Supplementary Online Content

Costain G, Walker S, Marano M, et al. Genome sequencing as a diagnostic test in children with unexplained medical complexity. *JAMA Netw Open.* 2020;3(9):e2018109. doi:10.1001/jamanetworkopen.2020.18109

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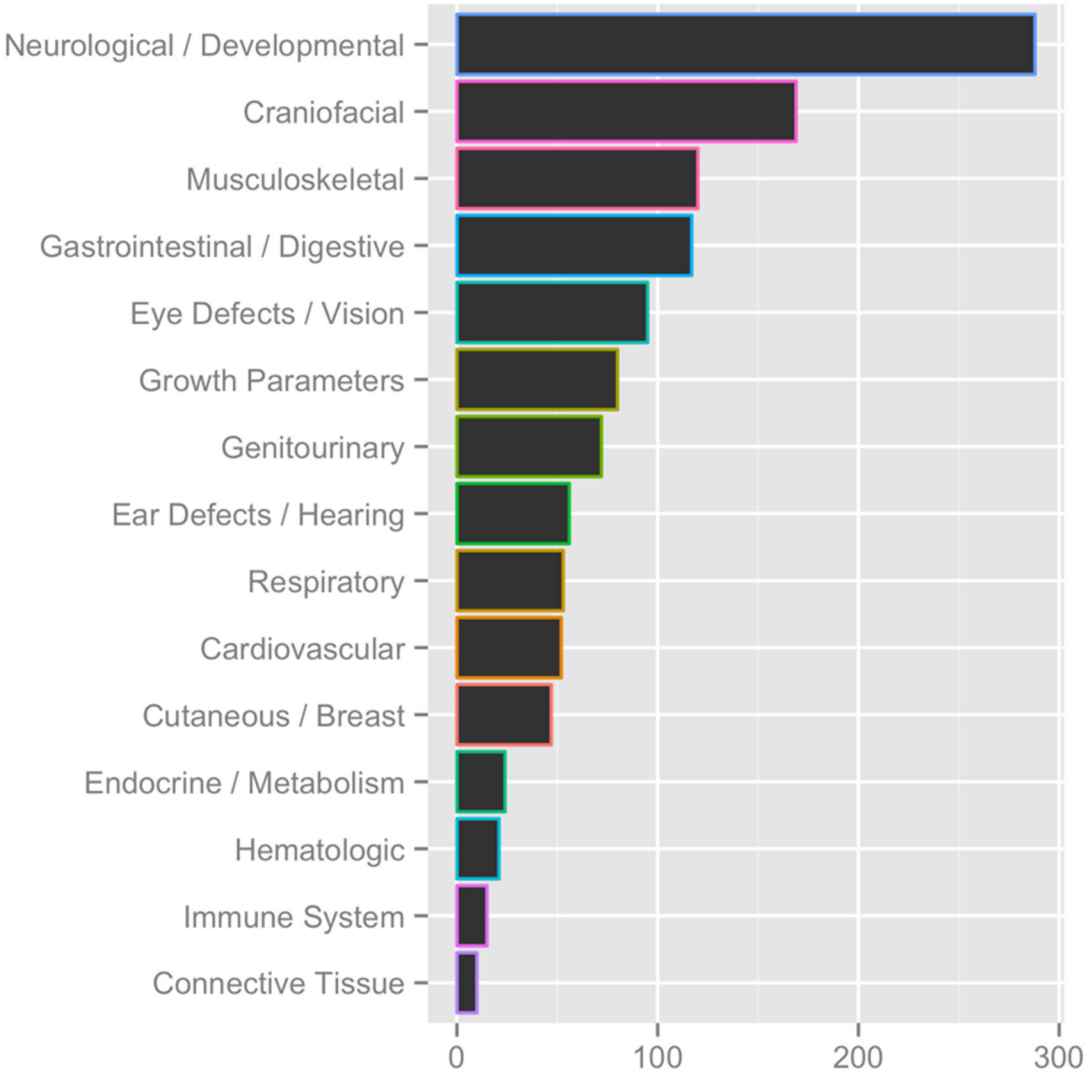
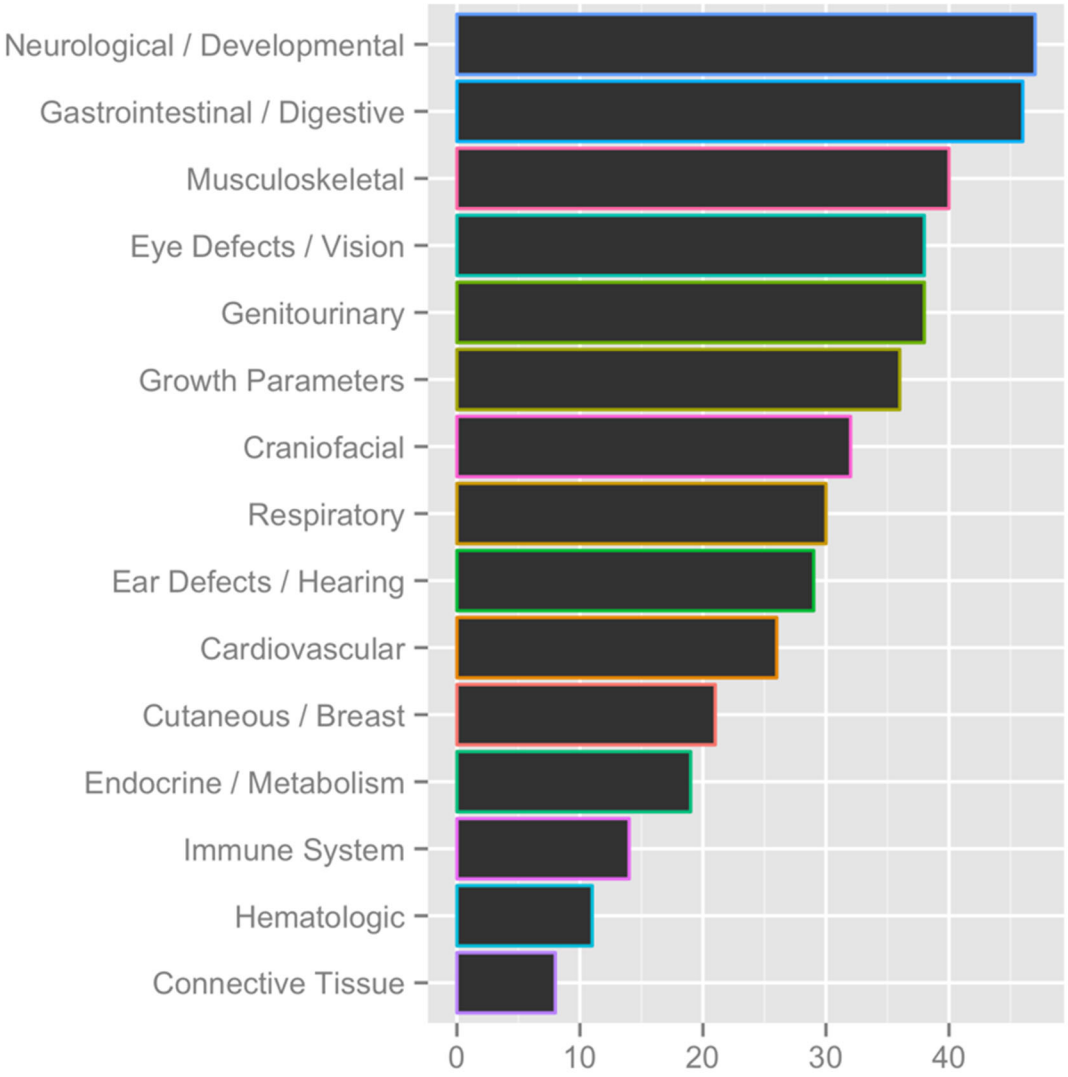
This supplementary material has been provided by the authors to give readers additional information about their work.

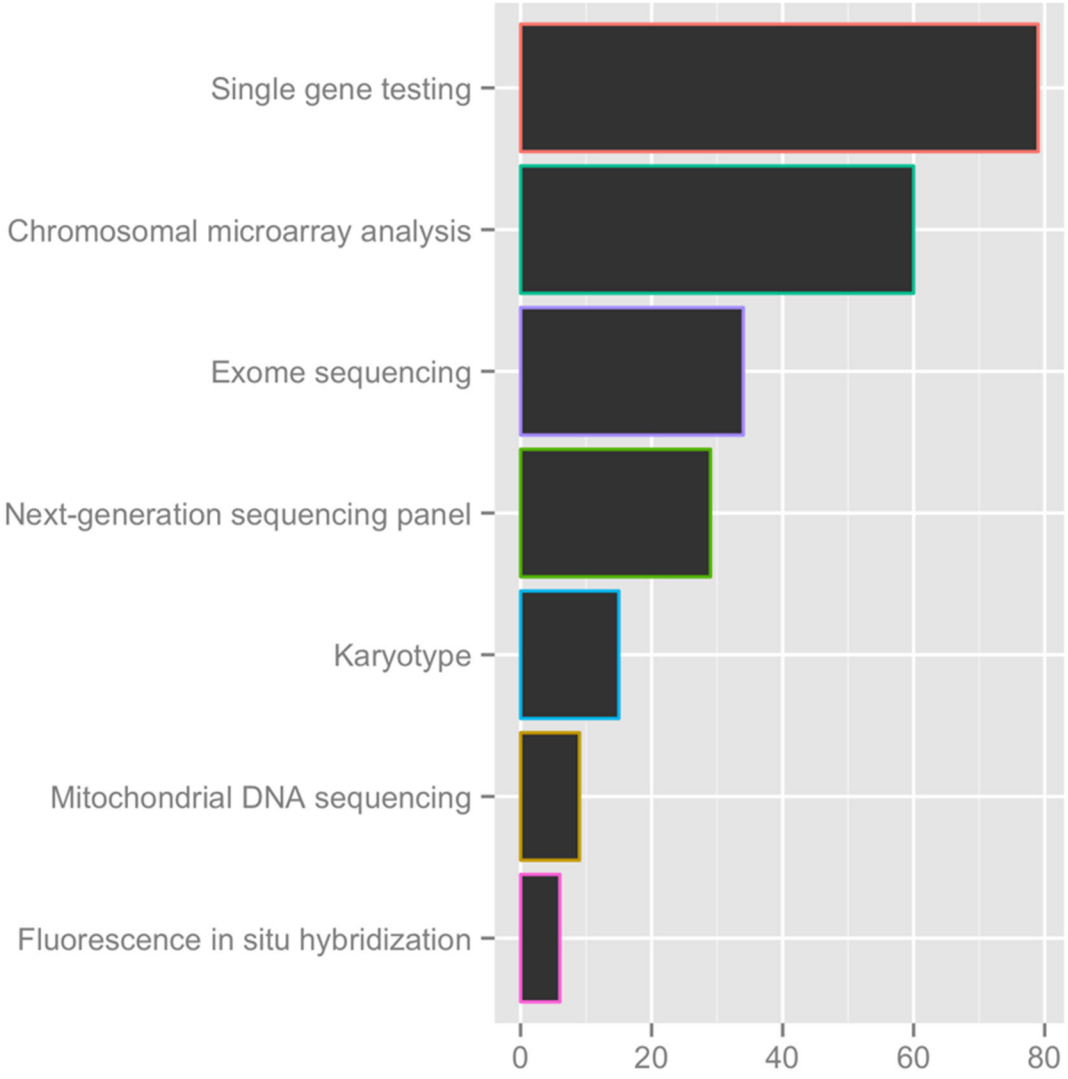
eFigure 1. Total Count of HPO Terms for the Proband Cohort (n=49), by Phenotype Category



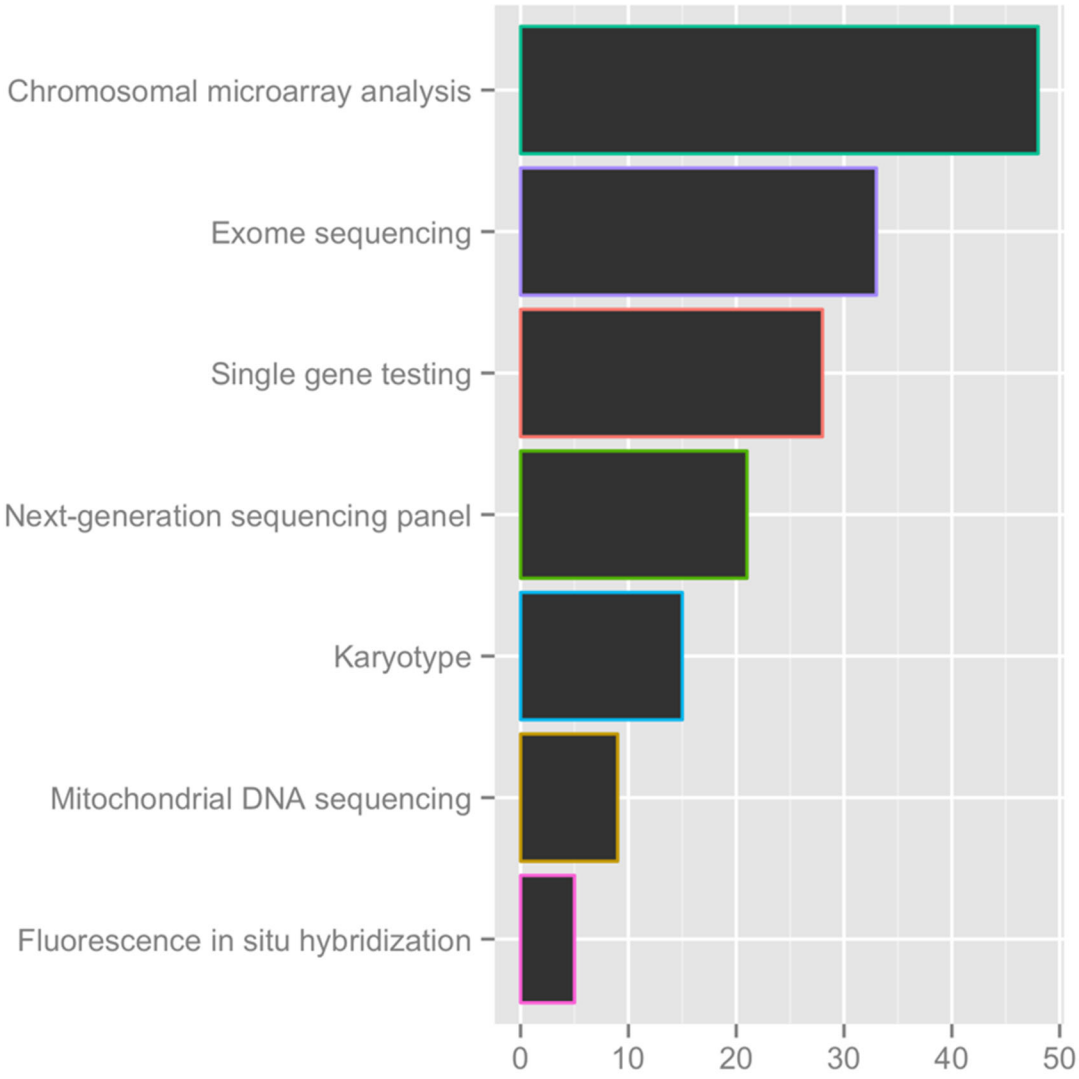
eFigure 2. Total Number of Probands With at Least One HPO Term in a Phenotype Category



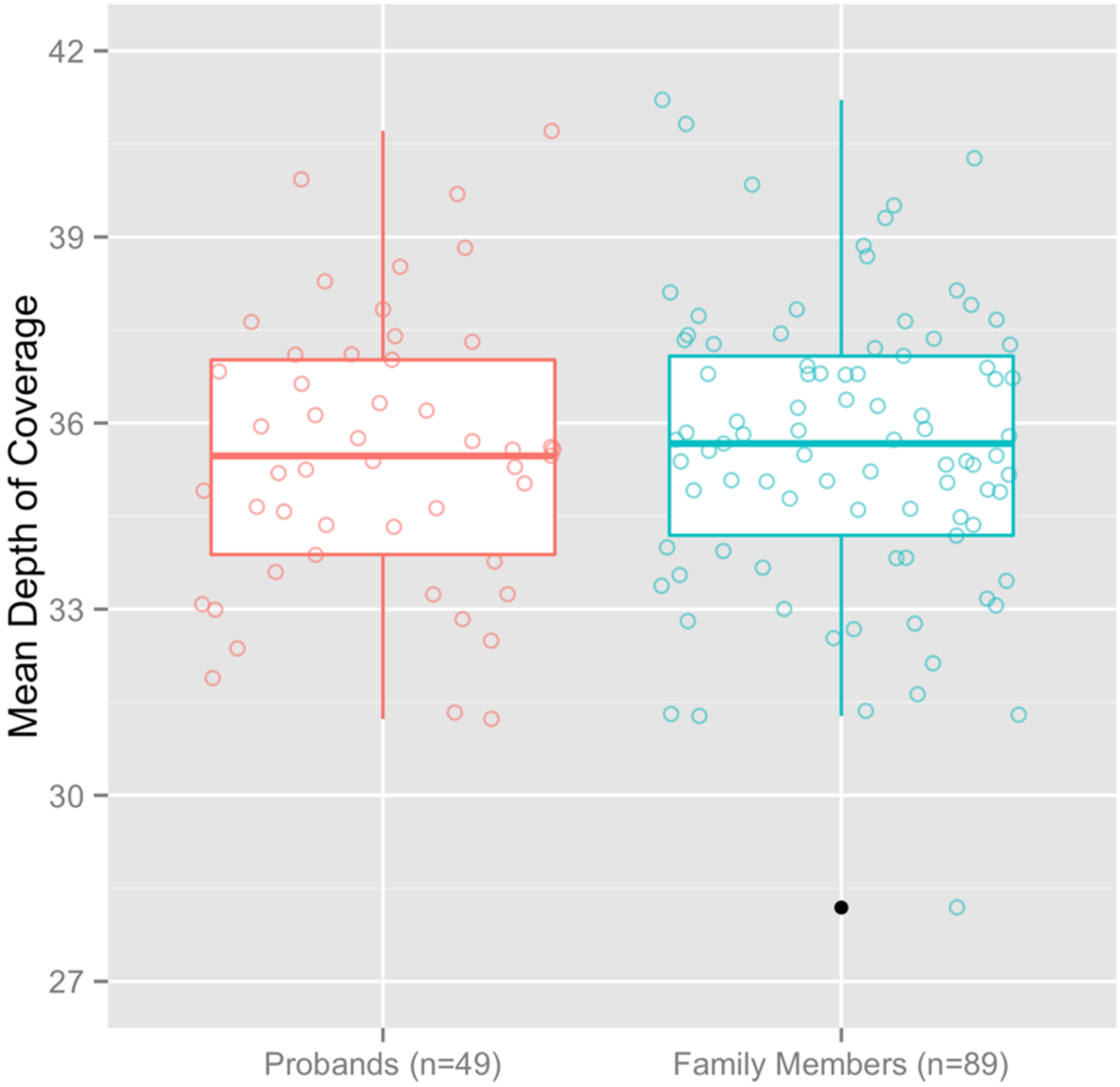
eFigure 3. Total Count of Clinical Genetic Tests in Each Category Performed in the Proband Cohort (n=49)



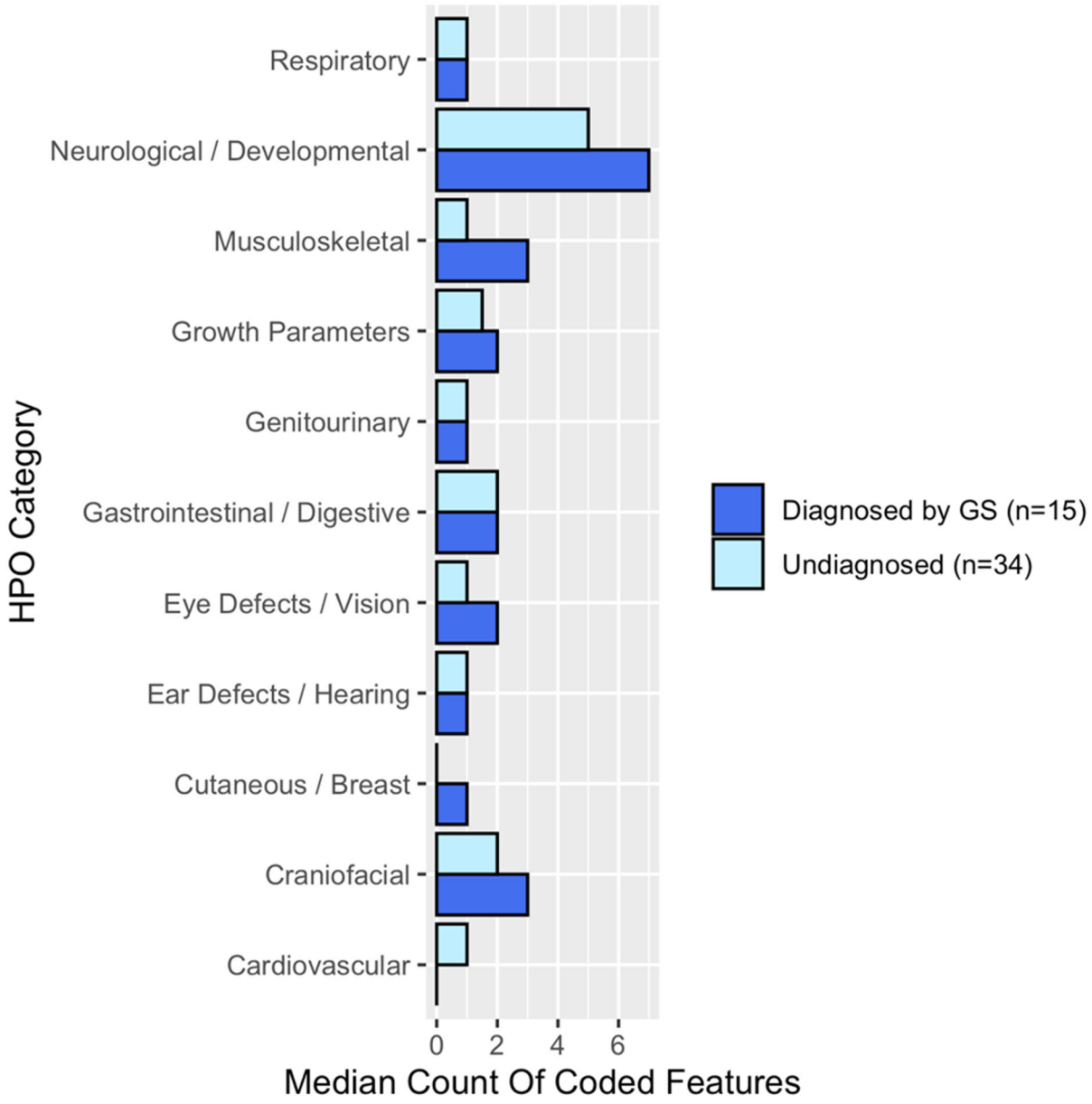
eFigure 4. Total Number of Probands With at Least One Test in a Clinical Genetic Test Category



eFigure 5. Mean Depth of Coverage of the Genome Sequencing Data



eFigure 6. Median Count of Features by HPO Category[a] in the Diagnosed and Undiagnosed Subgroups



[a]The categories "Hematologic", "Immune System", "Connective Tissue", and "Endocrine / Metabolism" are not displayed because the median count was 0 in both subgroups.

eTable 1. Individuals With Suspected or Partial Primary Diagnoses Known at the Time of Study Recruitment

Study ID	***Sex***	***Gene***	***Condition (MIM # Phenotype)***	***IP***	***Variant Details (Zygosity) [Transcript]***	***Origin***	***Reasons for further genetic testing***
CMC 02	M	-	Down syndrome (190685)	-	47,XY,+21	dn	Severity of developmental delays, seizure disorder, and feeding difficulties
CMC 23	F	*HNF4A*	Fanconi renotubular syndrome 4, with maturity-onset diabetes of the young (616026)	AD	c.187C>T / p.(R63W) (het) [NM_175914]	pat	Multiple major congenital anomalies
CMC 32	M	*SOX5*	Lamb-Shaffer syndrome (616803)	AD	c.928T>A / p.(C310S) (het) (NM_006940)	dn	Type I transferrin isoform profile, and variant not classified as pathogenic
CMC 43	M	*KRAS*	Noonan syndrome 3 (609942)	AD	c.40G>A / p.(V14I) (het) [NM_004985]	dn	Treatment-resistant hyperkinetic movement disorder
CMC 46	M	*ATP6AP2*	Mental retardation, X-linked, syndromic, Hedera type (300423)	XL	c.301-11_301-10delTT / p.(?) (hem) [NM_005765]	dn	Inflammatory bowel disease, and variant not classified as pathogenic
CMC 49	M	*EDA*	Ectodermal dysplasia 1, hypohidrotic, X-linked (305100)	XL	c.458G>A / p.(R153H) (hem) [NM_001005612]	mat	Multiple major congenital anomalies

AD, autosomal dominant; dn, *de novo*; F, female; hem, hemizygous; het, heterozygous; IP, inheritance pattern; M, male; mat, maternal; MIM, Mendelian Inheritance in Man® (www.omim.org); pat, paternal; XL, X-linked

eTable 2. Demographic and Clinical Features in the Proband Study Cohort

	Total (n=49)		**Diagnosed by GS (n=15)**		**No diagnosis by GS (n=34)**		**p-value**[a]
	Number	(%)	Number	(%)	Number	(%)	
Male sex	29	(59.1)	7	(46.6)	22	(64.7)	ns
Clinical ES	33	(67.3)	9	(60.0)	24	(70.5)	ns
Positive family history[b]	6	(12.2)	3	(20.0)	3	(8.8)	ns
Parental consanguinity	5	(10.2)	2	(13.3)	3	(8.8)	ns
European/Caucasian ethnicity	26	(53.0)	8	(53.3)	18	(52.9)	ns
	Median	(range)	Median	(range)	Median	(range)	
Age in years	6.9	(0.6-17.8)	8	(4.5-17.8)	4.9	(0.6-13.2)	0.003
Coded HPO terms[c]	24	(6-58)	26	(11-45)	23	(6-58)	ns

ES, exome sequencing; GS, genome sequencing; HPO, Human Phenotype Ontology; ns, not significant (p>0.05)

[a]Fisher's exact test for comparison of proportions, and Kruskal–Wallis test for comparison of medians.
[b]First-degree relative with at least partial phenotypic overlap
[c]See text for details.

eTable 3. Selected Variants of Uncertain Diagnostic Significance Identified in the Proband Study Cohort

Study ID	*Sex*	*Selected Features*	*Gene*	*Variant Details (Zygosity) [Transcript]*	*Origin*
Known disease genes					
CMC 01	F	Congenital microcephaly, GDD/ID, seizures	*MED23*	c.3957+5G>A (hom) [NM_015979]	mat/pat
CMC 03	F	SMMCI	*CDON*	c.2674G>A / p.(G892S) (het) [NM_016952]	mat[a]
CMC 04	M	GDD/ID, seizures, hypotonia, structural CNS anomalies	*PLCB1*	c.246+197G>A (hom) [NM_015192]	mat/pat
CMC 07	F	GDD, hypotonia, structural CNS anomalies	*KIF1A*	c.4566-66C>T (het) [NM_004321]	dn
CMC 28	M	GDD/ID, seizures, microcephaly, congenital anomalies	*THOC2*	c.623A>G / p.(N208S) (hem) [NM_001081550]	mat
CMC 29	M	GDD, congenital heart disease	*CTBP1*	c.107G>A / p.(R36Q) (het) [NM_001328][b]	dn
CMC 31	M	Intracranial hemorrhage, congenital cataracts	*JAM3*	c.410-1G>A (het) [NM_032801]	mat
				c.256+1260G>C (het) [NM_032801]	pat
CMC 37	M	Potter sequence, GDD	*POLA1*	c.3835T>G / p.(F1279V) (hem) [NM_016937]	mat
CMC 50	F	GDD, MCA	*TLK2*	c.532-1571_831+4del (het) [NM_006852][c]	uk[d]
Genes not yet associated with a Mendelian disorder					
CMC 04	M	GDD/ID, seizures, structural CNS anomalies	*MAGEE1*	c.877_984del / p.(293_328del) (hem) [NM_020932]	dn
CMC 18	F	GDD, MCA, nystagmus	*SF3B3*	c.2012A>G / p.(N671S) (het) [NM_012426]	dn
CMC 26	M	Severe achalasia	*ANKRD13B*	c.1761C>G / p.(D587E) (het) [NM_152345]	dn[e]
CMC 32	M	Seizures, GDD, hypotonia, sensorineural hearing loss	*DHRSX*	c.643C>T / p.(L215F) (het[f]) [NM_145177]	mat[e]

			DHRSY	c.541G>T / p.(V181F) (het[f]) [NM_145177]	pat[e]
CMC 33	M	Arthrogryposis, hypotonia	*CAPRIN1*	c.1338A>T / p.(Q446H) (het) [NM_005898]	dn
CMC 34	M	Neurodegenerative disorder, aplastic anemia	*KLHL12*	c.1700A>T / p.(D567V) (het) [NM_001303051]	dn
CMC 41	M	Congenital right cerebral hypoplasia, GDD/ID	*PLXNC1*	c.1400C>A / p.(A467D) (het) [NM_005761]	dn

CNS, central nervous system; dn, *de novo*; F, female; GDD, global developmental delay; hem, hemizygous; het, heterozygous; hom, homozygous; ID, intellectual disability; M, male; mat, maternal; MCA, multiple congenital anomalies; pat, paternal; SMMCI, solitary median maxillary central incisor syndrome; uk, unknown

[a]Mother does not have features of SMMCI on exam. We questioned whether there could be a gene–environment interaction with another risk factor present in this family (maternal type 1 diabetes mellitus).
[b]ClinVar Accession Number: VCV000426724.2 (same patient).
[c]chr17:60628092_60637491del
[d]Not inherited from mother.
[e]Variant also present in similarly affected brother.
[f]Gene is within the pseudoautosomal region.