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Supplementary Materials for

Effective diagnosis of genetic disease by computational phenotype analysis of the disease-associated genome

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Published 3 September 2014, *Sci. Transl. Med.* **6**, 252ra123 (2014)
DOI: 10.1126/scitranslmed.3009262

This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencetranslationalmedicine.org/cgi/content/full/6/252/252ra123/DC1)

Supplementary Materials

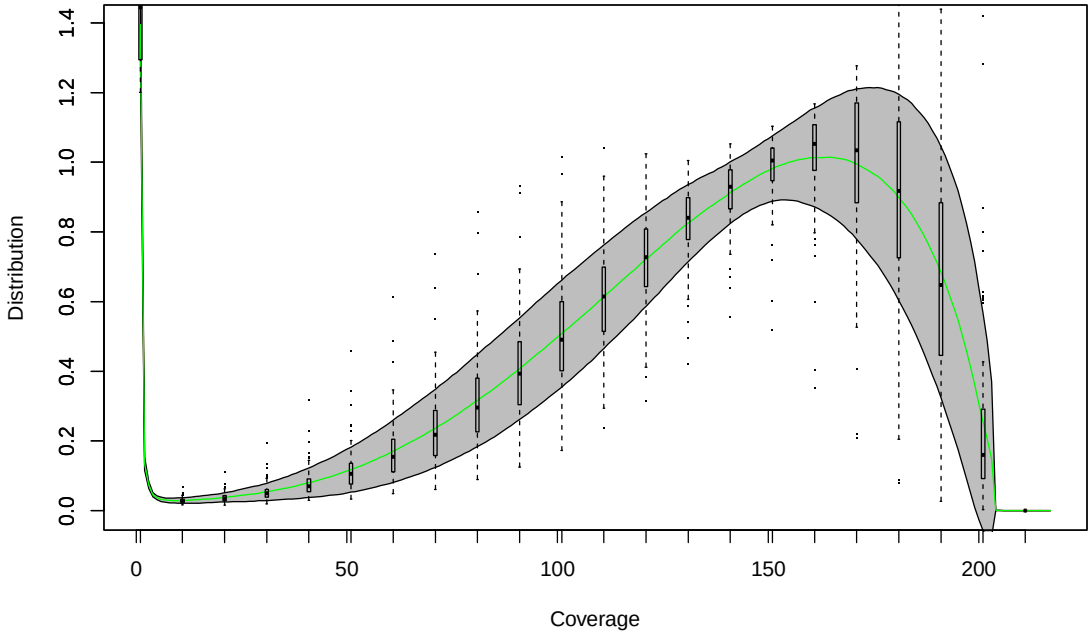
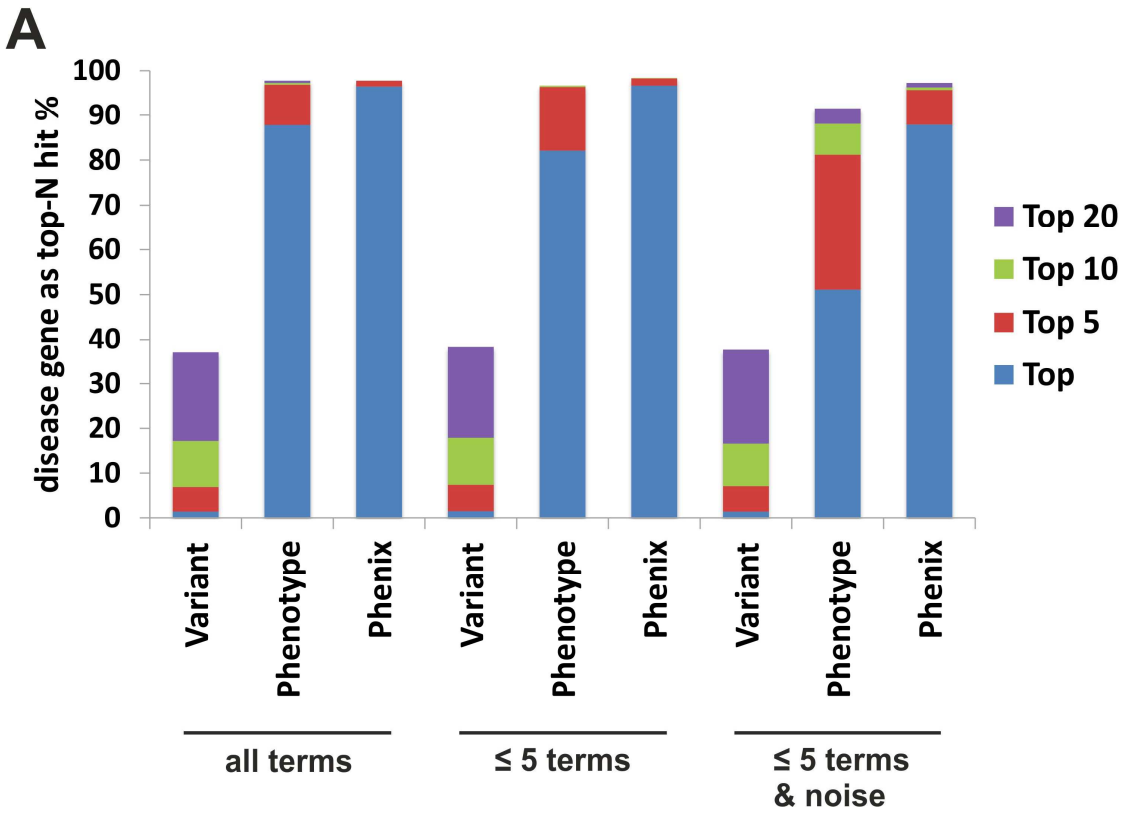
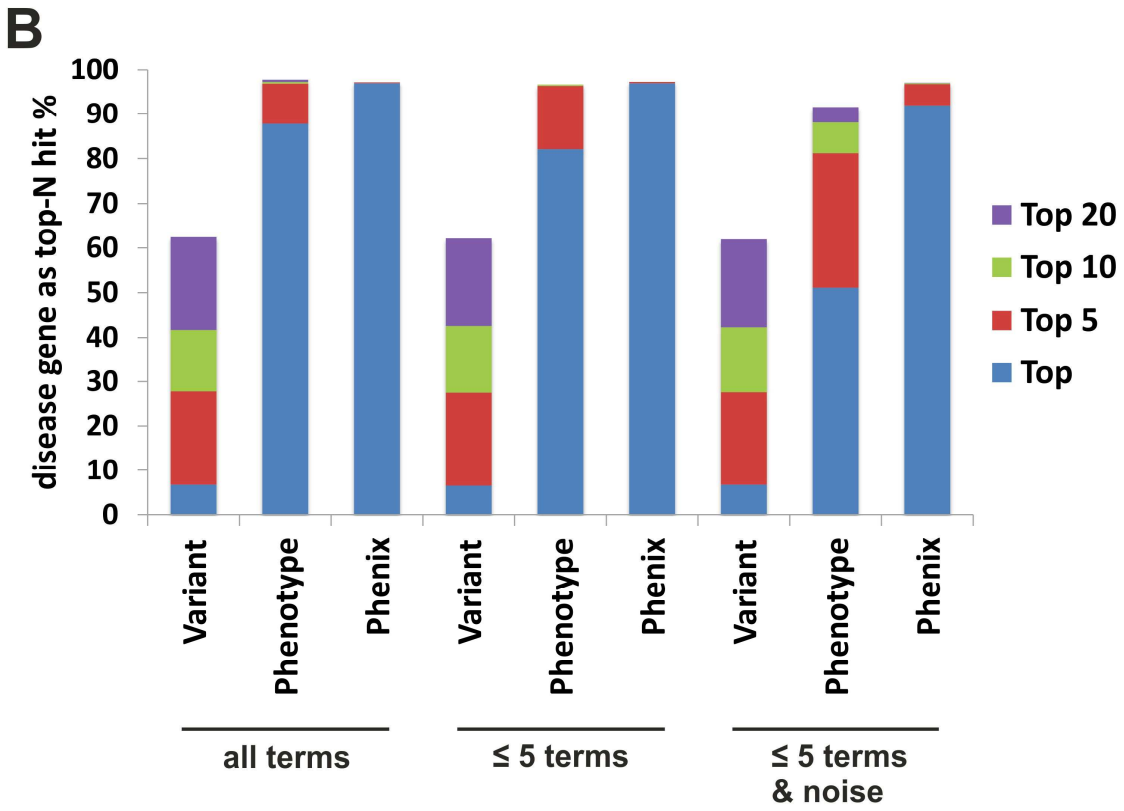


Fig. S1. Distribution of the coverage fraction for all sequenced 96 samples. The mean coverage is shown as a green line. The standard deviation is shown in gray. A box plot is shown for each 10th value; the box plot shows the median value of the data as a thick line, and the box itself shows the interquartile range, i.e., the range within which the middle 50% of the ranked data are found. Additional data are shown outside the box; the dashed lines indicate the values in the lower and upper range for the 1.5 rule or [Q1 - 1.5*IQR, Q3 + 1.5IQR] (where IQR is the Inter Quantile Range) and extreme datapoints (outliers) are shown as single points below or above these whiskers.


A
disease gene as top-N hit %
100
90
80
70
60
50
40
30
20
10
0
Variant
Phenotype
Phenix
Variant
Phenotype
Phenix
Variant
Phenotype
Phenix
all terms
≤ 5 terms
≤ 5 terms & noise
Top 20
Top 10
Top 5
Top


B
disease gene as top-N hit %
100
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50
40
30
20
10
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Variant
Phenotype
Phenix
Variant
Phenotype
Phenix
Variant
Phenotype
Phenix
all terms
≤ 5 terms
≤ 5 terms & noise
Top 20
Top 10
Top 5
Top

Figure S2. Computational evaluation of PhenIX. HGMD mutations were inserted into variant files from DAG panels from which the causative mutations had been removed and phenotypic annotations of the corresponding diseases were extracted from the HPO database. The genes were ranked using PhenIX. Results were simulated either on the entire disease set (All) (see Figure 1), or by filtering for known autosomal dominant (AD) or autosomal recessive (AR) diseases (panels A and B, respectively). A total of 8504 (All), 3471 (AD), and 5006 (AR) simulations were performed. Data are shown as the percentage of simulations in which the correct genes was ranked in first place. Variant, only variant scores used to rank candidate genes. All terms, All HPO terms used to annotate a disease were used for PhenIX analysis. ≤5 terms, Up to 5 HPO terms were chosen at random from the terms used to annotation the disease. ≤5 terms & noise, Up to 5 annotations are used, 2 of which are made imprecise by exchanging them with a more general parent term; additionally, two random "noise" terms were added. Results are shown for the correct gene being ranked as the single top hit, or being among the top 5, 10, or 20 hits for the three test scenarios.

Table S1. Percentage of target bases that exceed coverages of 10, 20, ..., 100 reads.

Minimal coverage	Percentage of base positions
10	98.2% ± 0.13%
20	97.8% ± 0.19%
30	97.4% ± 0.35%
40	96.7% ± 0.67%
50	95.8% ± 1.18%
60	94.4% ± 1.93%
70	92.4% ± 2.93%
80	89.7% ± 4.13%
90	86.1% ± 5.51%
100	81.6% ± 6.98%

Table S2. Read alignment and coverage summary statistics. 40 patients with unknown diagnosis P1-P40, 52 patients with known diagnosis C41-C92, 4 control samples R93-R96. Total reads=Total Pass filter reads - the total number of reads that pass the Illumina quality filters; Duplication rate - percentage of reads that are identified as duplicate reads (i.e., reads whose alignment location is identical to other reads from the same library); Uniquely aligned reads= number of reads after duplicate removal, that could be mapped to the target region (count and percentage); Mean coverage=per base mean coverage for the entire target region (after duplicate removal); B.20+cov=Base 20+ coverage [%] - fraction of bases with at least 20-fold coverage; (Analogous for B.40+cov, B.60+cov, B.80+cov, B.100+cov); E.10+cov and E.20+cov= Percentage of exons with overall per base coverage over 10-fold (20-fold), i.e., there was no base position with lower coverage. The mean mapping quality was analyzed for all 96 samples, with 0.296 ±0.184 percent of the reads exhibiting a mapping quality less than 30 (PHRED).

Patient ID	Total reads	Duplication rate[%]	Uniquely aligned reads	Mean coverage	B.20+cov	B.40+cov	B.60+cov	B.80+cov	B.100+cov	E.10+cov	E.20+cov
P1	62290776	43.97%	29573874 (85%)	145.23	98%	97%	95%	92%	86%	97%	97%
P2	65482190	47.95%	28845382 (85%)	143.38	98%	97%	96%	93%	87%	97%	97%
P3	54639206	46.27%	25247276 (86%)	133.44	98%	97%	94%	90%	81%	97%	96%
P4	56052330	44.51%	26982475 (87%)	136.07	98%	97%	95%	90%	83%	97%	96%
P5	45087680	41.90%	22467107 (86%)	128.13	98%	97%	94%	88%	78%	97%	96%
P6	77875220	53.37%	29714333 (82%)	148.48	98%	97%	96%	94%	89%	97%	97%
P7	57250042	46.45%	26062480 (85%)	132.27	98%	97%	94%	89%	80%	97%	97%
P8	76527678	51.34%	30065863 (81%)	148.04	98%	98%	96%	94%	88%	98%	97%
P9	58665278	46.05%	26396171 (83%)	135.90	98%	97%	95%	91%	83%	97%	97%
P10	53730860	44.25%	25566920 (85%)	128.68	98%	97%	94%	88%	78%	97%	96%
P11	64112406	49.75%	27516417 (85%)	138.14	98%	97%	95%	90%	83%	97%	97%
P12	41781802	40.29%	22014342 (88%)	118.85	97%	96%	91%	82%	69%	97%	96%
P13	47797112	42.07%	24236179 (88%)	127.07	98%	96%	93%	86%	76%	97%	96%
P14	64906410	47.22%	28979668 (85%)	142.21	98%	97%	96%	92%	86%	97%	97%
P15	63607178	45.58%	29453878 (85%)	141.75	98%	97%	95%	92%	86%	97%	97%

P16	62584840	46.43%	28481875 (85%)	141.33	98%	97%	95%	92%	85%	97%	97%
P17	60574714	47.50%	27302604 (86%)	140.13	98%	97%	95%	92%	85%	97%	97%
P18	72394308	48.37%	31141098 (83%)	147.76	98%	97%	96%	93%	88%	98%	97%
P19	53315984	44.28%	25494343 (86%)	134.34	98%	97%	94%	89%	80%	97%	96%
P20	66935954	46.64%	30095784 (84%)	145.86	98%	97%	96%	93%	88%	97%	97%
P21	79996308	51.98%	32091958 (84%)	153.28	98%	97%	96%	95%	91%	97%	97%
P22	56002138	42.87%	27148606 (85%)	136.65	98%	97%	95%	91%	84%	97%	97%
P23	60900124	46.54%	27864817 (86%)	138.48	98%	97%	95%	91%	84%	97%	97%
P24	49689092	42.11%	25257245 (88%)	129.30	98%	97%	94%	88%	78%	97%	96%
P25	63033168	46.65%	28333942 (84%)	142.35	98%	97%	96%	93%	87%	97%	97%
P26	43563468	40.12%	22434607 (86%)	122.54	98%	96%	92%	85%	73%	97%	96%
P27	42701178	38.60%	22702907 (87%)	121.84	98%	96%	93%	86%	74%	97%	96%
P28	42363964	39.36%	22524801 (88%)	120.55	98%	96%	92%	84%	72%	97%	96%
P29	47517530	41.93%	24060125 (87%)	126.52	98%	96%	93%	87%	76%	97%	96%
P30	67833304	47.70%	29962976 (84%)	145.60	98%	97%	96%	93%	88%	97%	97%
P31	59947064	49.25%	25765137 (85%)	135.12	98%	97%	94%	89%	81%	97%	96%
P32	45082340	39.98%	23536789 (87%)	125.77	98%	96%	93%	87%	77%	97%	96%
P33	68012532	46.76%	30111162 (83%)	145.23	98%	97%	96%	93%	87%	98%	97%
P34	30229960	31.79%	17923376 (87%)	103.67	97%	95%	88%	75%	57%	97%	95%
P35	52467982	42.06%	25975925 (85%)	103.67	98%	97%	94%	90%	81%	97%	96%
P36	59877862	46.72%	27489409 (86%)	140.98	98%	97%	94%	90%	82%	97%	97%
P37	71139068	47.31%	31686068 (85%)	132.85	98%	97%	96%	93%	88%	97%	97%
P38	56716568	42.72%	27644083 (85%)	137.50	98%	97%	95%	91%	84%	97%	97%
P39	72738144	48.92%	30952814 (83%)	149.34	98%	97%	96%	94%	89%	97%	97%
P40	62455678	46.84%	28003728 (84%)	141.18	98%	97%	96%	92%	86%	97%	97%
C41	90918934	53.42%	35355312 (83%)	157.74	98%	98%	97%	95%	92%	98%	97%
C42	82588486	52.63%	32099790 (82%)	151.59	98%	98%	96%	94%	90%	98%	97%
C43	46198520	40.76%	23608305 (86%)	128.95	98%	97%	94%	89%	79%	97%	96%
C44	70319668	48.79%	30148521 (84%)	147.02	98%	97%	96%	94%	89%	97%	97%
C45	61139016	45.72%	28130168 (85%)	137.61	98%	97%	95%	92%	85%	97%	97%
C46	62773488	45.68%	28655400 (84%)	141.88	98%	97%	96%	93%	87%	97%	97%

C47	67350342	48.37%	28727263 (83%)	142.35	98%	97%	95%	92%	86%	97%	97%
C48	49329306	42.28%	24097306 (85%)	131.30	98%	97%	95%	90%	81%	97%	96%
C49	38377102	37.92%	20367340 (85%)	115.43	98%	96%	91%	82%	68%	97%	96%
C50	65886630	49.51%	27877390 (84%)	142.91	98%	97%	96%	93%	87%	97%	97%
C51	59994804	44.58%	27938788 (84%)	139.18	98%	97%	95%	91%	84%	97%	97%
C52	46148884	38.87%	24431962 (87%)	123.96	98%	96%	93%	86%	75%	97%	96%
C53	60092968	45.92%	27686331 (85%)	138.58	98%	97%	95%	92%	85%	97%	97%
C54	66302380	47.50%	28869269 (83%)	146.33	98%	97%	96%	94%	89%	97%	97%
C55	54694250	45.46%	25167468 (84%)	135.42	98%	97%	95%	91%	83%	97%	96%
C56	51105516	43.80%	24425727 (85%)	133.43	98%	97%	95%	90%	82%	97%	96%
C57	60582984	46.25%	27148448 (83%)	140.82	98%	97%	95%	92%	85%	97%	97%
C58	53375038	45.22%	25113749 (86%)	131.06	98%	97%	94%	88%	79%	97%	96%
C59	50642058	44.20%	24815162 (88%)	130.70	98%	97%	94%	88%	79%	97%	96%
C60	28120002	30.78%	17502488 (90%)	98.59	97%	93%	84%	69%	0.51	96%	94%
C61	102143086	59.60%	33201741 (80%)	155.74	98%	97%	97%	95%	91%	97%	97%
C62	42257676	40.63%	21706213 (87%)	119.19	98%	96%	92%	84%	71%	97%	96%
C63	51664294	36.19%	28385482 (86%)	126.92	98%	97%	94%	88%	78%	97%	96%
C64	56763494	42.66%	27579907 (85%)	135.48	98%	97%	95%	90%	83%	97%	96%
C65	49299788	43.13%	24151454 (86%)	129.39	98%	97%	94%	88%	78%	97%	96%
C66	53035158	45.67%	25248435 (88%)	128.65	98%	96%	92%	86%	76%	97%	96%
C67	53312488	43.29%	25871033 (86%)	133.78	98%	97%	94%	90%	82%	97%	96%
C68	37866350	38.34%	20520653 (88%)	110.11	98%	95%	89%	78%	63%	97%	96%
C69	56840748	44.62%	27080959 (86%)	134.94	98%	97%	94%	90%	82%	97%	96%
C70	112176206	58.63%	36767995 (79%)	162.70	98%	98%	97%	96%	93%	98%	97%
C71	53632388	44.11%	25572459 (85%)	132.44	98%	97%	94%	89%	80%	97%	97%
C72	50699922	43.65%	24544419 (86%)	128.86	98%	97%	94%	88%	79%	97%	96%
C73	54822400	44.38%	25761754 (84%)	131.74	98%	97%	94%	89%	80%	97%	97%
C74	57826978	45.64%	26320639 (84%)	136.22	98%	97%	95%	90%	82%	97%	97%
C75	66487796	49.60%	27940494 (83%)	144.25	98%	97%	96%	93%	88%	97%	97%
C76	62535894	46.17%	27773214 (83%)	137.88	98%	97%	95%	92%	84%	97%	97%
C77	51076476	42.63%	24643746 (84%)	133.97	98%	97%	95%	90%	82%	97%	96%

C78	65615014	52.09%	26626137 (85%)	138.22	98%	97%	95%	90%	83%	97%	96%
C79	71472892	51.51%	28983331 (84%)	145.29	98%	97%	96%	93%	87%	97%	97%
C80	67086790	51.04%	27105252 (83%)	145.35	98%	97%	96%	93%	88%	97%	97%
C81	52229586	44.06%	24671796 (84%)	129.26	98%	97%	94%	88%	78%	97%	96%
C82	61819428	49.00%	26277531 (83%)	139.98	98%	97%	95%	91%	85%	97%	97%
C83	52182388	46.27%	24159940 (86%)	132.46	98%	97%	95%	90%	81%	97%	96%
C84	57870846	46.11%	26198278 (84%)	138.20	98%	97%	95%	92%	85%	97%	97%
C85	56286554	49.16%	24846389 (87%)	131.77	97%	96%	93%	87%	78%	97%	96%
C86	52127672	43.93%	25094366 (86%)	133.28	98%	97%	94%	89%	81%	97%	97%
C87	43167002	39.42%	22039567 (84%)	124.64	98%	96%	93%	87%	76%	97%	96%
C88	51720430	44.44%	24123973 (84%)	132.93	98%	97%	94%	89%	81%	97%	97%
C89	39500556	40.25%	20587183 (87%)	116.99	98%	96%	91%	82%	69%	97%	96%
C90	51915414	45.52%	24999672 (88%)	131.08	98%	96%	93%	87%	77%	97%	96%
C91	57701590	46.74%	25738238 (84%)	139.41	98%	97%	95%	92%	85%	97%	97%
C92	58109680	46.71%	26095366 (84%)	138.91	98%	97%	95%	91%	84%	97%	97%
R93	79860340	55.64%	29864618 (84%)	147.24	98%	97%	96%	93%	88%	97%	97%
R94	53671250	45.46%	24923776 (85%)	136.46	98%	97%	95%	91%	84%	97%	97%
R95	69151142	51.13%	28572387 (85%)	144.38	98%	97%	96%	92%	86%	97%	97%
R96	47599196	42.32%	23951726 (87%)	124.22	98%	96%	92%	84%	73%	97%	96%

Table S3. Average number of variants called only from the original BAM files from the DAG panels. These were not in simulated BAM files generated down-sampling reads to a typical exome coverage (100x). Random down-sampling of the reads was performed to achieve the indicated average coverage, and the variants called in the original BAM file were compared with those called in the down-sampled files. This procedure was performed once for each of the 48 original DAG panel BAM files, and the mean and standard deviations are shown in the table.

Variant	Original only (80x)
Missense	24.0 ± 6.9
Stopgain	0.7 ± 0.6
Splicing	4.1 ± 1.0
Frameshift deletion	12.5 ± 2.3
Frameshift insertion	13.7 ± 1.9
Nonframeshift deletion	25.1 ± 3.6
Nonframeshift insertion	23.6 ± 3.4
UTR3	7.2 ± 1.9
Synonymous	15.4 ± 4.1
Intronic	18.9 ± 2.8
dbSNP	117.8 ± 10.5
HGMD	5.4 ± 2.1
ClinVar	5.1 ± 1.9

Table S4. Detailed clinical and molecular findings for the 11 individuals in whom a previously unknown diagnosis was clarified by PhenIX analysis. HPO terms shown in bold match with the disease profiles in the HPO database for these diseases.

Patient ID	Age & sex	Phenotypic Features (match in bold)	Diagnosis	Gene, Mutation, Segregation	Rank	Pathogenicity Assessment
P1	3y (f)	**Global developmental delay (HP:0001263)** **Delayed speech and language development (HP:0000750)** Motor delay (HP:0001270) **Proportionate short stature (HP:0003508)** Microcephaly (HP:0000252) **Feeding difficulties (HP:0011968)** Congenital megaloureter (HP:0008676) Cone-shaped epiphysis of the phalanges of the hand (HP:0010230) **Sacral dimple (HP:0000960)** Hyperpigmentated/hypopigmentated macules (HP:0007441) **Hypertelorism (HP:0000316)** Abnormality of the midface (HP:0000309) **Flat nose (HP:0000457)** Thick lower lip vermilion (HP:0000179) Thick upper lip vermilion (HP:0000215) Full cheeks (HP:0000293) Short neck (HP:0000470)	Wiedemann-Steiner syndrome	*KMT2A* NM_001197104 Exon 27 c.7264G>T p.G2422* *de novo*	2	Multiple nonsense mutations have been identified previously in individuals with Wiedemann-Steiner syndrome (*54*). Cosegregation studies in the family demonstrated a de novo nonsense mutation in the affected child.
P2	5y (f)	**Global developmental delay (HP:0001263)** Delayed speech and language development (HP:0000750) Growth delay (HP:0001510) **Stereotypical hand wringing (HP:0012171)** **Muscular hypotonia of the trunk (HP:0008936)** Strabismus (HP:0000486) Short philtrum (HP:0000322)	Mental retardation, autosomal dominant 5	*SYNGAP1* uc010juz.3, exon1, c.302C>A, p.S101* *de novo*	4	De novo truncating mutations in SYNGAP1 have been identified in nonsyndromic mental retardation (*55*). Cosegregation studies in the family demonstrated a de novo nonsense mutation in the affected child.

		Aggressive behavior (HP:0000718) **Self-injuries behavior (HP:0100716)** Widely spaced teeth (HP:0000687)				
P3	6y (f)	**2-3 toe cutaneous syndactyly (HP:0005709)** Proximal/middle symphalangism of 3rd Finger (HP:0009482) Proximal/middle symphalangism of 2nd Finger (HP:0009579) Distal/middle symphalangism of 2nd finger (HP:0009563) Short proximal phalanx of 2nd finger (HP:0009597) Aplasia/Hypoplasia of the middle phalanx of the 2nd finger (HP:0009568) Aplasia/Hypoplasia of the middle phalanx of the 3rd finger (HP:0009437) Aplasia/Hypoplasia of the middle phalanx of the 4th finger (HP:0009299) Aplasia/Hypoplasia of the middle phalanx of the 5th finger (HP:0009161) **Broad proximal phalanx of the thumb (HP:0009630)** **Short proximal phalanx of the thumb (HP:0009638)** **Abnormality of the philtrum (HP:0000288)** **Thin upper lip vermilion(HP:0000219)** **Broad toe (HP:0001837)**	Pfeiffer syndrome	*FGFR2* uc010qtl.2, exon5, c.515C>T, p.A172F *de novo*	1	The mutation identified is identical with the mutation originally identified in Pfeiffer syndrome (*56*).
P4	Death at 5.5m (f)	Supravalvular aortic stenosis (HP:0004381) Abnormality of the ventricular septum (HP0010438) Junctional ectopic tachycardia (HP:0011716) Persistent left superior vena cava (HP:0005301) **Congenital glaucoma (HP:0001087)** Sclerocornea (HP:0000647) Retinal detachment (HP:0000541)	Frank-ter Haar syndrome	*SH3PXD2B* uc003mbr.3, exon4, c.250C>T, p.R84* Autosomal recessive	6	Mutations in *SH3PXD2B* Including premature truncation mutations, are associated with Frank-ter Haar syndrome (*57*). Cosegregation studies in the family demonstrated a homozygous nonsense mutation in the affected child, and heterozygous carrier status in each parent.

		Talipes equinovarus (HP:0001762) Radial deviation of finger (HP:0009466) Joint contracture of the hand (HP:0009473) Feeding difficulties (HP:0011968) Cholestasis (HP:0001396) Delayed CNS myelination (HP:0002188) Flat occiput (HP:0005469) **Microretrognathia (HP:0000308)** Microcephaly (HP:0000252) **Wide anterior fontanel (HP:0000260)** Broad prominent forehead (HP:0200061) Thick eyebrows (HP:0000574) **Hypertelorism (HP:0000316)** Depressed nasal bridge (HP:0005280) **Full cheeks (HP:0000293)** Pilonidal sinus (HP:0010769)				
P5	6m (f)	**Muscle stiffness (HP:0003552)** **Generalized dystonia (HP:0007325)** **Opisthotonus (HP:0002179)** **Global developmental delay (HP:0001263)** Blue irides (HP:0000635)	Infantile Parkinsonism dystonia	*SLC6A3* uc003jck.3 exon 13 c.1639_1640insC p.H547fs & exon 10 c.1297G>A p.G433R Autosomal recessive (compound het.)	1	Loss of function mutations in SLC6A3 were shown to cause infantile Parkinson dystonia syndrome (*58*). We identified one frameshift mutation and one missense mutation. The glycine residue affected by the missense mutation is conserved in mouse, pufferfish (*T. rubripes*), zebrafish, and frog (*X. tropicalis*) and is predicted disease causing by MutationTaster (*16*).
P6	Fetus (m) Death at 22w of gestation	**Multiple prenatal fractures (HP:0005855)** **Short lower limb (HP:0006385)** **Femoral bowing (HP:0002980)** **Short femur (HP:0003097)** Short humerus (HP:0005792) Premature birth (HP:0001622)	Infantile hypophos-phatasia,	*ALPL* uc010odp.2 exon 9 c.1052G>C p.R351P & exon 10 c.1132G>A	2	Ultrasound findings at the 18th week of pregnancy led to genetic testing. Both *ALPL* mutations have been previously published in individuals with infantile hypophosphatasia (*65-67*).

				p.G378S Autosomal recessive (compound het.)		
P7	7y (m)	Oligohydramnios (HP:0001562) Muscular hypotonia of the trunk (HP:0008936) **Congenital cataract (HP:0000519)** **Glaucoma (HP:0000501)** Optic atrophy (HP:0000648) Esotropia (HP:0000565) **Nystagmus (HP:0000639)** **Visual impairment (HP:0000505)** Band-shaped corneal dystrophy (HP:0007709) Hypermetropia (HP:0000540) **Microphthalmos (HP:0000568)** Finger joint hypermobility (HP:0006094) Joint hypermobility (HP:0001382) Constipation (HP:0002019) Recurrent viral infections (HP:0004429) Deeply set eyes (HP:0000490) Thick lower lip vermilion (HP:0000179) Thick upper lip vermilion (HP:0000215) Short philtrum (HP:0000322) Downslanted palpebral fissure (HP:0000494) Abnormality of the pupil (HP:0000615) Abnormality of the thorax (HP:0000765)	Nance-Horan Syndrome / Cataract 40, X-linked	*NHS* uc004cya.3, exon6, c.2403delC, p.N802fs *de novo* (X chromosomal)	2	Truncating mutations in *NHS* were shown to cause Nance Horan syndrome (*60*). Cosegregation studies in the family demonstrated a de novo frameshift mutation in the affected child.
P8	14y (m)	Macrocephaly (HP:0000256) **Delayed speech and language development (HP:0000750)** **Global developmental delay (HP:0001263)** **Blepharophimosis (HP:0000581)** **Bilateral ptosis (HP:0001488)** **Abnormality of the helix (HP:0011039)** Paroxysmal vertigo (HP:0010532) Inappropriate sexual behavior	Wiedemann-Steiner syndrome	*KMT2A* uc001pta.3 exon 27 c.10343delA p.E3448fs7 *de novo*	1	Multiple nonsense mutations have been identified previously in individuals with Wiedemann-Steiner syndrome (*54*). Cosegregation studies in the family demonstrated a de novo frameshift mutation in the affected child.

		(HP:0008769) Lymphedema (HP:0001004) Hyperhidrosis (HP:0000975) **Epicanthus inversus (HP:0000537)** **Attached earlobe (HP:0009907)** Long toe (HP: 0010511)				
P9	6y (f)	**Global developmental delay (HP:0001263)** **Motor delay (HP:0001270)** **Patent ductus arteriosus (HP:0001643)** Muscular hypotonia (HP:0001252) **Autism spectrum disorder (HP:0000717)** **Seizures (HP:0001250)** Fair hair (HP:0002286) Blue irides (HP:0000635) Sparse scalp hair (HP:0002209)	Mental retardation, autosomal dominant 7	*DYRK1A* uc011aei.2, exon7, c.961C>T, p.Q321* *de novo*	4	Truncating, de novo mutations in DYRK1A were shown to cause intellectual disability with microcephaly and epilepsy (*61*). Cosegregation studies in the family demonstrated a de novo nonsense mutation in the affected child.
P10	4 children between 1 ½ and 7y	**Intellectual disability (HP:0001249)** **Global developmental delay (HP:0001263)** **Hypoplasia of the corpus callosum (HP:0002079)** Feeding difficulties (HP:0011968) Scoliosis (HP:0002650) Brisk reflexes (HP:0001348) Microcephaly (HP:0000252) Low-set ears (HP:0000369) **Retinitis pigmentosa (HP:0000510)**	Type IV mucolipidosis	*MCOLN1* uc002mgp.3, exon9, c.1103G>T, p.R368L Autosomal recessive (hom.)	1	Type IV mucolipidosis is caused by mutations in a gene encoding a novel transient receptor potential channel (*62*). Mutations in transmembrane regions can be disease causing (*68*). The affected arginine residue is conserved in mouse, chicken, pufferfish (*T. rubripes*), zebrafish, fruitfly (*D. melanogaster*), worm (*C. elegans*), and frog (*X. tropicalis*) and is predicted disease causing by MutationTaster (*16*).
P11	3y (m)	Growth delay (HP:0001510), Myoclonic spasm (HP:0003739), Cerebral sinus thrombosis (HP:0005305) Porencephaly (HP:0002132) Feeding difficulties (HP:0011968) Muscular hypotonie (HP:0001252) Delayed speech and language development (HP:0000750) Motor delay (HP:0001270) **Persistent left superior vena cava (HP:0005301)** **Patent foramen ovale (HP:0001655)**	TARP syndrome	*RBM10* uc004dhf.3 exon 11 c.1160G>A p.R387K *de novo* (X chromosomal)	3	TARP syndrome is caused by mutations in *RBM10* (*69*). The mutation occurs in the last nucleotide of exon 11. The mutation, which was shown to be de novo, is predicted to cause a splice defect (*70*).

	Defect in the atrial septum (HP:0001631) Myopia (HP:0000545) Trigonocephaly (HP:0000243) Microcephaly (HP:0000252) Low anterior hairline (HP:0000294) Epicanthus (HP:0000286) Upslanted palpebral fissures (HP:0000582) Hypertelorism (HP:0000316) Downslanted corners of the mouth (HP:0002714) **High palate (HP:0000218)** Wide intermamillary distance (HP:0006610) Absent middle phalanx of the 5th finger (HP:0009162) Short middle phalanx of the 2nd finger (HP:0009577) Broad toe (HP:0001837) Cryptorchidism (HP:0000028)

Table S5. Clinical presentation of 29 patients for whom PhenIX analysis failed to reveal a molecular diagnosis.

Patient ID	Global developmental delay (HP:0001263)	Neuropsychological Features	Musculosceletal Features	Growth abnormalities	Craniofacial abnormalities	Malformations	Skin/connective tissue Features	Eye/Ear Features
P12	+	Autism spectrum disorder (HP:0000729) Stereotypic behaviour (HP:0000733) Sleep-wake cycle disturbances (HP:0006979)						
P13	+	Tremor (HP:0001337)	Slender build (HP:0001533) Arachnodactyly (HP:0001166) Joint laxity (HP:0001388)	Tall stature (HP:0000098)	Microtia (HP:0008551)			
P14	+		Short neck (HP:0000470)	Growth delay (HP:0001510)	Microcephaly (HP:0000252) Brachycephaly (HP:0000248) Synophrys (HP:0000664)		Low anterior hairline (HP:0009890) Thick hair (HP:0100874)	
P15	+	Feeding difficulties (HP:0011968)	Muscular hypotonia of the trunk (HP:0008936)	Short stature (HP:0004322)	Microcephaly (HP:0000252) Brachycephaly (HP:0000248) Thin upper lip vermilion (HP:0000219) Thin lower lip vermilion (HP:0010282)			
P16	+				Macrotia (HP:0000400) Wide nose (HP:0000445)	Vesicoureteral reflux (HP:0000076)		
P17	+	Seizures			Postnatal		Small nail	

		(HP:0001250)			microcephaly (HP:0005484) Everted lower lip vermilion (HP:0000232) Everted upper lip vermilion (HP:0010803)		(HP:0001792)	
P18	+	Tremor (HP:0001337)						
P19	+	Feeding difficulties (HP:0011968) Sleep disturbance (HP:0002360)	Finger joint hyperextensibility (HP:0006158) Hyperextensibility of the knee (HP:0010500) Muscular hypotonia (HP:0001252) Myalgia (HP:0003326) Increased muscle fatiguability (HP:0003750)			Urinary incontinence (HP:0000020)		
P20	+		Broad neck (HP:0000475) Pectus carinatum (HP:0000768)	Growth delay (HP:0001510)	Epicanthus inversus (HP:0000537) Large posterior fontanel (HP:0004491)		Abnormal hair whorl (HP:0010721)	Blepharophimosis (HP:0000581) Congenital sensorineural hearing impairment (HP:0008527)
P21	+	Hypoplasia of the corpus callosum (HP:0002079) Hypoplasia of the frontal lobes (HP:0007333)	Muscular hypotonia (HP:0001252)		Hypertelorism (HP:0000316)			
P22	+	Generalized tonic-clonic seizures (HP:0002069) Stereotypic behaviour (HP:0000733)				Renal agenesis (HP:0000104)	Supernumerary nipples (HP:0002558)	
P23	+	Agenesis of corpus callosum (HP:0001274)	Duplication of phalanx of thumb (HP:0009942) Broad hallux		Microcephaly (HP:0000252)			

			(HP:0010055) 2-3 toe syndactyly (HP:0004691)					
P24	+	Hypoplasia of the corpus callosum (HP:0002079) Feeding difficulties (HP:0011968)	Broad thumb (HP:0011304)	Proportionate short stature (HP:0003508)	Wide cranial sutures (HP:0010537) Large fontanelles (HP:0000239) Hypertelorism (HP:0000316) Downslanted palpebral fissure (HP:0000494)			Congenital conductive hearing impairment (HP:0008591)
P25			Postaxial hand polydactyly (HP:0001162) Postaxial foot polydactyly (HP:0001830) 2-3 toe cutaneous syndactyly (HP:0005709)		Wide mouth (HP:0000154)	Common atrium (HP:0011565)		
P26		Feeding difficulties (HP:0011968)	Short neck (HP:0000470)		Microtia (HP:0008551) Epicanthus inversus (HP:0000537)	Abnormality of the ventricular septum (HP:0001629) Patent foramen ovale (HP:0001655) Cryptorchidism (HP:000028)	Edema of the dorsum of feet (HP:0012098) Palpebral edema (HP:0100540) Cutis laxa (HP:0000973)	Bilateral ptosis (HP:0001488) Myopia (HP:0000545) Periauricular skin pits (HP:0100277)
P27		Seizures (HP:0001250) Specific learning disability (HP:0001328)			Highly arched eyebrow (HP:0002553) Low-set ears (HP:0000369)		Hyperpigmentated/ hypopigmentated macules (HP:0007441)	
P28			Cleft palate (HP:0000175) Short neck (HP:0000470) Long fingers (HP:0100807)		Telecanthus (HP:0000506) Prominent eyelashes (HP:0011231)	Absent uvula (HP:0010292)	Low anterior hairline (HP:0009890) Low posterior hairline (HP:0002162)	Hearing impairment (HP:0000365)
P29			Short metatarsal (HP:0010743) Broad toe (HP:0001837)		Flat nose (HP:0000457)			

			Ectopic calcification (HP:0010766)					
P30	+	Neonatal breathing dysregulation (HP:0002790)	Muscular hypotonia (HP:0001252)	Growth delay (HP:0001510)	Microcephaly (HP:0000252) Flat occiput (HP:0005469) Triangular face (HP:0000325) Broad forehead (HP:0000337) Microtia (HP:0008551) Low-set ears (HP:0000369) Narrow mouth (HP:0000160) Thin upper lip vermilion (HP:0000219) Thin lower lip vermilion (HP:0010282)			Blepharophimosis (HP:0000581)
P31	+	Feeding difficulties (HP:0011968) Inappropriate laughter (HP:0000748)						
P32	+		Long fingers (HP:0100807) Long toes (HP:0010511)		Microcephaly (HP:0000252)			
P33	+		Short neck (HP:0000470) Talipes calcaneovalgus (HP:0001884)	Growth delay (HP:0001510)	Wide anterior fontanel (HP:0000260) Hypertelorism (HP:0000316) Wide nose (HP:0000445)	Interrupted aortic arch type A (HP:0011612) Abnormality of the ventricular septum (HP:0001629)		Bilateral ptosis (HP:0001488)
P34	+		Muscular hypotonia (HP:0001252)		Microcephaly (HP:0000252) Dolichocephaly (HP:0000268) Long face (HP:0000276) Sparse eyebrow		Sparse hair (HP:0008070)	Blepharophimosis (HP:0000581)

					(HP:0000535) Downslanted palpebral fissure (HP:0000494) Telecanthus (HP:0000506)		
P35		Cerebellar vermis hypoplasia (HP:0006855)	Cervical vertrebral fusion (C2/C3) (HP:0004602) Short clavicles (HP:0000894) Camptodactyly of finger (HP:0100490)	Short stature (HP:0004322)	Microtia (HP:0008551) Low-set ears (HP:0000369) Hypertelorism (HP:0000316) Abnormality of the cranial sutures (HP:0011329)		Blepharophimosis (HP:0000581) Hearing impairment (HP:0000365) Hypoplasia of the cochlea (HP:0008586) Stenosis of the external auditory canal (HP:0000402)
P36		Delayed speech and language development (HP:0000750)					Bilateral ptosis (HP:0001488) Blepharophimosis (HP:0000581) Hearing impairment (HP:0000365)
P37		Generalized tonic-clonic seizures (HP:0002069) Hypoplasia of the corpus callosum (HP:0002079)	Congenital finger flexion contracture (HP:0005879) Hip dislocation (HP:0002827) Muscular hypotonia (HP:0001252)	Short stature (HP:0004322)	Microcephaly (HP:0000252) High palate (HP:0000218) Retrognathia (HP:0000278) Tented upper lip vermilion (HP:0010804)		Microphthalmos (HP:0000568) Congenital cataract (HP:0000519) Hearing impairment (HP:0000365)
P38	+	Developmental regression (HP:0002376) Aggressive behaviour (HP:0000718) Self-injurious behaviour (HP:0100716)	Muscular hypotonia (HP:0001252)	Tall stature (HP:0000098)			
P39	+		Arachnodactyly (HP:0001166) Adducted thumb (HP:0001181) Camptodactyly 2nd-		Macrocephaly at birth (HP:0004488) Short philtrum (HP:0000322)		

	5th finger (HP:0001215) Bilateral talipes equinovarus (HP:0001776)	
P40	Missing ribs (HP:0000921) Aplasia/hypoplasia of the radius (HP:0006501) Aplasia/hypoplasia of the ulna (HP:0006495) Oligodactyly (hands) (HP:0001180) Aplasia/hypoplasia of the femur (HP:0002823) Rudimentary to absent tibiae (HP:0006426) Rudimentary to absent fibulae (HP:0004986) Oligodactyly (feet) (HP:0001180)	Microtia (HP:0008551) Wide nose (HP:0000445) Microretrognathia (HP:0000308)