The American Journal of Human Genetics, Volume *88*

Supplemental Data

NEK1 Mutations Cause

Short Rib-Polydactyly Syndrome Type Majewski

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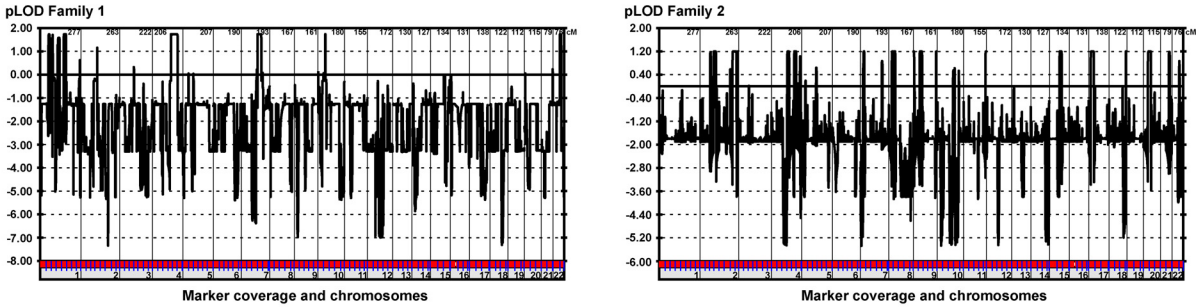
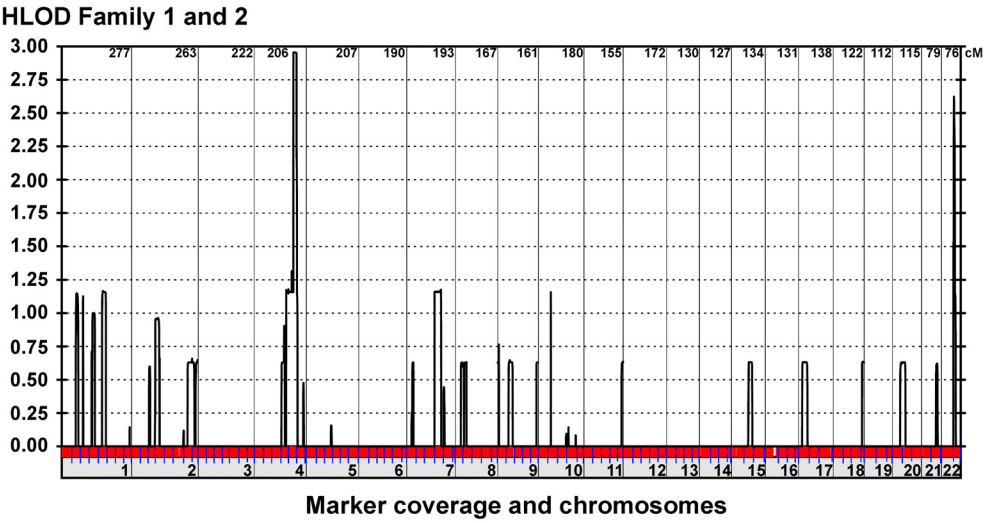
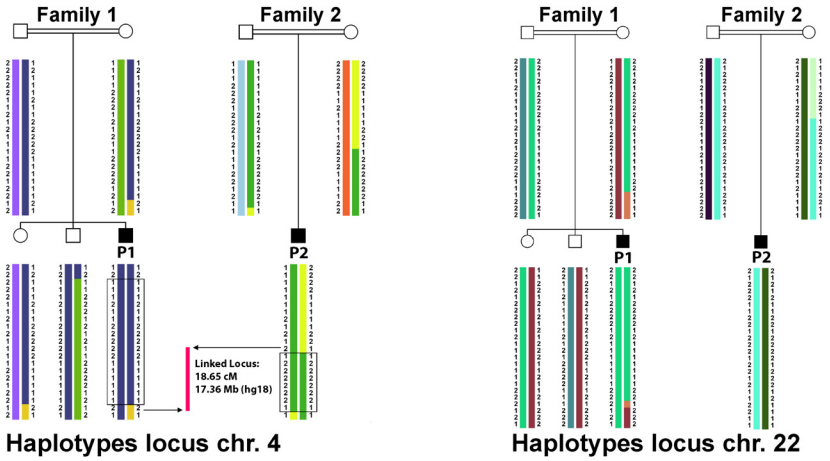
a


pLOD Family 1
pLOD Family 2
Marker coverage and chromosomes
Marker coverage and chromosomes

b


HLOD Family 1 and 2
Marker coverage and chromosomes

c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Family 2
P1
P2
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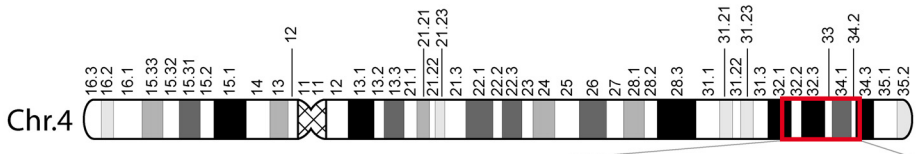
17.36 Mb (hg18)
Haplotypes locus chr. 4
Family 1
Family 2
P1
P2
Haplotypes locus chr. 22

d


Chr.4

e

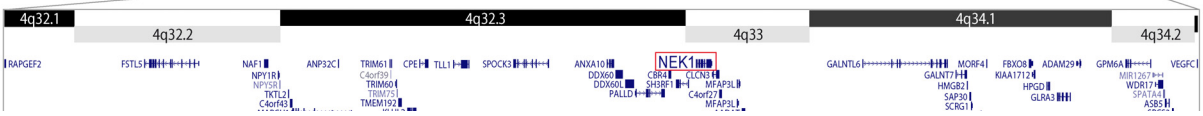
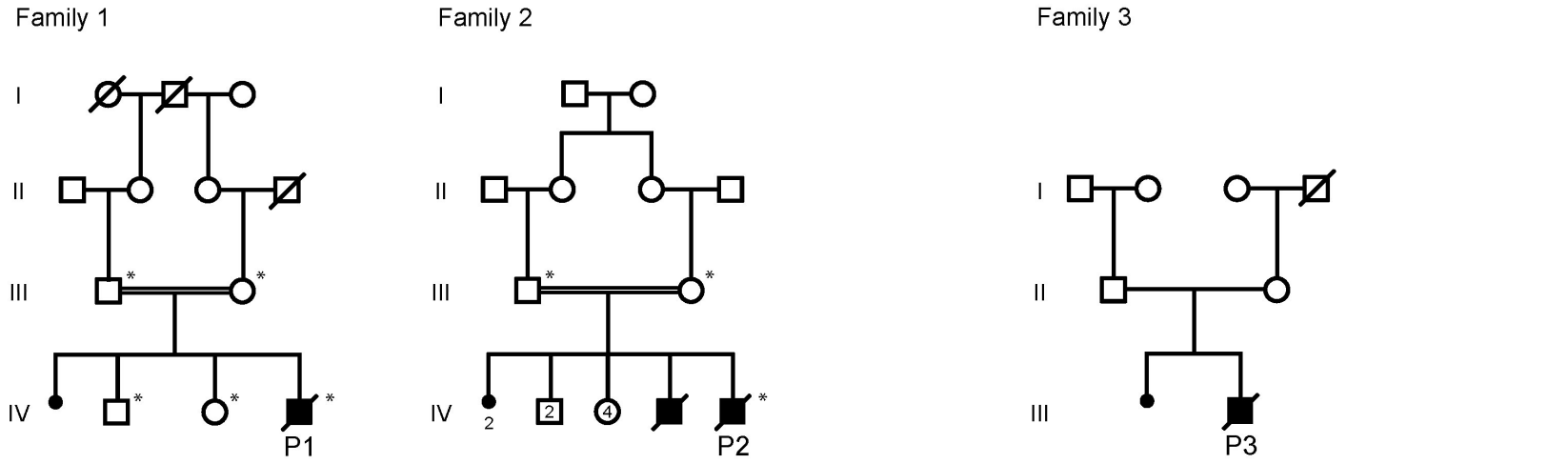

4q32.1
4q32.2
4q32.3
4q33
4q34.1
4q34.2
NEK1

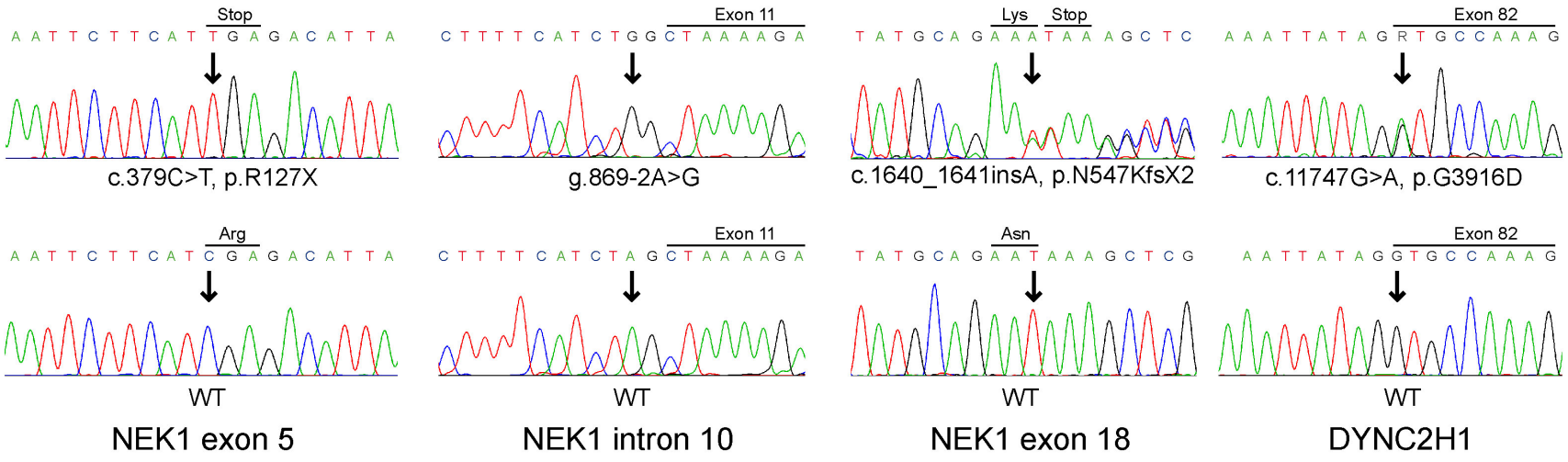
Figure S1. Positional Cloning of *NEK1*

(A) Individual parametric mutlipoint LOD scores for the two consanguineous families 1 and 2. (B) SRPS Majewski type homozygosity mapping detected a linked locus on 4q32.1–34.3 with a HLOD of 2.95 and a second possible locus on chr. 22. (C) Haplotype structure analysis in the two consanguineous families confirmed and refined the locus to 18.65 cM / 17.36 Mb (D, E) including 54 RefSeq and hypothetical genes on chr. 4.

a


Family 1
Family 2
Family 3
I
II
III
IV
P1
P2
P3

b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Exon 11
Lys
Stop
Exon 82
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g.869-2A>G
c.1640_1641insA, p.N547KfsX2
c.11747G>A, p.G3916D
Arg
Exon 11
Asn
Exon 82
WT
WT
WT
WT
NEK1 exon 5
NEK1 intron 10
NEK1 exon 18
DYNC2H1

Figure S2. Pedigrees of Investigated Families and the Respective Mutations in the *NEK1* and *DYNC2H1* Genes

(A) Families 1 and 2 were used for the whole-genome scan. Individuals marked with asterisks were included in the linkage analysis. (B) The individuals of the consanguineous families 1 and 2 were homozygous for a nonsense and a splice/site mutation, respectively. The affected individual of family 3 showed a heterozygous mutation of the *NEK1* and the *DYNC2H1* genes.

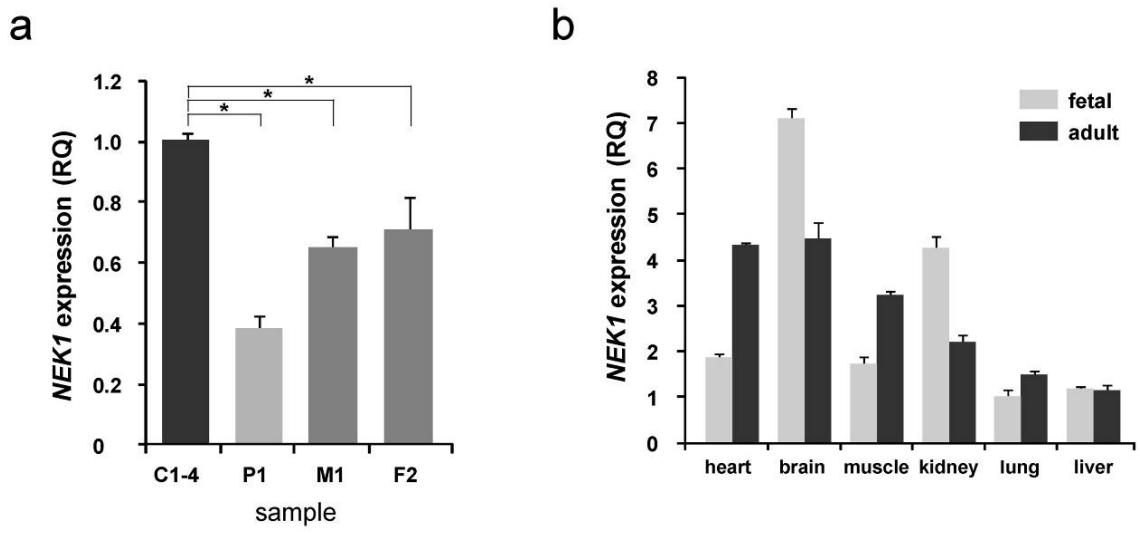

Figure S3. *NEK1* Gene Expression

(A) Relative mRNA expression levels of the *NEK1* gene in lymphoblastoid cell lines of the patient (P1), the mother (M1) , the father (F2) of family 1 and 4 healthy controls (C1-4). Note significant decrease of NEK1 expression levels in the patient and the parents (*t test $p < 0.001$). (B) Relative expression levels of *NEK1* in different human fetal and adult tissues. Note the highest fetal expression levels in the brain and kidney, and the highest adult levels in brain, heart and muscle.

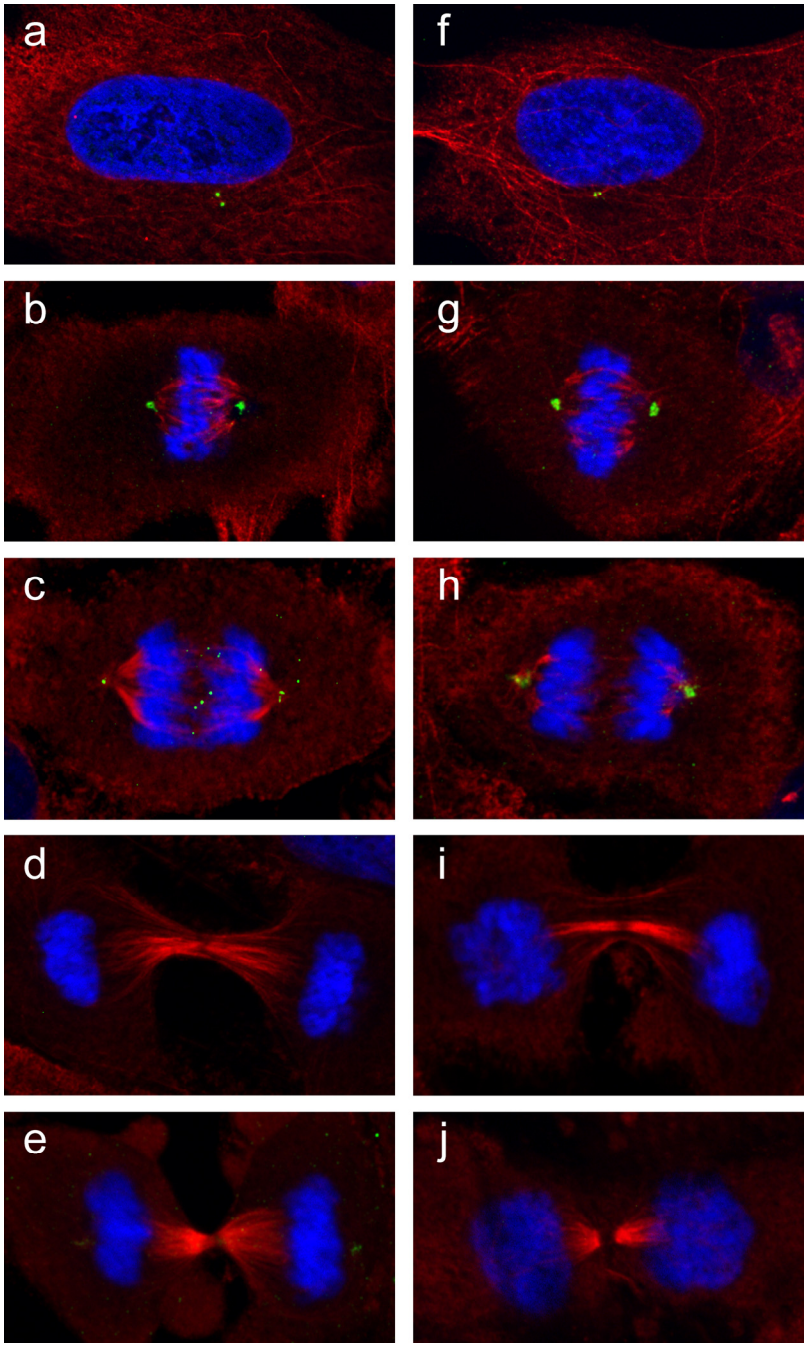

Figure S4. Mitotic Morphology of Patient Fibroblasts

Immunofluorescence images of fibroblast cells with antibodies against Pericentrin (PCNT, green) and α-tubulin (red), and 4′,6′-diamino-2-phenylindole (DAPI) staining of chromosoms (blue). (A-E) Morphology of fibroblasts from a healthy control individual during (A) interphase, (B) metaphase, (C) anaphase, (D) telophase, and (E) cytokinesis. (F-J) Cell cycle morphology of the affected individual P1 [(F) interphase, (G) metaphase, (H) anaphase, (I) telophase, (J) cytokinesis]. Note the same cell cycle morphology of both samples.

Table S1. Main Characteristics of Human Ciliopathies

	Alstrom syndrome[1]	Bardet-Biedl syndrome[2]	Joubert syndrome[3]	Meckel-Gruber syndrome[4]	Nephro-nophthisis[5]	Oral-facial-digital syndrome[6]	Primary Ciliary Dyskinesia[7]	Polycystic kidney disease[8]	Retinitis pigmentosa[9]	Senior-Loken syndrome[10]
Inheritance	autosomal recessive	autosomal recessive	autosomal recessive	autosomal recessive	autosomal recessive	X-linked dominant	autosomal recessive	autosomal recessive / dominant	autosomal / X-linked recessive	autosomal recessive
Genes	ALMS1	BBS1-14	AHI1, NPHP1, NPHP6, TMEM67, RPGRIP1L	MKS1, 3-6	NPHP1-9	OFD1	DNAI1, DNAH5, DNAHI1, DNAI2, TXNDC3, RSPH9, RSPH4A, KTU, LRRC50	PKD1, PKD2, PKHD1	RP1, RPGR	NPHP1, 4 - 6
Brain malformation			+	+		+				
Polydactyly		+	+	+		+				
Kidney disease	+	+	+	+	+	+		+		+
Retinal degeneration	+	+	+		+				+	+
Skeletal abnormalities	+	+		+		+				
Developmental delay	+	+	+			+				
Cardiac anomalies	+	+	+	+						
Hepatic abnormalities	+	+	+	+	+			+		
Short stature	+				+	(+)				
Obesity	+	+	+							
Hearing impairment	+	+					+			
Deafness	+									
Diabetes mellitus / Insulin resistance	+	+								
Male infertility/ Hypogonadism	+	+					+			
Situs inversus		+					+			
Pulmonary disease / recurrent infection							+	(+)		
Vascular abnormalities								+		

Table S2A. SRPSs Classification and Macroscopic Findings

SRPS Classification	Majewski	Non-Majewski						Ellis v. Crefeld
		Saldino-Noonan	Verma-Naumhoff	Jeune	Beemer	Le Marec	Yang	
Sillence[11]	Type II	Type I	Type III		Type IV			
Spranger-Maroteaux[12]	Type VI	Type I	Type II	Type V	Type VII	Type III	Type IV	
Known Genes			*DYNC2H1 IFT80*	*IFT80*				*EVC1 EVC2*
Hydrops	+	+	+		+			
Short stature	+	+	+	+	+	+	+	+
Narrow Thorax	+	+	+	+	+	+	+	(+)
Polydaktyly	pre- and postaxial	postaxial	(postaxial)	(postaxial)	(pre- and postaxial)	postaxial	postaxial	postaxial
Depressed Nasal bridge		+	+					
Median Cleft Palate	+				+			
Additional Facial features		+ [a]			+ [b]			
Hypoplastic Lungs	+	+	+		+		+	
Cardiac abnormalities	(+)	(+)	(+)	(+)	(+)	(+)	+	+
Genito-uretral abnormalities	(+)	+	+	(+)	(+)	(+)	(+)	(+)
Cerebral abnormalities	(+)	(+)	+		+ [c]	+		

Note. [a] Retrognathia, [b] Prominent forehead, flat face, hypertelorism, [c] Holoprosencephaly, Anencephaly

Table S2B. SRPSs Radiographic Characteristics

SRPS Classification	Majewski	Non-Majewski						Ellis v. Crefeld
		Saldino-Noonan	Verma-Naumhoff	Jeune	Beemer	Le Marec	Yang	
Rib anomaly	+	+	+	+	+	+	+	+
Platyspondyly		+	+			+		
Pelvis								
small				+		+	+	
small iliac bones only		+	+		+			
flattened acetabular roof		+	+					
Limbs								
Short tubular bones	+							
"torpede shaped" bones		+	+					+
Incomplete ossification		+	+					
"banana-peel" metaphysis			+	+		+	+	
Bowed radius / ulna					+	+	+	
Shortening of the tibia	+	(+)						+
Incomplete ossification of the metacarpal/ metatarsal bones		+	+					[a]
Further anomalies								[b]

Note. [a] Additional carpal bone, fusion of os capitatum and hamatum, [b] Hypoplastic nails

Table S3. Results of Candidate Gene Priorisation (ENDEAVOUR Software)

	Global prioritization		Annotation EnsemblEst		Annotation GeneOntology		Annotation Interpro		Annotation Kegg		Annotation Swissprot		Blast		CisRegModule		Expression SonEtAl		Expression SuEtAl		Interaction Bind		Interaction BioGrid		Interaction Hprd		Interaction InNetDb		Interaction Intact		Interaction Mint		Interaction String		Motif		Precalc. Ouzounis		Precalc. Prospectr		Text	
Gene symbol	score	rank	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score	rank	score
HMGB2	1	0.0166	9	1.14E-25	3	1.39E-35	4	1	12	none	5	0.0276	11	86.7	23	none	18	0.798	4	0.646	1	1.5	2	13	6	13	8	4.65	1	1.5	2	none	7	1.9	7	0.946	12	0.454	19	0.52	11	0.571
NEK1	2	0.0397	4	1.76E-26	13	6.63E-14	12	1	17	none	3	0.00326	2	2.00E-11	12	none	4	0.622	13	0.768	3	5	4	none	5	4.67	2	1.75	3	6	3	none	9	1.94	14	0.967	21	0.71	22	0.59	5	0.407
SC4MOL	3	0.0602	7	7.03E-26	12	2.22E-14	11	1	3	1	12	1	23	666	2	0.0482	13	0.734	15	0.8	15	none	16	none	2	2	9	5.88	2	2	1	2	16	3.2	2	0.842	8	0.375	13	0.475	22	0.734
CPE	4	0.062	5	6.45E-26	20	0.00122	19	1	5	1	14	1	8	22.4	8	0.0891	14	0.735	8	0.72	9	none	10	none	7	18	5	3.04	4	10	4	none	8	1.92	16	0.97	7	0.352	12	0.474	15	0.648
CLCN3	5	0.0912	6	7.03E-26	4	8.75E-23	5	1	13	none	6	0.0276	27	982	6	0.059	3	0.609	10	0.741	11	none	12	none	14	none	7	3.39	9	none	9	none	13	2.06	4	0.939	1	0.217	6	0.402	19	0.704
TLL1	6	0.114	16	9.85E-23	28	0.347	27	1	27	none	9	0.0276	5	0.00001	9	0.131	23	none	26	none	26	none	26	none	26	none	14	24	14	none	14	none	6	1.56	6	0.944	4	0.324	1	0.225	13	0.631
PALLD	7	0.147	1	1.22E-27	1	3.22E-67	2	1	10	none	27	none	22	665	28	none	11	0.726	23	none	23	none	23	none	24	none	25	none	25	none	25	none	12	2	22	0.979	16	0.588	18	0.5	8	0.499
KLHL2	8	0.164	13	3.91E-25	2	2.01E-40	3	1	11	none	10	0.0635	25	700	1	0.0395	7	0.704	21	none	21	none	21	none	22	none	1	1.69	5	none	5	none	5	1.48	25	0.983	20	0.703	5	0.393	16	0.672
C4orf27	9	0.195	17	1.23E-22	9	1.83E-16	28	none	28	none	26	1	16	335	27	none	5	0.633	1	0.596	2	2	3	none	1	2	15	none	15	none	15	none	23	none	23	0.98	11	0.45	16	0.477	7	0.494
TKTL2	10	0.217	11	3.54E-25	14	1.19E-10	13	1	4	1	13	1	21	529	16	none	25	none	27	none	27	none	27	none	27	none	27	none	27	none	27	none	15	2.4	18	0.975	6	0.333	10	0.447	1	0.307
GK3P	11	0.227	18	1.37E-21	6	5.94E-21	6	1	14	none	4	0.00378	10	57.1	22	none	27	none	12	0.756	13	none	14	none	16	none	21	none	21	none	21	none	20	8.5	19	0.976	14	0.483	11	0.457	28	none
SH3RF1	12	0.25	15	4.17E-25	15	3.82E-08	14	1	18	none	20	1	4	0.00000141	5	0.0561	16	0.749	24	none	24	none	24	none	3	2.67	16	none	16	none	16	none	24	none	12	0.965	15	0.583	4	0.392	6	0.474
GALNT7	13	0.282	8	1.14E-25	21	0.00122	20	1	6	1	15	1	17	381	10	0.168	20	0.849	2	0.601	4	none	5	none	9	none	3	2.05	6	none	6	none	17	3.33	1	0.821	17	0.592	7	0.418	23	0.75
SAP30	14	0.298	3	1.46E-26	5	3.31E-21	1	0.564	9	none	18	1	28	none	3	0.0507	2	0.579	3	0.619	5	none	6	none	8	19.5	11	8.33	11	none	11	none	14	2.3	21	0.979	9	0.399	27	0.686	21	0.726
CBR4	15	0.348	14	4.17E-25	26	0.084	25	1	25	none	25	1	19	496	15	none	15	0.737	5	0.654	6	none	7	none	10	none	10	7	10	none	10	none	19	6.6	5	0.94	10	0.449	9	0.443	3	0.337
TRIM75	16	0.362	28	none	18	2.85E-07	17	1	21	none	28	none	1	1.41E-11	18	none	26	none	28	none	28	none	28	none	28	none	28	none	28	none	28	none	28	none	20	0.978	28	none	2	0.361	27	none
GK	17	0.377	26	none	7	5.94E-21	7	1	1	1	1	5.08E-09	9	57.1	13	none	12	0.727	11	0.754	12	none	13	none	15	none	4	2.57	7	none	7	none	22	none	3	0.854	25	none	21	0.567	24	0.756
SPOCK3	18	0.386	20	1.02E-20	25	0.0118	24	1	24	none	24	1	12	156	7	0.0726	6	0.647	14	0.769	14	none	15	none	17	none	6	3.25	8	none	8	none	10	2	17	0.973	27	none	8	0.427	9	0.547
HAND2	19	0.448	19	1.73E-21	8	3.70E-19	8	1	15	none	19	1	20	528	4	0.0517	17	0.778	19	0.902	19	none	1	6	4	4.67	17	none	17	none	17	none	3	1.42	10	0.951	3	0.262	25	0.617	18	0.695
MFAP3L	20	0.512	2	3.61E-27	19	0.000292	18	1	22	none	22	1	26	727	11	0.195	10	0.726	7	0.702	8	none	9	none	12	none	19	none	19	none	19	none	25	none	13	0.966	26	none	15	0.475	10	0.558
AADAT	21	0.533	10	1.59E-25	10	1.54E-15	9	1	2	1	11	1	7	8.83	21	none	22	0.917	25	none	25	none	25	none	25	none	26	none	26	none	26	none	18	3.38	24	0.98	13	0.463	14	0.475	2	0.33
TRIM60	22	0.6	25	0.00672	17	2.85E-07	16	1	20	none	2	1.56E-07	3	3.87E-07	19	none	1	0.486	20	none	20	none	20	none	21	none	23	none	23	none	23	none	26	none	8	0.95	24	0.762	23	0.59	4	0.379
ANXA10	23	0.603	23	1.49E-12	11	3.54E-15	10	1	16	none	7	0.0276	13	231	24	none	19	0.838	18	0.887	18	none	19	none	20	none	12	10	12	none	12	none	1	1.25	9	0.95	23	0.745	17	0.482	17	0.673
NPY1R	24	0.741	21	7.65E-19	24	0.00173	23	1	8	1	17	1	24	699	17	none	21	0.854	17	0.844	17	none	18	none	19	none	13	24	13	none	13	none	2	1.36	27	0.991	2	0.243	3	0.392	12	0.617
MARCH1	25	0.744	12	3.55E-25	22	0.00122	21	1	23	none	23	1	14	264	25	none	9	0.726	9	0.721	10	none	11	none	13	none	20	none	20	none	20	none	11	2	11	0.963	19	0.615	24	0.615	26	0.875
TRIM61	26	0.871	22	1.06E-14	16	2.85E-07	15	1	19	none	21	1	6	0.008	20	none	8	0.715	22	none	22	none	22	none	23	none	24	none	24	none	24	none	27	none	15	0.968	22	0.725	26	0.643	20	0.72
NPY5R	27	0.883	24	6.95E-10	23	0.00135	22	1	7	1	16	1	18	451	14	none	24	none	6	0.699	7	none	8	none	11	none	18	none	18	none	18	none	4	1.45	28	0.992	5	0.329	20	0.566	14	0.636
ANP32C	28	0.994	27	none	27	0.181	26	1	26	none	8	0.0276	15	293	26	none	28	none	16	0.817	16	none	17	none	18	none	22	none	22	none	22	none	21	10.3	26	0.983	18	0.595	28	0.909	25	0.777

Note. Red box indicating the *NEK1* gene.

Table S4A. *In silico* Splice Site Prediction Analysis of Identified Variants

Gene	Nucleotide change	Amino acid change	Position	HSF 2.4		Splice-View		NetGene2		BDGP	
				wild-type	mutated	wild-type	mutated	wild-type	mutated	wild-type	mutated
NEK1	c.869-2A>G	none	acceptor	score 82.88	score 53.93	score 84	**no site**	score 0.62	**no site**	score 0.98	**no site**
	c.2680+5G>A	none	donor	score 90.24	score 78.08	score 84	score 77	score 0.63	**no site**	score 0.99	score 0.50
DYNC2H1	c.11747G>A	p.Gly3916Asp	acceptor	n.d.	n.d.	score 79	score 77	score 0.25	score 0.19	score 0.87	score 0.75

Note. Donor and acceptor splice site prediction for nucleotide changes in *NEK1* and *DYNC2H1* were calculated using the programs HSF 2.4, Splice-View, NetGene2, and Berkeley Drosophila Genome Project (BDGP). High and low scores indicate strong and weak recognition splice sites, respectively. Loss of splice site is denoted by "no site".

Table S4B. *In silico* Protein Analysis of Amino Acid Substitutions

Gene	Nucleotide change	Amino acid change	SIFT		PolyPhen		Panther	
			score	prediction	score	prediction	score	prediction
NEK1	c.379C>T	p.Arg127X	N/A	N/A	N/A	N/A	N/A	N/A
	C.1640dup	p.Asn547LysfsX2	N/A	N/A	N/A	N/A	N/A	N/A
DYNC2H1	c.11747G>A	p.Gly3916Asp	0.72	tolerated	0.227	benign	**-4.06593**	**0.74382**

Note. Amino acid substitutions were analyzed by the SIFT, PolyPhen, and PANTHER programs. When the program offered a choice of parameter settings, defaults were used. Tolerated and benign indicate that the amino acid substitution is unlikely to affect protein function. Panther: A cutoff of -3 corresponds to a 50% probability that a score is deleterious.

Table S4C. Open Reading Frame of Nonsense Mutations

Gene	Nucleotide change	Amino acid change	mRNA	Protein	Domains
NEK1	wild type	wild type	3777 bp	1258 aa	Kinase, basic, Coiled-coil, nuclear export sequence 1 &2
	c.379C>T	p.Arg127X	381 bp	126 aa	N-terminal part of kinase domain
	C.1640dup	p.Asn547LysfsX2	1644 bp	547 aa	Kinase, basic, n-terminal part of coiled-coil

Supplemental References

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2. Beales, P.L., Elcioglu, N., Woolf, A.S., Parker, D., and Flinter, F.A. (1999). New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. J Med Genet 36, 437-446.
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