Supplementary Materials

Manual Curation of Genetic Variants at Clinical Sites

Each variant of interest undergoes manual curation at the gene and variant level, although specific processes vary by UDN clinical site. The function of the gene and associated role in human disease, if any, are examined using web sources such as OMIM (https://omim.org/), PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), GeneCards (https://www.genecards.org/), and UniProt (https://www.uniprot.org/). The variant of interest is queried in human disease databases such as ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), HGMD (http://www.hgmd.cf.ac.uk/), OMIM (https://omim.org/), Geno2MP (https://geno2mp.gs.washington.edu/), and DECIPHER (https://decipher.sanger.ac.uk/), and reported associations with disease are investigated to the extent possible. Control databases such as gnomAD (https://gnomad.broadinstitute.org/) and DGV (http://dgv.tcag.ca/dgv) are queried, as well as bioinformatic tools to assess conservation and predicted pathogenicity, depending on the type of variant, such as RVIS (http://genic-intolerance.org/), CADD (https://cadd.gs.washington.edu/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and many others. Further steps include the consideration of alternative transcripts (https://www.ncbi.nlm.nih.gov/refseq/) and differential expression patterns for genes of interest (https://gtexportal.org/). The MARRVEL tool (http://marrvel.org/) is a publicly available resource created by the UDN which allows users to efficiently search multiple databases simultaneously especially for model organisms (other similar tools used include Franklin (https://franklin.genoox.com/) and Varsome (https://varsome.com/)), and all of this information is synthesized by the UDN clinical sites independent of the sequencing core laboratory's interpretation to determine whether the variant is diagnostically relevant.

Figure S1. Types of diagnoses achieved in prior non-diagnostic ES and ES-naïve settings are similar, indicating that an in-depth N-of-1 approach is crucial to resolving diagnoses in all UDN participants. Note that 15 of 90 diagnoses made after prior nondiagnostic ES were with non-NGS approaches, emphasizing the careful formulation of differential diagnoses with complementation/supplementation of the phenotype and then customizing the next steps.

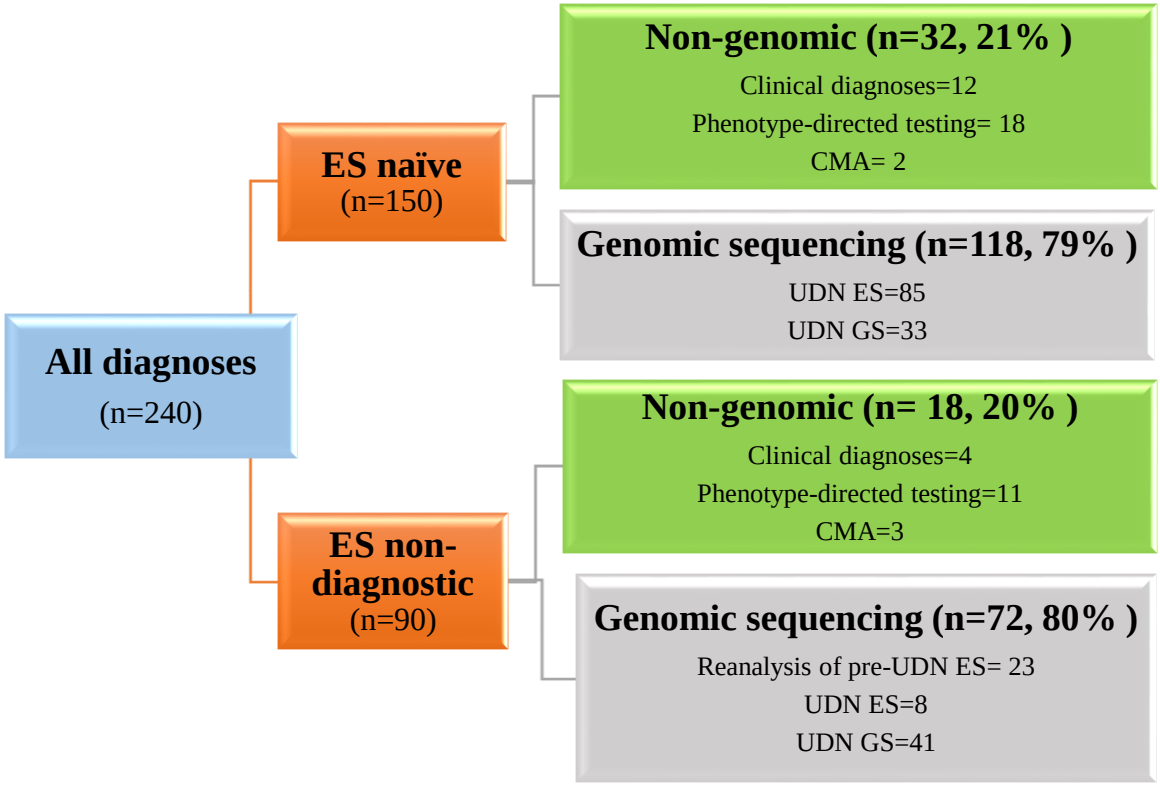

Figure S2. Travel distances to UDN sites compared to the clinical genetics practices at the same medical centers, demonstrating the greater catchment area served by the UDN clinical sites.

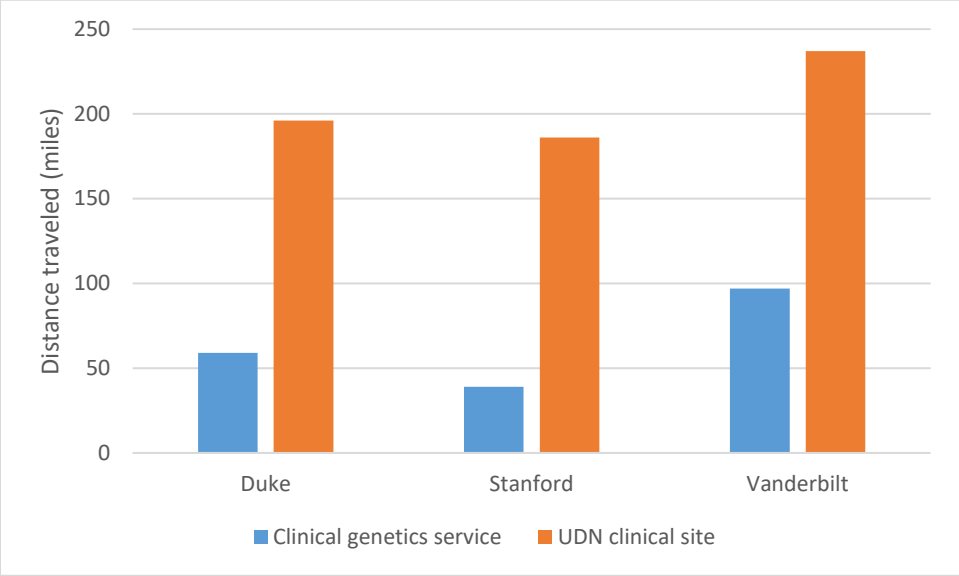

Table S1. Details of the 240 diagnoses made by the four clinical sites.

Diagnosis	Participant age at evaluation (years)	Gene	Gene OMIM #	Was a Pre-UDN ES performed?	Were additional UDN-specific investigation (beyond ES/GS) required in order to achieve diagnosis?
Clinical diagnosis					
Orofaciodigital syndrome, type unknown	6	n/a	n/a	Yes	Complementation/ supplementation of prior clinical data
Lewy body dementia, Parkinson disease	69	n/a	n/a	No	Complementation/ supplementation of prior clinical data
You-Hoover-Fong syndrome	3	n/a	n/a	Yes	Complementation/ supplementation of prior clinical data
Recurrent autoimmune thrombocytopenic purpura	18	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Primary progressive multiple sclerosis	62	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Adiposis dolorosa	31	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Necrotizing myopathy due to anti-HMGCR antibodies	47	n/a	n/a	Yes	Complementation/ supplementation of prior clinical data
Multiple sclerosis	33	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Hughes-Stovin syndrome	26	n/a	n/a	No	Complementation/ supplementation of prior clinical data

Multiple pterygium syndrome, Escobar variant	10	n/a	n/a	Yes	Complementation/ supplementation of prior clinical data
Plastic bronchitis	14	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Ehlers-Danlos syndrome, hypermobility type	52	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Brown-Vialetto-Van Laere syndrome 1	38	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Nontuberculous mycobacterial infection	63	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Multiple sclerosis	28	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Schnitzler syndrome	70	n/a	n/a	No	Complementation/ supplementation of prior clinical data, Collaborative investigations
Directed clinical testing					
IgG4-related condition	49	n/a	n/a	No	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Peroxisomal disorder, NOS	6	*PEX1*	602136	No	Complementation/supplementation of prior clinical data
Autoimmune myopathy with HMGCR and MDA5 antibodies	25	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Congenital prion disease	39	*PRNP*	176640	No	Generation of new genomic data, Complementation/ supplementation of

					prior clinical data
Alpha-1-antitrypsin deficiency	36	*SERPINA1*	107400	Yes	Complementation/ supplementation of prior clinical data
Systemic lupus erythematosus	18	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Multiple sclerosis	53	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Sjogren syndrome	58	n/a	n/a	No	Complementation/ supplementation of prior clinical data
Megalencephalic leukoencephalopathy with subcortical cysts 2B, remitting, with or without mental retardation	6	*HEPACAM*	611642	Yes	Complementation/ supplementation of prior clinical data
Mitochondrial disease	7	n/a	n/a	Yes	Complementation/ supplementation of prior clinical data
Sjogren syndrome	28	n/a	n/a	Yes	Complementation/ supplementation of prior clinical data
Mitochondrial disorder of unknown etiology	4	n/a	n/a	Yes	Complementation/ supplementation of prior clinical data
Single gene testing					
Pseudohypoparathyroidism 1B	46	*GNAS*	139320	No	Generation of new genomic data
Huntington disease	7	*HTT*	613004	Yes	Generation of new genomic data
Dystrophic epidermolysis bullosa	3	*COL7A1*	120120	No	Generation of new genomic data
Infantile-onset	11	*SPTBN2*	604985	No	Generation of new genomic data,

spinocerebellar ataxia 5					collaborative investigations
Temple syndrome	9	n/a	n/a	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Hyaline fibromatosis syndrome	2	ANTXR2	608041	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data
Infantile neuroaxonal dystrophy 1	3	*PLA2G6*	603604	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Action myoclonus-renal failure syndrome	31	*SCARB2*	602257	No	Generation of new genomic data, Complementation/supplementation of prior clinical data
Frontotemporal dementia and/or amyotrophic lateral sclerosis 1	44	*C9orf72*	614260	Yes	Generation of new genomic data
Pseudohypoparathyroidism	7	*GNAS*	139320	No	Generation of new genomic data
Epileptic encephalopathy, early infantile, 38	16	*ARV1*	611647	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Glomerulocystic kidney disease with hyperuricemia and isosthenuria	58	*UMOD*	191845	No	Generation of new genomic data
Poretti-Boltshauser syndrome	22	*LAMA1*	150320	No	Complementation/supplementation of prior clinical data

Mosaic *GNAQ*-related disorder: phakomatosis pigmentovascularis	12	*GNAQ*	600998	No	Generation of new genomic data
Rett syndrome	5	*MECP2*	300005	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Shwachman-Diamond syndrome 2	17	*EFL1*	617538	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Hypercoagulable state secondary to prothrombin mutation	48	*F2*	176930	Yes	Complementation/supplementation of prior clinical data
Chromosomal Microarray					
Chromosome 16p11.2 deletion	27	n/a	n/a	Yes	Generation of new genomic data
Chromosome 1p13.3 deletion	40	n/a	n/a	Yes	Generation of new genomic data
Wieacker-Wolff syndrome	10	*ZC4H2*	300897	Yes	Generation of new genomic data
Developmental delay, intellectual disability, obesity and dysmorphism (DIDOD)	19	*PHIP*	612870	No	Generation of new genomic data
Chromosome 1q21.1 duplication	28	n/a	n/a	No	Generation of new genomic data
Reanalysis of prior data					
Neurodegeneration, childhood-onset, with cerebellar atrophy	1	*AGTPBP1*	606830	Yes	Generation of new genomic data, collaborative investigations
Neurodevelopmental disorder with cataracts,	5	Novel gene (unpublished)	n/a	Yes	Generation of new genomic data, collaborative investigations

hearing loss, microcephaly and dysmorphic features					
Nephronophthisis 1	13	*NPHP1*	607100	Yes	Generation of new genomic data
MAPK8IP3-related neurodevelopmental disorder with or without variable brain abnormalities	4	*MAPK8IP3*	605431	Yes	Generation of new genomic data
Mitochondrial disorder	7	*ATP5F1D*	603150	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
EIF2AK2-related condition	12	*EIF2AK2*	176871	Yes	Generation of new genomic data, collaborative investigations
mTOR-related disorder	3	*MTOR*	601231	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data
CACNA1A-related condition	18	*CACNA1A*	601011	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data
Baraitser-Winter syndrome	15	*ACTG1*	102560	Yes	Complementation/supplementation of prior clinical data, collaborative investigations
Hypotonia, ataxia, and delayed development syndrome	7	*EBF3*	607407	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
ZNF526-related condition	2	*ZNF526*	614387	Yes	Generation of new genomic data, collaborative investigations

HUWE1-related intellectual disability	15	*HUWE1*	300697	Yes	Generation of new genomic data
GP130-deficient hyper-IgE syndrome	8	*IL6ST*	600694	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Neurodevelopmental disorder with epilepsy, cataracts, feeding difficulties, and delayed brain myelination	2	*NACC1*	610672	Yes	Generation of new genomic data, collaborative investigations
Cohen-Gibson syndrome	9	*EED*	605984	Yes	Generation of new genomic data
Galloway-Mowat syndrome	14	*WDR73*	616144	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data
CACNA1C-related condition	7	*CACNA1C*	114205	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data
Glycogen storage disease XV: Polyglucosan Body Myopathy	66	*GYG1*	603942	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Shwachman-Diamond syndrome 2	14	*EFL1*	617538	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Neurodevelopmental disorder with hypotonia, seizures, and absent language	6	*HECW2*	617245	Yes	Complementation/supplementation of prior clinical data, collaborative investigations

Neurodevelopmental disorder with regression, abnormal movements, loss of speech, and seizures (NEDAMSS)	5	*IRF2BPL*	611720	Yes	Generation of new genomic data, Complementation/supplementation of prior clinical data, collaborative investigations
MYBPC1-related disorder	1	*MYBPC1*	160794	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
IRF2BPL-related condition	2	IRF2BPL	611720	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
UDN Exome sequencing					
Pontocerebellar hypoplasia, type 6	12	*RARS2*	611524	No	None
Hypotonia, infantile, with psychomotor retardation and characteristic facies 3	1	*TBCK*	616899	No	None
Spinocerebellar ataxia 28 (SCA28)	66	*AFG3L2*	604581	No	None
Ehlers-Danlos syndrome, classic type, 2	34	*COL5A2*	120190	No	Generation of new genomic data
Basal ganglia calcification, idiopathic, 1	58	*SLC20A2*	158378	No	None
Spastic paraplegia 9A, autosomal dominant	35	*ALDH18A1*	138250	No	None
Severe progeria (atypical)	4	*ZMPSTE24*	606480	No	None
Coffin-Siris syndrome 1	5	*ARID1B*	614556	No	None
Farber lipogranulomatosis	43	*ASAH1*	613468	No	Collaborative investigations

Aicardi-Goutieres syndrome	28	*RNASEH2A*	606034	No	Complementation/supplementation of prior clinical data
Congenital disorder of glycosylation, type IIj	3	*COG4*	606976	No	Generation of new genomic data, collaborative investigations
TRIM8-related epileptic encephalopathy	11	*TRIM8*	606125	No	None
UBAP1 related spastic paraplegia	12	*UBAP1*	609787	No	Collaborative investigations
Cardiofaciocutaneous syndrome	18	*BRAF*	164757	No	None
Mitochondrial DNA depletion syndrome, NOS	62	*POLG*	174763	No	None
Mental retardation, autosomal dominant 31	9	*PURA*	600473	No	Generation of new genomic data, Complementation/supplementation of prior clinical data
Stankiewicz-Isidor syndrome	12	*PSMD12*	604450	No	None
Intellectual developmental disorder with dysmorphic facies and behavioral abnormalities	4	*FBXO11*	607871	Yes	None
Kohlschutter-Tonz syndrome	19	*ROGDI*	614574	No	None
Epilepsy-aphasia syndrome	13	*GRIN2A*	138253	No	None
DYRK1A-related intellectual disability syndrome	3	*DYRK1A*	600855	No	None
Peroxisome biogenesis disorder 14B	38	*PEX11B*	603867	No	None
Osteopathia striata with cranial sclerosis	10	*AMER1*	300647	No	None

Mental retardation, X-linked, with cerebellar hypoplasia and distinctive facial appearance	13	*OPHN1*	300127	No	Generation of new genomic data
Bainbridge-Ropers syndrome	16	*ASXL3*	615115	No	None
Marfan syndrome	18	*FBN1*	134797	No	None
Basal ganglia calcification, idiopathic, 1	58	*SLC20A2*	158378	No	None
Stickler syndrome, type 1	15	*COL2A1*	120140	No	None
Roifman syndrome	4	*RNU4ATAC*	601428	Yes	None
ZNF292-related Neurodevelopmental disorder	4	*ZNF292*	616213	Yes	Collaborative investigations
DSP-related arrhythmogenic cardiomyopathy	33	*DSP*	125647	No	None
Epileptic encephalopathy, early infantile, 33 (*EIEE33*)	11	*EEF1A2*	602959	No	None
Deafness, autosomal recessive 2 (DFNB2)	13	*MYO7A*	276903	Yes	None
Bohring-Opitz syndrome	3	*ASXL1*	612990	No	None
Mitochondrial encephalomyopathy	26	*SLC25A42*	610823	No	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Mental retardation, autosomal dominant, 26	14	*AUTS2*	607270	No	Collaborative investigations
Progressive Myoclonic Epilepsy	29	*KCNC1*	176258	No	None

Polycystic Kidney Disease	64	*PKD1*	601313	No	None
CAPOS syndrome / Fever-induced paroxysmal weakness and encephalopathy (FIPWE)	8	*ATP1A3*	182350	No	None
Mucopolysaccharidosis type IIIB (Sanfilippo B Syndrome)	19	*NAGLU*	609701	No	None
Metabolic encephalomyopathic crises, recurrent, with rhabdomyolysis, cardiac arrhythmias, and neurodegeneration (MECRCN)	4	*TANGO2*	616830	No	None
Helsmoortel-Van der Aa Syndrome	5	*ADNP*	611386	No	None
USP7-Related Condition	8	*USP7*	602519	No	Collaborative investigations
KMT2C-related disorder	18	*KMT2C*	606833	No	None
CHRNA3-related disease	66	*CHRNA3*	118503	No	Complementation/supplementation of prior clinical data
Spastic paraplegia 35	8	*FA2H*	611026	No	None
Mental retardation, autosomal dominant 26 (MRD26)	10	*AUTS2*	607270	No	None
Autoinflammatory syndrome	72	*NLRP12*	609648	No	Complementation/supplementation of prior clinical data
Retinitis pigmentosa 71	10	*IFT172*	607386	No	Generation of new genomic data, collaborative investigations
Kleefstra syndrome	33	*EHMT1*	607001	No	Complementation/supplementation of

					prior clinical data
Basal cell nevus syndrome	12	*PTCH1*	601309	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Noonan syndrome	36	*PTPN11*	176876	No	None
Epileptic encephalopathy, early infantile, 47 (EIEE47)	7	*FGF12*	601513	No	None
Pitt-Hopkins syndrome	10	*TCF4*	602272	No	None
FOXG1 syndrome	5	*FOXG1*	164874	No	None
Marfan syndrome	4	*FBN1*	134797	No	None
Trichorhinophalangeal syndrome	4	*TRPS1*	604386	No	None
Mental retardation, autosomal dominant 18	4	*GATAD2B*	614998	Yes	Generation of new genomic data, collaborative investigations
Hypotonia, infantile, with psychomotor retardation and characteristic facies 3 (IHPRF3)	3	*TBCK*	616899	Yes	None
Aortic valve disease	28	*NOTCH1*	190198	No	Generation of new genomic data, complementation/ supplementation of prior clinical data
Scoliosis	28	*POC5*	617880	No	Complementation/supplementation of prior clinical data
Autism	28	*MSL2*	614802	No	Complementation/supplementation of prior clinical data
Poretti-Boltshauser syndrome	6	*LAMA1*	150320	No	None

Epileptic encephalopathy, early infantile, 36	2	*ALG13*	300776	No	None
Idiopathic basal ganglia calcification (Fahr disease)	17	*SLC20A2*	158378	No	None
SanFilippo Syndrome	41	*HGSNAT*	610453	No	None
Familial cold-induced autoinflammatory syndrome	31	*NLRP3*	606416	No	Complementation/supplementation of prior clinical data
Short stature, onychodysplasia, facial dysmorphism, and hypotrichosis (SOFT syndrome)	7	*POC1A*	614783	No	Collaborative investigations
Hypokalemic periodic paralysis	7	*SCN4A*	603967	No	None
Proximal myopathy & ophthalmoplegia	49	*MYH2*	160740	No	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
TAX1BP3-related arrhythmogenic right ventricular cardiomyopathy	15	*TAX1BP3*	616484	No	Generation of new genomic data, collaborative investigations
Mental retardation, autosomal dominant 13 (MRD13)	8	*DYNC1H1*	600112	No	None
Coffin-Siris Syndrome 8 (CSS8)	11	*SMARCC2*	601734	No	Collaborative investigations
Mandibulofacial dysostosis, Guion-almeida type	4	*EFTUD2*	603892	No	None
Neurodevelopmental disorder with or without anomalies of the brain,	21	*RERE*	605226	No	None

eye, or heart					
GTP cyclohydrolase I deficiency	4	*GCH1*	600225	No	None
Alagille syndrome	32	*JAG1*	601920	No	None
Stormorken syndrome	6	*STIM1*	605921	No	Generation of new genomic data, complementation/ supplementation of prior clinical data
PIGQ-related condition	2	*PIGQ*	605754	No	Complementation/supplementation of prior clinical data, collaborative investigations
SCN2A-related condition	15	*SCN2A*	182390	No	None
Cardiofaciocutaneous syndrome 3	4	*MAP2K1*	176872	No	None
Bethlem myopathy 1	15	*COL6A1*	120220	No	Complementation/supplementation of prior clinical data
ATP1A3-related disorder	7	*ATP1A3*	182350	No	None
TSPEAR-related disorder of tooth and hair follicle morphogenesis	7	*TSPEAR*	612920	No	None
Shashi-Pena syndrome	9	*ASXL2*	612991	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
PIK3CA-related overgrowth spectrum	1	*PIK3CA*	171834	No	None
UDN Genome sequencing					
Idiopathic basal ganglia calcification (Fahr disease)	61	*SLC20A2*	158378	No	None

Galactosialidosis	16	*CTSA*	613111	No	None
KLF7-related syndrome	7	*KLF7*	604865	No	Complementation/supplementation of prior clinical data
Kilquist syndrome	5	*SLC12A2*	600840	Yes	Complementation/supplementation of prior clinical data
UBA5-related disorder(s): spinocerebellar ataxia & epileptic encephalopathy early infantile, 44	4	*UBA5*	610552	Yes	Complementation/supplementation of prior clinical data
ADNP syndrome (Helsmoortel-van der Aa syndrome)	8	*ADNP*	611386	Yes	None
Early infantile epileptic encephalopathy 4	7	*STXBP1*	602926	No	Generation of new genomic data
Mental retardation, autosomal dominant 6, with or without seizures (MRD6)	16	*GRIN2B*	138252	Yes	None
Paroxysmal dyskinesia	4	*KCNMA1*	600150	No	None
Diploid/triploid mosaicism	3	n/a	n/a	No	Generation of new genomic data
Van Maldergem syndrome 2	3	*FAT4*	612411	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Coffin-Lowry syndrome	2	*RPS6KA3*	300075	No	Complementation/supplementation of prior clinical data
Coffin-Siris syndrome 1	2	*ARID1B*	614556	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data
Senior-Loken syndrome 5	32	*IQCB1*	609237	No	None

Epileptic encephalopathy, early infantile, 64	2	*RHOBTB2*	607352	Yes	None
Rett syndrome, congenital variant	18	*FOXG1*	164874	No	None
Charcot-Marie-Tooth disease, axonal, 2s	11	*IGHMBP2*	600502	Yes	Generation of new genomic data, collaborative investigations
Kleefstra syndrome 2	6	*KMT2C*	606833	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data
COL4A1-related disease	55	*COL4A1*	120130	No	None
Myopathy, distal, 5	33	*ADSSL1*	612498	Yes	None
Spastic paraplegia, 76	41	*CAPN1*	114220	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Cornelia de Lange syndrome 5	10	*HDAC8*	300269	Yes	Generation of new genomic data
FAM177A1-related disorder	6	*FAM177A1*	n/a	Yes	Generation of new genomic data, collaborative investigations
NR2F2-associated congenital heart defects	11	*NR2F2*	107773	Yes	None
POLR3-related leukodystrophy	5	*POLR3A*	614258	Yes	None
Leukoencephalopathy, brain calcifications, and cysts	45	*SNORD118*	616663	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Peroxisome biogenesis disorder	11	*PEX6*	601498	Yes	None
Dermatitis, atopic, susceptibility to, 2	4	*FLG*	135940	Yes	None
Rett syndrome	3	*MECP2*	300005	No	None

Complex phenotype with hypopigmentation, developmental delay and organomegaly	2	*CLCN7*	602727	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Telangiectasia, hereditary hemorrhagic, 1 (HHT1)	18	*ENG*	131195	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Hypotonia, infantile, with psychomotor retardation and characteristic facies 3	4	*TBCK*	616899	No	Generation of new genomic data
SYNGAP1-related Intellectual disability and epilepsy	9	*SYNGAP1*	603384	Yes	None
Muscular dystrophy, limb-girdle, 2Y	16	*TOR1AIP1*	614512	No	Complementation/supplementation of prior clinical data
Spastic paraplegia, 7	53	*SPG7*	602783	No	None
Epileptic encephalopathy, early infantile, 2	3	*CDKL5*	300203	Yes	Generation of new genomic data
Microcephaly 17, primary, autosomal recessive (MCPH17)	7	*CIT*	605629	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data
SCN8A-related epilepsy with encephalopathy	11	*SCN8A*	600702	No	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Developmental delay, intellectual disability, obesity, and dysmorphic features	14	*PHIP*	612870	No	None
Ataxia-telangiectasia-like disorder 1 (ATLD1)	31	*MRE11*	600814	Yes	Generation of new genomic data

Intellectual developmental disorder with or without epilepsy or cerebellar ataxia	7	*RORA*	600825	Yes	None
PURA Syndrome	4	*PURA*	600473	Yes	None
Joubert syndrome 30	7	*ARMC9*	617612	Yes	None
NADK2 deficiency	15	*NADK2*	615787	No	None
TBX2-related disorder	8	*TBX2*	600747	Yes	Collaborative investigations
Paragangliomas 1	58	*SDHD*	602690	No	Generation of new genomic data
Titinopathy	10	*TTN*	188840	Yes	Complementation/supplementation of prior clinical data
Epileptic encephalopathy, early infantile, 35	2	*ITPA*	147520	Yes	Generation of new genomic data
Congenital disorder of glycosylation, Ik	4	*ALG1*	605907	Yes	Collaborative investigations
Smith-Magenis syndrome	25	*RAI1*	607642	No	None
KMT2B-related dystonia	18	*KMT2B*	606834	No	Generation of new genomic data
Epilepsy, hearing loss, and mental retardation syndrome	10	*SPATA5*	613940	Yes	Generation of new genomic data
Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 5 (MDDGC5)	26	*FKRP*	606596	Yes	Generation of new genomic data, Collaborative investigations
Muscular dystrophy rigid spine and myopathy, congenital with fiber type disproportion	36	*SELENON*	606210	No	Complementation/supplementation of prior clinical data
Progressive external ophthalmoplegia with	40	*TOP3A*	601243	No	Generation of new genomic data, Complementation/supplementation of

mitochondrial DNA deletions					prior clinical data
Cystinosis	18	*CTNS*	606272	No	Generation of new genomic data
Rett syndrome	7	*MECP2*	300005	Yes	None
Dystonia, childhood-onset, with optic atrophy and basal ganglia abnormalities	5	*MECR*	608205	Yes	Generation of new genomic data, collaborative investigations
Charcot-Marie-Tooth disease, axonal, 2T	31	*MME*	120520	Yes	None
HNRNPA1-related multisystem proteinopathy	32	*HNRNPA1*	164017	Yes	Generation of new genomic data, collaborative investigations
Mental retardation, autosomal dominant 18	9	*GATAD2B*	614998	Yes	None
Au-Kline Syndrome	10	*HNRNPK*	600712	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Retinal dystrophy and obesity	49	*TUB*	601197	No	None
RNH1-associated disorder	22	*RNH1*	173320	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Muscular dystrophy, limb-girdle, autosomal recessive 10	15	*TTN*	188840	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Diagnoses due to clinical site dual analysis of UDN Exome sequencing data					
3-Methylglutaconic aciduria type 8	11	*HTRA2*	606441	No	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative

					investigations
Mental retardation, AD58	24	*SET*	600960	No	Generation of new genomic data
Fanconi anemia, complementation group R (FANCR)	10	*RAD51*	179617	No	Generation of new genomic data
KIDINS220-related condition	2	*KIDINS220*	615759	No	Generation of new genomic data, collaborative investigations
Spastic paraplegia 7	31	*SPG7*	602783	No	Generation of new genomic data
Progressive myoclonic epilepsy 3	12	*KCTD7*	611725	No	Generation of new genomic data, collaborative investigations
PUS7-related syndrome	5	*PUS7*	616261	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations
Diagnoses due to clinical site dual analysis of Genome sequencing data					
KMT2B-related dystonia	9	*KMT2B*	606834	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data
CACNA1A-related disease	2	*CACNA1A*	601011	No	Generation of new genomic data, complementation/supplementation of prior clinical data
AIFM1-related hypomyelination with spondylometaphyseal dysplasia	8	*AIFM1*	300169	Yes	Generation of new genomic data
NBEA-related developmental delay and	6	*NBEA*	604889	Yes	Generation of new genomic data, complementation/supplementation of

generalized epilepsy					prior clinical data, collaborative investigations
Neurodevelopmental disorder with epilepsy, microcephaly, and dysmorphic features	2	Novel gene (unpublished)	n/a	Yes	Generation of new genomic data, collaborative investigations
Early infantile epileptic encephalopathy, 58	22	*NTRK2*	600456	No	Generation of new genomic data
ADGRV1-related myoclonic epilepsy	11	*ADGRV1*	602851	No	Generation of new genomic data
Primary congenital glaucoma 3	31	*TEK*	600221	No	Generation of new genomic data, complementation/supplementation of prior clinical data
Neurodevelopmental disorder with epilepsy and dysmorphic features	16	Novel gene (unpublished)	n/a	Yes	Generation of new genomic data, complementation/supplementation of prior clinical data, collaborative investigations

HMGCR= 3-hydroxy-3-methylglutaryl-coenzyme A reductase; NOS= not otherwise specified

Table S2 Novel disease gene associations (n=17) described by the four UDN clinical sites, achieved by additional phenotyping, innovative genomic analyses, and internal and external collaborations.

Disorder (OMIM #)	Gene	Description	Reference (Pubmed ID)
Shashi-Pena syndrome (617190)	*ASXL2*	IDD, hypotonia, macrocephaly, and dysmorphic features including hypertelorism and glabellar nevus flammeus	27693232
Neurodevelopmental disorder with epilepsy, cataracts, feeding difficulties, and delayed brain myelination (617393)	*NACC1*	Microcephaly, IDD, epilepsy, cataracts, feeding difficulties, cyclic severe irritability, and delayed brain myelination	28132692
Hypotonia, ataxia, and delayed development syndrome (617330)	*EBF3*	Congenital hypotonia, IDD, structural CNS malformations, ataxia, and genitourinary abnormalities	28017372
Mitochondrial complex V (ATP synthase) deficiency (618120)	*ATP5F1D*	Metabolic disorder with episodic lethargy, 3-methylglutaconic aciduria, and hyperammonemia	29478781
Kilquist syndrome[a]	*SLC12A2*	IDD, sensorineural hearing loss, gastrointestinal abnormalities, and absent salivation	30740830
Neurodegeneration, childhood-onset, with cerebellar atrophy (618276)	*CCP1*	Infantile-onset neurodegeneration primarily affecting the cerebellum, spinal motor neurons, and peripheral nerves	30420557
Neurodevelopmental disorder with regression, abnormal movements, loss of speech, and seizures (618276)	*IRF2BPL*	Neurodevelopmental regression following typical early development, ataxia, dystonia, choreoathetosis, and seizures	30057031
Coffin-Siris syndrome 8 (618362)	*SMARCC2*	IDD, hypotonia, feeding difficulties, and behavioral abnormalities	30580808
Intellectual developmental disorder with cardiac defects and dysmorphic facies (618316)	*TMEM94*	IDD, congenital cardiac malformations, dysmorphic features, variable skeletal anomalies	30526868
Vertebral anomalies and variable endocrine and T-cell dysfunction (618223)	*TBX2*	Skeletal malformations, dysmorphic features, congenital heart defect, and variable thymus aplasia/hypoplasia and growth hormone abnormality	29726930
Myopathy, congenital, with tremor (618524)	*MYBPC1*[b]	Infantile hypotonia and tremor, delayed walking, unsteady gait, proximal muscle weakness, and	31264822

		high-frequency tremor of the limbs, with normal cognition	
Neurooculocardiogenitourinary syndrome (618562)	*WDR37*	IDD, epilepsy, ophthalmologic anomalies including colobomas and microphthalmia, dysmorphic features, cerebellar hypoplasia	31327508
Leukoencephalopathy, motor delay, spasticity, and dysarthria syndrome (618878)	*EIF2AK1*	Delayed motor development, white matter abnormalities, dysarthria, and progressive spasticity	32197074
Leukoencephalopathy, developmental delay, and episodic neurologic regression syndrome (618877)	*EIF2AK2*	IDD, white matter abnormalities, ataxia, abnormal tone, variable movement disorder, and neurologic regression with febrile illness or infection	32197074
Saul-Wilson syndrome (618150)	*COG4*	Skeletal dysplasia with speech delay, short stature, dysmorphic features, and cataracts, with normal cognition	30290151
Neurodevelopmental disorder with speech delays, altered behavior, and neurologic anomalies[a]	*USP7*	IDD, autism, seizures, ophthalmologic anomalies, hypogonadism, and white matter changes	30679821
Hypopigmentation, organomegaly, delayed myelination and development (618541)	*CLCN7*	IDD, hypopigmentation, organomegaly, delayed myelination, and biopsy consistent with lysosomal storage disorder	31155284

[a] Not yet been assigned an MIM number.
[b] Represents phenotypic expansion

IDD = intellectual and developmental disability; CNS = central nervous system

Table S3. Articles led by or with contributions from the clinical sites

Artlcle	**Pubmed ID**
Cope H, Spillmann R., Rosenfeld J.A., et al. Missed Diagnoses: Clinically relevant lessons learned through medical mysteries solved by the Undiagnosed Diseases Network. *Mol Genet Genomic Med,* in press.	In press
Schoch K, Tan QK, Stong N, et al. Alternative transcripts in variant interpretation: the potential for missed diagnoses and misdiagnoses [published online ahead of print, 2020 May 5]. *Genet Med*. 2020;10.1038/s41436-020-0781-x.	32366967
Burdick KJ, Cogan JD, Rives LC, et al. Limitations of exome sequencing in detecting rare and undiagnosed diseases. *Am J Med Genet A*. 2020;182(6):1400-1406.	32190976
Mao D, Reuter CM, Ruzhnikov MRZ, et al. De novo *EIF2AK1* and *EIF2AK2* Variants Are Associated with Developmental Delay, Leukoencephalopathy, and Neurologic Decompensation. *Am J Hum Genet*. 2020;106(4):570-583.	32197074
Shieh C, Jones N, Vanle B, et al. *GATAD2B*-associated neurodevelopmental disorder (GAND): clinical and molecular insights into a NuRD-related disorder. *Genet Med*. 2020;22(5):878-888.	31949314
Mirzaa GM, Chong JX, Piton A, et al. De novo and inherited variants in *ZNF292* underlie a neurodevelopmental disorder with features of autism spectrum disorder. *Genet Med*. 2020;22(3):538-546.	31723249
Gu S, Chen CA, Rosenfeld JA, et al. Truncating variants in *UBAP1* associated with childhood-onset nonsyndromic hereditary spastic paraplegia. *Hum Mutat*. 2020;41(3):632-640.	31696996
McConkie-Rosell A, Schoch K, Sullivan J, et al. The genome empowerment scale: An assessment of parental empowerment in families with undiagnosed disease. *Clin Genet*. 2019;96(6):521-531.	31448412

Geng LN, Kohler JN, Levonian P, et al. Genomics in medicine: a novel elective rotation for internal medicine residents. *Postgrad Med J*. 2019;95(1128):569-572.	31439813
Kanca O, Andrews JC, Lee PT, et al. De Novo Variants in *WDR37* Are Associated with Epilepsy, Colobomas, Dysmorphism, Developmental Delay, Intellectual Disability, and Cerebellar Hypoplasia. *Am J Hum Genet*. 2019;105(2):413-424.	31327508
Johnson BV, Kumar R, Oishi S, et al. Partial Loss of *USP9X* Function Leads to a Male Neurodevelopmental and Behavioral Disorder Converging on Transforming Growth Factor β Signaling. *Biol Psychiatry*. 2020;87(2):100-112.	31443933
Bhatia A, Mobley BC, Cogan J, et al. Magnetic Resonance Imaging characteristics in case of *TOR1AIP1* muscular dystrophy. *Clin Imaging*. 2019;58:108-113.	31299614
Frésard L, Smail C, Ferraro NM, et al. Identification of rare-disease genes using blood transcriptome sequencing and large control cohorts. *Nat Med*. 2019;25(6):911-919.	31160820
Nicoli ER, Weston MR, Hackbarth M, et al. Lysosomal Storage and Albinism Due to Effects of a De Novo *CLCN7* Variant on Lysosomal Acidification. *Am J Hum Genet*. 2019;104(6):1127-1138.	31155284
Shashi V, Geist J, Lee Y, et al. Heterozygous variants in *MYBPC1* are associated with an expanded neuromuscular phenotype beyond arthrogryposis. *Hum Mutat*. 2019;40(8):1115-1126.	31264822
Cassini TA, Duncan L, Rives LC, et al. Whole genome sequencing reveals novel *IGHMBP2* variant leading to unique cryptic splice-site and Charcot-Marie-Tooth phenotype with early onset symptoms. *Mol Genet Genomic Med*. 2019;7(6):e00676.	31020813
Newman JH, Shaver A, Sheehan JH, et al. IgG4-related disease: Association with a rare gene variant expressed in cytotoxic T cells. *Mol Genet Genomic Med*. 2019;7(6):e686.	30993913

Zastrow DB, Kohler JN, Bonner D, et al. A toolkit for genetics providers in follow-up of patients with non-diagnostic exome sequencing. *J Genet Couns*. 2019;28(2):213-228.	30964584
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Hom J, Marwaha S, Postolova A, et al. A Patient with Sjogren's Syndrome and Subsequent Diagnosis of Inclusion Body Myositis and Light-Chain Amyloidosis. *J Gen Intern Med*. 2019;34(6):1058-1062.	30887439
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Macnamara EF, Koehler AE, D'Souza P, et al. Kilquist syndrome: A novel syndromic hearing loss disorder caused by homozygous deletion of *SLC12A2*. *Hum Mutat*. 2019;40(5):532-538.	30740830
Grove ME, White S, Fisk DG, et al. Developing a genomics rotation: Practical training around variant interpretation for genetic counseling students. *J Genet Couns*. 2019;28(2):466-476.	30706981
Fountain MD, Oleson DS, Rech ME, et al. Pathogenic variants in *USP7* cause a neurodevelopmental disorder with speech delays, altered behavior, and neurologic anomalies. *Genet Med*. 2019;21(8):1797-1807.	30679821
Machol K, Rousseau J, Ehresmann S, et al. Expanding the Spectrum of BAF-Related Disorders: De Novo Variants in *SMARCC2* Cause a Syndrome with Intellectual Disability and Developmental Delay. *Am J Hum Genet*. 2019;104(1):164-178.	30580808

Stephen J, Maddirevula S, Nampoothiri S, et al. Bi-allelic *TMEM94* Truncating Variants Are Associated with Neurodevelopmental Delay, Congenital Heart Defects, and Distinct Facial Dysmorphism. *Am J Hum Genet*. 2018;103(6):948-967.	30526868
Deisseroth CA, Birgmeier J, Bodle EE, et al. ClinPhen extracts and prioritizes patient phenotypes directly from medical records to expedite genetic disease diagnosis. *Genet Med*. 2019;21(7):1585-1593.	30514889
Shashi V, Magiera MM, Klein D, et al. Loss of tubulin deglutamylase *CCP1* causes infantile-onset neurodegeneration. *EMBO J*. 2018;37(23):e100540.	30420557
Ferreira CR, Xia ZJ, Clément A, et al. A Recurrent De Novo Heterozygous *COG4* Substitution Leads to Saul-Wilson Syndrome, Disrupted Vesicular Trafficking, and Altered Proteoglycan Glycosylation. *Am J Hum Genet*. 2018;103(4):553-567.	30290151
Walley NM, Pena LDM, Hooper SR, et al. Characteristics of undiagnosed diseases network applicants: implications for referring providers. *BMC Health Serv Res*. 2018;18(1):652. Published 2018 Aug 22.	30134969
Marcogliese PC, Shashi V, Spillmann RC, et al. *IRF2BPL* Is Associated with Neurological Phenotypes. *Am J Hum Genet*. 2018;103(2):245-260.	30057031
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