_000404.2 NM_000404.2	c.931G>A c.75+1delG	p.Gly311Arg ---	Missense Splice site	Compound heterozygous	Reported Novel	Thick NF, ascites, scalp edema, VSD, pelvic kidney, SUA	Pregnancy termination
H054	GUSB	Mucopoly-saccharidosis type VII	7:65,447,136 7:65,446,960	A/G C/T	NM_000181.3 NM_000181.3	c.35T>C c.210+1G>A	p.Leu12Pro ---	Missense Splice site	Compound heterozygous	Novel Novel	Thick NF, pleural effusions, pericardial effusion, ascites, skin edema, unilateral MCDK, oligohydramnios	Stillbirth
H068	GUSB	Mucopoly-saccharidosis type VII	chr7:65,435,095-65,435,534	---	---	Homozygous exon 9 deletions (chr7:65,435,095-65,435,534)	---	Deletions	Homozygous	Novel	Pericardial effusion, ascites, skin edema, oligohydramnios	Living infant
Primarily musculoskeletal disorders												
H026	MYH3	Multiple pterygium syndrome	17:10,544,453	A/G	NM_002470.3	c.2114T>C	p.Ile705Thr	Missense	Heterozygous	Novel	Pleural effusions, skin edema, absent CSP, absent stomach, short long bones, scoliosis, abnormal vertebrae, clubbed hands and feet	Stillbirth
H034	FGFR3	Thanatophoric	4:1,803,564	C/T	NM_001163213.1	c.742C>T	p.Arg248Cys	Missense	Heterozygous	Reported	CH, Dandy Walker malformation, absent	Pregnancy termination

		dysplasia type I									nasal bone, aberrant right subclavian artery, short long bones	
H055	KLHL40	Nemaline myopathy	3:42,730,455	A/C	NM_152393.3	Homozygous c.1516A>C	Homozygous p.Thr506Pro	Missense	Homozygous	Reported	Pleural effusions, pericardial effusion, ascites, skin edema, clenched hands, polyhydramnios	Stillbirth
H085	SF3B4	Nager syndrome (acrofacial dysostosis)	1:149,899,650	A/G	NM_005850.4	c.2T>C	p.Met1?	Start loss	Heterozygous	Novel	CH, micrognathia, mesomelia, abnormal positioning of extremities	Pregnancy termination
Lymphedema disorders												
H044	FOXC2	Lymphe-dema-distichiasis	16:86,601,968	G/T	NM_005251.2	c.1027G>T	p.Glu343*	Nonsense	Heterozygous	Novel	CH, abnormal kidneys	Pregnancy termination
H050	FLT4	Milroy disease	5:180,043,465	G/A	NM_182925.5	c.3121C>T	p.Arg1041Trp	Missense	Heterozygous	Reported	Pleural effusions, ascites, skin edema	Living infant
H072	PIEZO1	Generalized lymphatic dysplasia	16:88,787,086 16:88,783,084	23bp del A/G	NM_001142864.2 NM_001142864.2	c.5716_5738del c.6809T>C	p.Pro1906Lysfs*55 p.Ile2270Thr	Frameshift Missense	Compound heterozygous	Novel Novel	Pleural effusions, skin edema, polyhydramnios	Neonatal death
Primarily neurodevelopmental disorders												

H018	WAC	Desanto-Shinawi syndrome	10:28,903,527	G/-	NM_016628.4	c.1335delG	p.Ser491fs*9	Frameshift	Heterozygous	Novel	CH, two VSDs, severe aortic coarctation	Pregnancy termination
H063	ZEB2	Mowat-Wilson syndrome	2:145,156,671	G/A	NM_014795.3	c.2083C>T	p.Arg695*	Nonsense	Heterozygous	Reported	CH, scalp edema	Stillbirth
H083	DHCR24	Desmo-sterolosis	1:55,319,709 1:55,319,724	C/T G/A	NM_014762.3 NM_014762.3	c.1218+1G>A c.1204C>T	--- p.Gln402*	Splice site Nonsense	Compound heterozygous	Novel Novel	CH, skin edema, abnormal skull shape, abnormal extremity positioning, contractures	Pregnancy termination
Primarily cardiovascular disorders												
H007	ACAD9	Mitochon-drial complex I deficiency	3:128,623,307 3:128,618,292	C/- C/T	NM_014049.5 NM_014049.5	c.1109delC c.796C>T	p.Pro370fs*13 p.Arg266Trp	Frameshift Missense	Compound heterozygous	Novel Novel	Pericardial effusion, ascites, cardiomegaly, short long bones, small thorax	Neonatal death
H058	NEXN	Dilated and hypertro-phic cardiomyo-pathy	1:78,392,255 1:78,407,837	C/T AA/-	NM_144573.3 NM_144573.3	c.646C>T c.1606_1607del	p.Arg216* p.Lys536fs	Nonsense, Frameshift	Heterozygous or compound heterozygous ‡	Novel Novel	Pericardial effusion, ascites, cardiomegaly, dilation and hypertrophy of cardiac ventricles, hypoplastic and dysplastic aortic valve, diminished systolic function, FGR	Stillbirth

H080	MYRF	Cardiac-urogenital syndrome	11:61,539,012	-/C	NM_001127392.1	c.789dupC	p.Ser264fs	Frameshift	Heterozygous	Reported	Pleural effusions, pericardial effusion, ascites, small bilateral jugular sacs, AV canal defect, small aorta, possible persistent left SVC, heterotaxy, umbilical cyst	Pregnancy termination
Primarily hematologic disorders												
H027	RPL11	Diamond Blackfan anemia	1:24,021,197	TT/-	NM_001199802.1	c.314_315delTT	p.Phe105fs*15	Frameshift	Heterozygous	Novel	CH, thick NF, pericardial effusion, skin edema, single UA, elevated MCA Dopplers	Living infant
H094	PIEZO1	Dehydrated hereditary stomato-cytosis	16:88,799,740	C/T	NM_001142864.2	c.2610G>A	p.Met870Ile	Missense	Heterozygous	Reported	Pleural effusions, ascites, skin edema, scalloping of frontal skull, polyhydramnios, normal MCA Dopplers	Living infant
H126	PIEZO1	Dehydrated hereditary stomato-cytosis	16:88,801,339	C/T	NM_001142864.2	c.1792G>A	p.Val598Met	Missense	Heterozygous	Reported	CH, pleural effusions, pericardial effusion, ascites, skin edema, hypoplastic nasal bone, brachycephaly,	Ongoing pregnancy

											SUA, elevated MCA Dopplers, spherocytes in fetal blood	
Primarily immunologic disorders												
H056	STAT3	Hyper-IgE recurrent infection syndrome, multisystem infantile-onset autoimmune disease	17:40,485,718	G/A	NM_213662.1	c.1022C>T	p.Thr341Ile	Missense	Heterozygous	Reported	Pleural effusions, ascites	Pregnancy termination
H113	FOXP3	Immuno-dysregulation, polyendo-crinopathy, and enteropathy, X-linked (IPEX syndrome)	X:49,112,265	C/T	NM_014009.3	c.543-2A>G	---	Splice site acceptor	X-linked	Novel	Pleural effusions, pericardial effusion, ascites, skin edema, absent stomach, clubbed feet, SUA	Pregnancy termination
Primarily renal disorders												

H107	CEP55	Multinucleated neurons, anhydramnios, renal dysplasia, cerebellar hypoplasia, and hydranencephaly	10:95,262,876 10:95,287,888	C/T A/G	NM_001127182.1 NM_001127182.1	c.190C>T c.1373A>G	p.Arg64* p.His458Arg	Nonsense Missense	Compound heterozygous	Novel Novel	CH, skin edema, hydrocephaly, VSD, overriding aorta, kidneys not visualized, arthrogryposis, clubbed feet, FGR, low normal amniotic fluid	Stillbirth
Ciliopathies												
H030	DNAH9	Primary ciliary dyskinesia	17:11,572,741	G/-	NM_001372.3	Homozygous c.2984delG	Homozygous p.Arg995fs*5	Frameshift	Homozygous	Novel	Ascites, skin edema, left atrial isomerism, ventricular non-compaction, AV canal defect, arrhythmia, polyhydramnios	Neonatal death
Overgrowth disorders												
H109	SUZ12	Imagawa-Matsumoto syndrome	17:30,321,594	G/-	NM_015355.2	c.1451delG	p.Gly484fs	Frameshift	Heterozygous	Novel	Thick NF, pleural effusion, skin edema, suspected pulmonary lymphangiectasia	Ongoing pregnancy
Other disorders												
H124	CHD7	CHARGE syndrome	8:61,741,262	TG/-	NM_017780.3	c.3422_3423delTG	p.Val1141fs	Frameshift	Heterozygous	Novel	Increased NT (4.5 mm)	Pregnancy termination

* Reference nucleotide in the genome/alternate nucleotide seen in the proband (fetus or neonate).

† Previously reported is noted if the variant has been documented in ClinVar or the literature in prenatal or postnatal cases.

‡ Two *NEXN* variants were identified, one of maternal inheritance and the other de novo, for which cis versus trans phase could not be definitively determined due to the distance at which these variants were spaced from each other.

AV, atrioventricular. CH, cystic hygroma. CSP, cavum septum pellucidum. DV, ductus venosus. FGR, fetal growth restriction. MCA, middle cerebral artery. MCDK, multicystic dysplastic kidney. NT, nuchal translucency. NF, nuchal fold. NIHF, non-immune hydrops fetalis. NT, nuchal translucency. SUA, single umbilical artery. SVC, superior vena cava. VSD, ventricular septal defect.

Table S3. Variants of uncertain significance in NIHF cases.

Case	Gene	Genetic disorder	Protein alteration	Inheritance
Neurodevelopmental disorders				
H021	POU3F3	Developmental delay	p.His180Pro	Suspected parental mosaicism (AD)
H032	KMT2D	Kabuki syndrome	p.Ala4568Pro	Paternal (AD)
H070	DYNC1H1	Spinal muscular atrophy, lower extremity predominant; neuronal migration defects	p.Arg2091Trp	De novo or paternal (duo, AD)
H075	FBXW11	FBXW11-related neurodevelopmental, digital, jaw, and eye anomalies	p.Arg98*	De novo or paternal (duo, AD)
H099	ERCC5	Cerebrooculofacioskeletal syndrome	p.Asp730Gly	Maternal and paternal (AR)
H106	EP300	Rubenstein-Taybi syndrome	p.Ser111Asn	De novo (AD)
H120	WDFY3	Primary microcephaly	p.Phe2825Cys	De novo (AD)
Lymphedema disorders				
H088	PIEZO1	Generalized lymphatic dysplasia	p.Arg2336Trp p.Phe1247Cys	Maternal and paternal (AR)
H096	FLT4	Milroy disease	p.Ser1275Gly	De novo (AD)
RASopathies				
H118	LZTR1	Noonan syndrome	p.Arg412Cys	Maternal (AD)
Hematologic disorders				
H035	GATA1	Anemia with or without neutropenia and with or without platelet abnormality	p.Pro385Leu	Maternal (AD)
Other disorders				
H009	LRP6	Tooth agenesis, coronary artery disease	p.Tyr373Cys	Maternal (AD)

AD, autosomal dominant. AR, autosomal recessive. NIHF, non-immune hydrops fetalis.