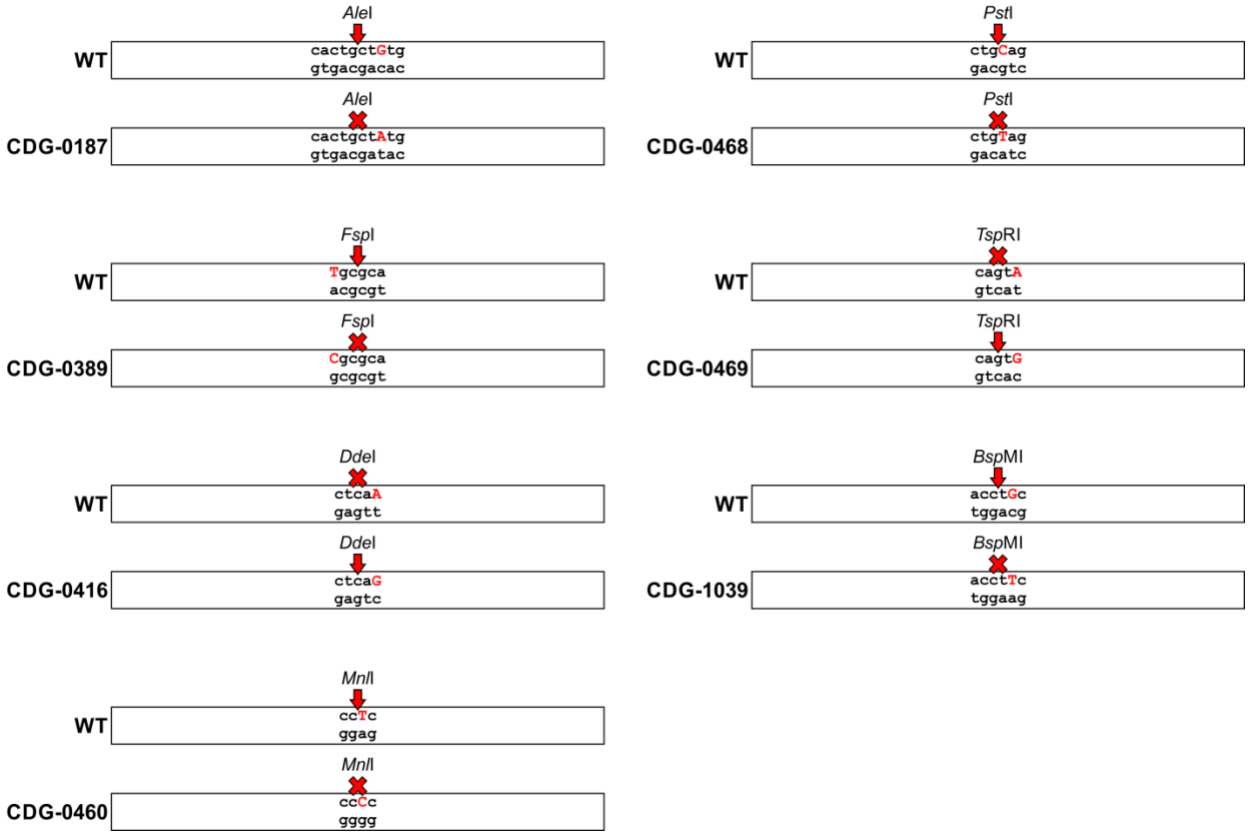
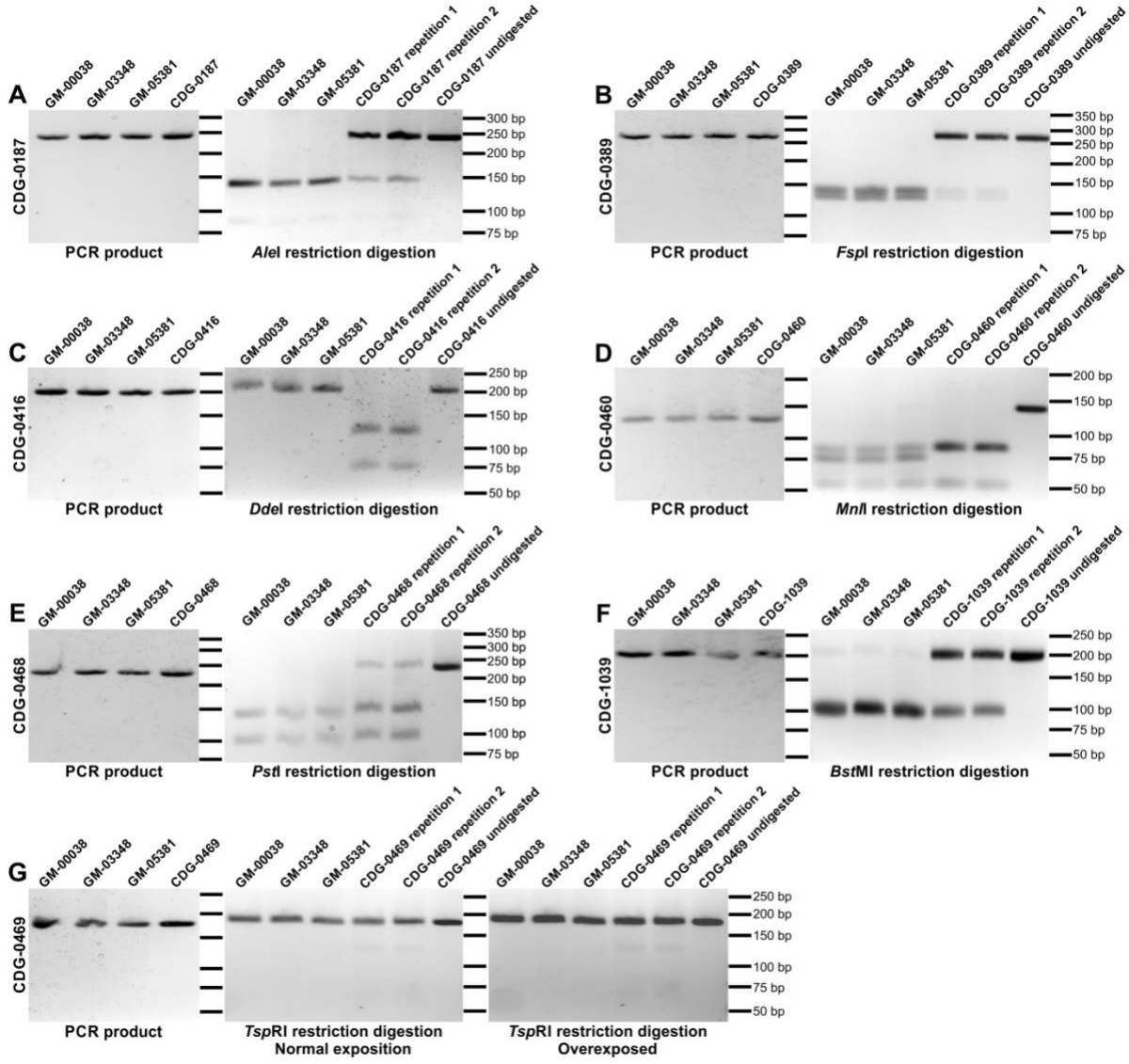
Supporting Figure S1



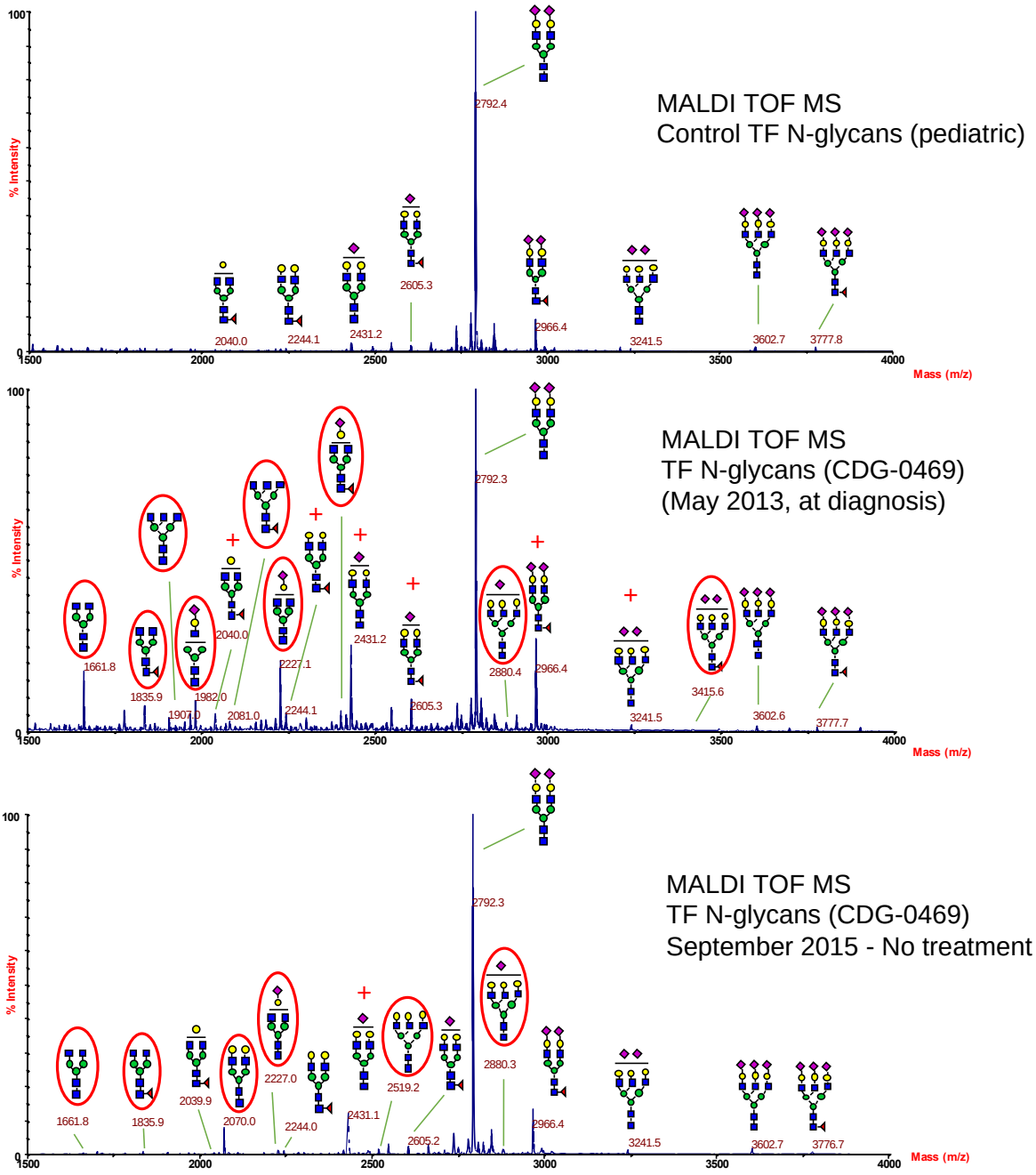
Supp. Figure S1. Strategy of *SLC35A2* cDNA allele ratios assay. Schematic representation of the effect of each variant present in the primary fibroblast cells from each individual on the gain/loss of particular restriction site.

Supporting Figure S2



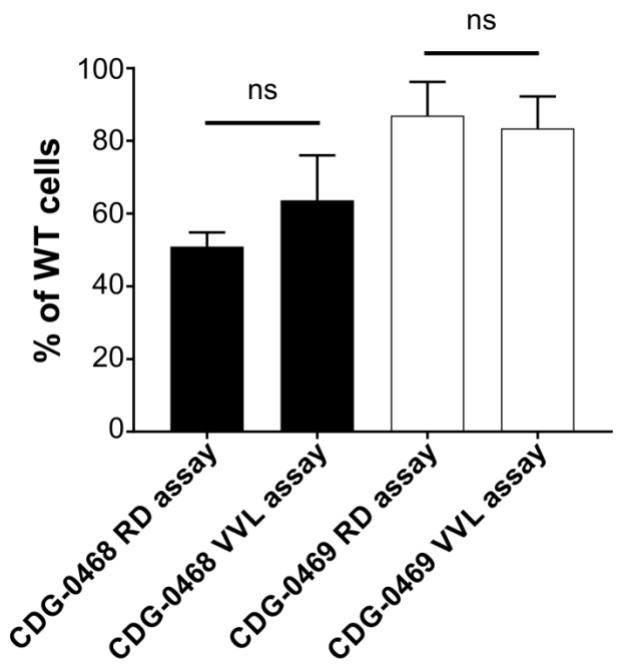
Supp. Figure S2. *SLC35A2* cDNA allele ratios assay summary. Agarose gels were used to quantify the cDNA allele ratio. Each panel represents a different individual.

Supporting Figure S3



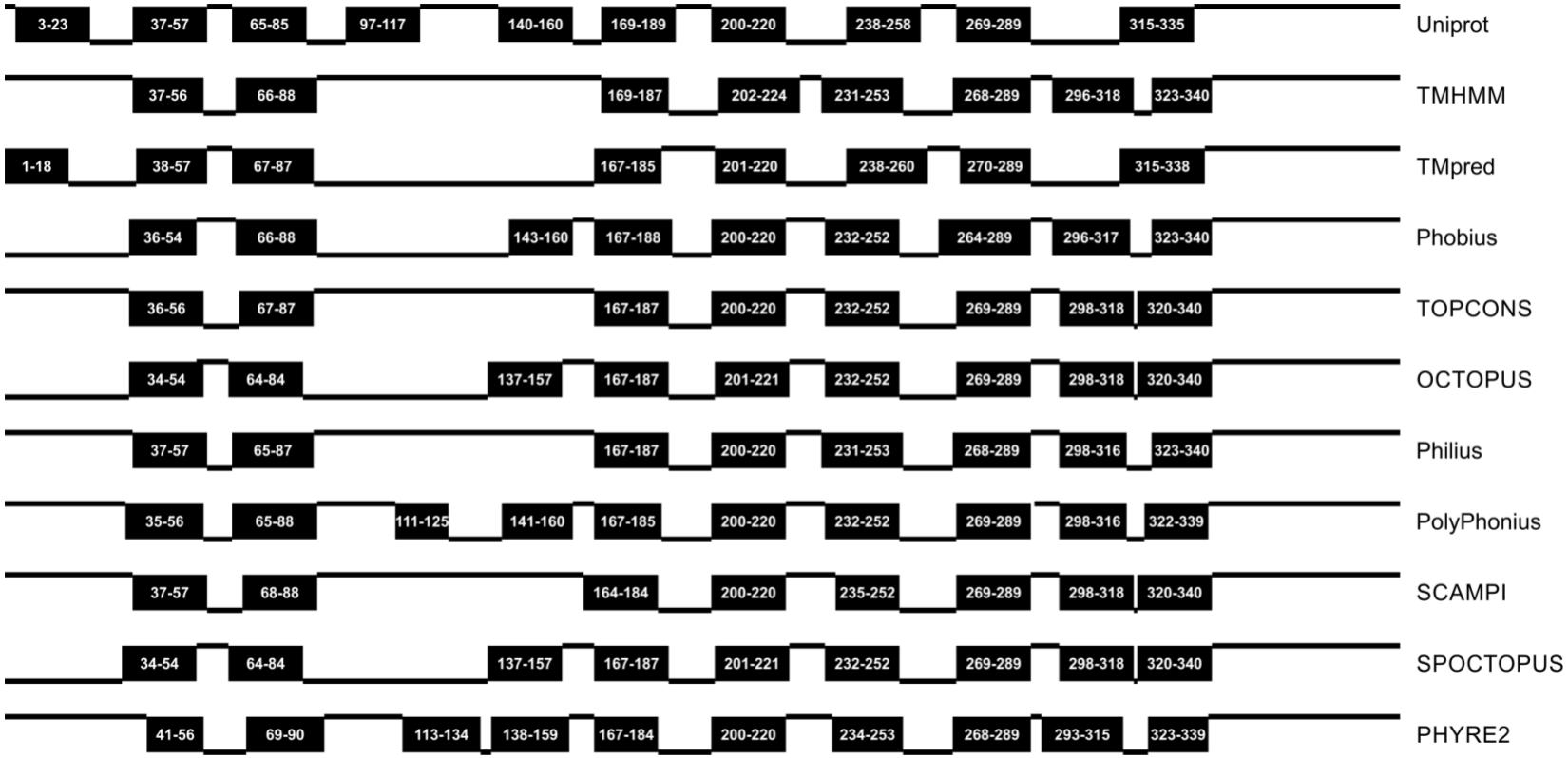
Supp. Figure S3. MALDI MS analysis of N-glycans released from serum Tf from CDG-0469 over a period of 28 months showing improvement of her CDT without treatment.

Supporting Figure S4



Supp. Figure S4. Comparison of wild-type to mutant cDNA allele ratios determined using restriction digestion-based assay (RD assay) with the percentage of VVL positive cells calculated based on the immunofluorescence staining (VVL assay). Data are presented as mean ± SD.

Supporting Figure S5



Supp. Figure S5. *In silico* topology prediction of UDP-galactose transporter using different tools.

Supporting Table S1 – Summary of 32 previously reported SLC35A2-CDG individuals.

Individual	Gender	cDNA position	Protein Position	Variant Status	Detection Method	CDT results	Reference
1	Male	c.15_91+48 delinsA	p.Gly8Serfs*9	Novel	Sanger	Abnormal	Ng et al., 2013
2	Male	c.991G>A	p.Val331Ile	Known	WES	Abnormal	Ng et al., 2013
3	Female	c.3G>A	p.Met1?	Novel	WES	Abnormal	Ng et al., 2013
4	Female	c.433_434del	p.Tyr145Profs∗76	Novel	WES	Normal	Kodera et al., 2013
5	Female	c.972del	p.Phe324Leufs∗25	Novel	WES	Normal	Kodera et al., 2013
6	Female	c.638C>T	p.Ser213Phe	Novel	WES	Normal	Kodera et al., 2013
7	Female	c.502C>T	p.Gln168Ter	Novel	WES	Normal	EuroEPINOMICS-RES Consortium, Epilepsy Phenome/Genome Project & Epi4K Consortium, 2014
8	Female	c.683C>A	p.Ser228Ter	Novel	WES	Normal	EuroEPINOMICS-RES Consortium, Epilepsy Phenome/Genome Project & Epi4K Consortium, 2014
9	Female	c.797G > T	p.Gly266Val	Novel	WES	Abnormal	Dorre et al., 2015
10	Male	c.800A>G	p.Tyr267Cys	Known	WES	Normal	Bosch et al., 2016
11	Female	c.800A>G	p.Tyr267Cys	Known	WES	Normal	Lelieveld et al., 2016
12	NA	c.508G>C	p.Ala170Pro	Novel	WES	Normal	Lelieveld et al., 2016
13	NA	c.616G>A	p.Val206Ile	Novel	WES	Normal	Lelieveld et al., 2016
14	Female	c.950del	p.Gly317Alafs*32	Novel	WES	Normal	Kimizu et al., 2017
15	Female	c.991G>A	p.Val331Ile	Known	WES	Abnormal**	Westenfield et al., 2018
16	Female	c.910T>C	p.Ser304Pro	Novel	WES	NT	Winawer et al., 2018
17	Male	c.339_340insCTC	p.Leu113dup	Novel	WES	NT	Winawer et al., 2018
18	Male	c.634_635del	p.Ser212Leufs*9	Novel	WES	NT	Winawer et al., 2018
19	Male	c.164G>T	p.Arg55Leu	Novel	WES	NT	Winawer et al., 2018

20	Male	c.747_757dup	p.Ala253Glyfs*100	Novel	WES	NT	Winawer et al., 2018
21	Female	c.262G>C	p.Ala88Pro	Novel	Panel	Abnormal	Bruneel et al., 2018
22	Female	c.889A>G	p.Lys297Glu	Novel	WES	Normal	Yates et al., 2018
23	Female	c.327T>G	p.Tyr106Ter	Novel	WES	Normal	Yates et al., 2018
24	Female	c.195C>A	p.Phe65Leu	Novel	WGS	Normal	Yates et al., 2018
25	Female	c.515T>C	p.Leu172Pro	Novel	Panel	Normal	Yates et al., 2018
26	Female	c.923C>T	p.Ser308Phe	Novel	WES	Normal	Yates et al., 2018
27	Male	c.589C>T	p.Gln197*	Novel	WES	NT	Sim et al., 2018
28	Male	c.760G>T	p.Glu254*	Novel	WES	NT	Sim et al., 2018
29	Female	c.703T>G	p.Asn235Gln	Novel	Targeted Amplicon	NT	Sim et al., 2018
30	Male	c.502C>T	p.Gln168*	Recurrent	Targeted Amplicon	NT	Sim et al., 2018
31	Female	c.553C>T	p.Gln185*	Novel	Targeted Amplicon	NT	Sim et al., 2018
32	Male	Acceptor Splice site	p.?	Novel	Targeted Amplicon	NT	Sim et al., 2018

Supp. Table S1 – General information for the 32 previously reported individuals with SLC35A2-CDG. Individual ID, gender, genotypes, CDT results, detection method and reference are provided for the 32 individuals found to carry *de novo* variants within *SLC35A2*. Nucleotide numbering for cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1. *SLC35A2* NCBI Accession (NM_001042498.2) and for ENSEMBL (ENST00000376521.6). All cDNA to protein translations were confirmed using https://mutalyzer.nl/. ** Not consistent with SLC35A2-CDG, NA – Not Available, NT – Not tested

Supporting Table S2 – Expanded clinical summary for 30 unreported SLC35A2-CDG individuals.

Clinical Phenotype	Number of affected / Total Subjects (%)
Facial dysmorphisms	26/30 (87%)
	Microcephaly 13/30 (43%)
	Prominent facial features 21/30 (70%)
	Downslanting palpebral fissures 6/30 (20%)
	Large fontanelle 2/30 (7%)
	High palate 11/30 (37%)
	Other 15/30 (50%)
Neurological	30/30 (100%)
	Intellectual disability 28/29 (97%)
	Seizures / epilepsy 25/30 (83%)
	Hypotonia 28/30 (93%)
	Autistic features 9/30 (30%)
	Behavioral changes 7/30 (23%)
Brain structure	25/30 (83%)
	Cerebellar Atrophy or dysplasia 17/30 (57%)
	Dandy-Walker 2/30 (7%)
	Thinning of corpus callosum 9/30 (30%)
	Cerebellar vermis hypoplasia 4/30 (13%)
	Polymicrogyria 1/30 (3%)
	White matter abnormalities 16/30 (53%)
Ocular	20/30 (67%)
	Cataracts / glaucoma 1/30 (3%)

	Retinitis Pigmentosa 1/30 (3%)
	Strabismus 8/30 (27%)
	Nystagmus 4/30 (13%)
	Optic nerve atrophy 2/30 (7%)
	Cortical visual impairment 13/30 (43%)
Skin	18/30 (60%)
	Ichthyosis / Dermatitis 5/30 (17%)
	Inverted nipples 8/30 (27%)
	Nail abnormalities 3/30 (10%)
	Differential pigmentation 4/30 (13%)
Skeletal	25/30 (83%)
	Shortened limbs 9/30 (30%)
	Contractures 8/30 (27%)
	Dysplasia 3/30 (10%)
	Osteopenia 5/30 (17%)
	Scoliosis 15/30 (50%)
	Hip dislocation 9/30 (30%)
	Arthrogryposis 4/30 (13%)
	Hand or finger abnormalities 16/30 (53%)
	Foot abnormalities 10/30 (33%)
	Hyperextension of joints 9/30 (30%)
Heart	8/30 (27%)
	Structural abnormalities 4/30 (13%)
	Cardiomyopathy 1/30 (3%)
	Arrhythmia 1/30 (3%)
	Bradycardia 2/30 (7%)
	Failure 1/30 (3%)
Respiratory	10/30 (33%)

	Respiratory difficulties 6/30 (20%)
	Apnea 3/30 (10%)
	Recurrent respiratory infections 6/30 (20%)
Liver	12/30 (40%)
	Hepatomegaly 3/30 (10%)
	Hypoglycemia 1/30 (3%)
	Failure (Acute or Chronic)1/30 (3%)
	Elevated ALT or AST 8/30 (27%)
	Hypercholesterolemia 1/30 (3%)
	Triglyceridemia 2/30 (7%)
Gastrointestinal	24/30 (80%)
	G-Tube 20/30 (67%)
	Vomiting 4/30 (13%)
Immunological	10/30 (33%)
	Thrombocytopenia 2/30 (7%)
	Recurrent infections 6/30 (20%)
	Anemia 4/30 (13%)
Other Organ systems	Genital 5/30 (17%)
	Kidney 1/30 (3%)
	Hearing Loss 7/30 (23%) (3 - sensorineural)

Supp. Table S2 – Expanded clinical summary for 30 unreported individuals with *de novo* variants in *SLC35A2*.

Supporting Table S3 – Strategy of restriction digestion-based SLC35A2 cDNA allele ratios assay

	Restriction enzyme	**Gain / Loss of restriction site**	**PCR product length**	**Length of WT PCR product after the digestion**	**Length of mutated PCR product after the digestion**
CDG-0187	*Ale*I	Loss	237bp	145bp + 92bp	237bp
CDG-0389	*Fsp*I	Loss	259bp	135bp + 124bp	259bp
CDG-0416	*Dde*I	Gain	199bp	199bp	75bp + 124bp
CDG-0460	*Mnl*I	Loss	134bp	51bp + 73bp + 10bp	51bp + 83bp
CDG-0468	*Pst*I	Loss	222bp	95bp + 127bp	222bp
CDG-0469	*Tsp*RI	Gain	177bp	177bp	62bp + 115bp
CDG-1039	*Bsp*MI	Loss	199bp	96bp + 103bp	199bp

Supp. Table S3 – Strategy of restriction digestion-based SLC35A2 cDNA allele ratios assay in fibroblasts from indicated SLC35A2 individuals.