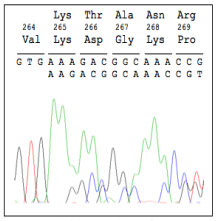
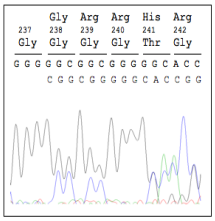
Supplementary Figure S1 Sanger Sequencing results of NKX2-1 intragenic mutations No. 11-27.

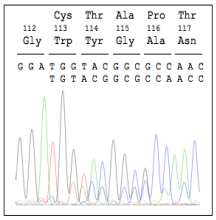
Reference sequence Isoform 2 REFSEQ NM_003317.3



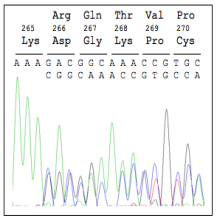
case 11 c793delA / p.Lys265LysfsX87



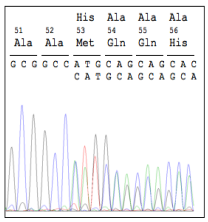
case 12 c.712delGG / p.Gly238ArgfsX170



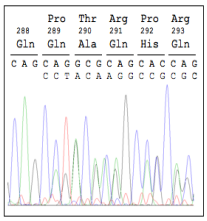
case 13 c.339delG / p.Trp113CysfsX16



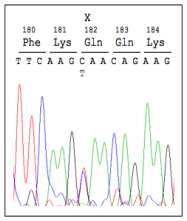
case 14 c.796delGA / p.Asp266ArgfsX142



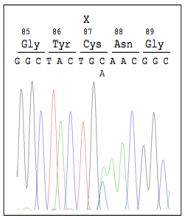
case 15 c.157insC / p.Met53HisfsX355



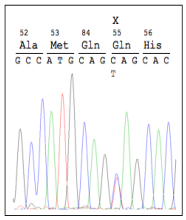
case 16 c.866_883delinsCTACA / p.Gln289ProfsX59



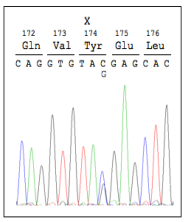
case 17 c.544C>T / p.Gln182X



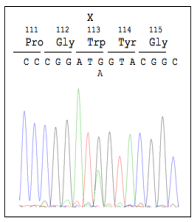
case 18 c.261C>A / p.Cys87X



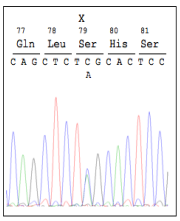
case 19 c.160C>T / p.Gln55X



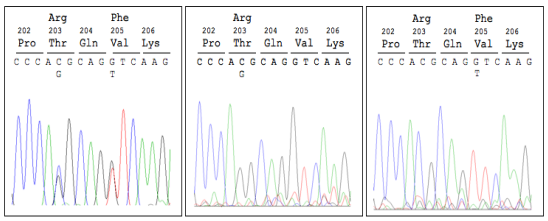
case 20 c.522C>G / p.Tyr174X



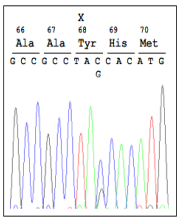
case 21 c.338G>A / p.Trp113XX



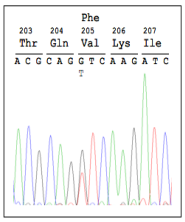
case 22 c.236C>A / p.Ser79X



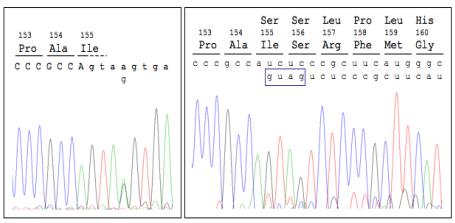
case 24 [c.608C>G]+[c.613G>T] / p.[Thr203Arg]+[Val205Phe]



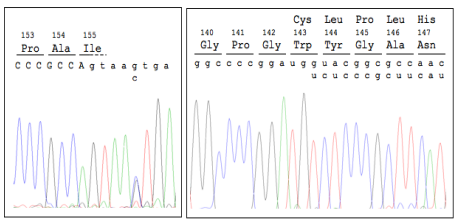
case 23 c.204C>G / p.Tyr68X



case 25 c.613G>T / p.Val205Phe



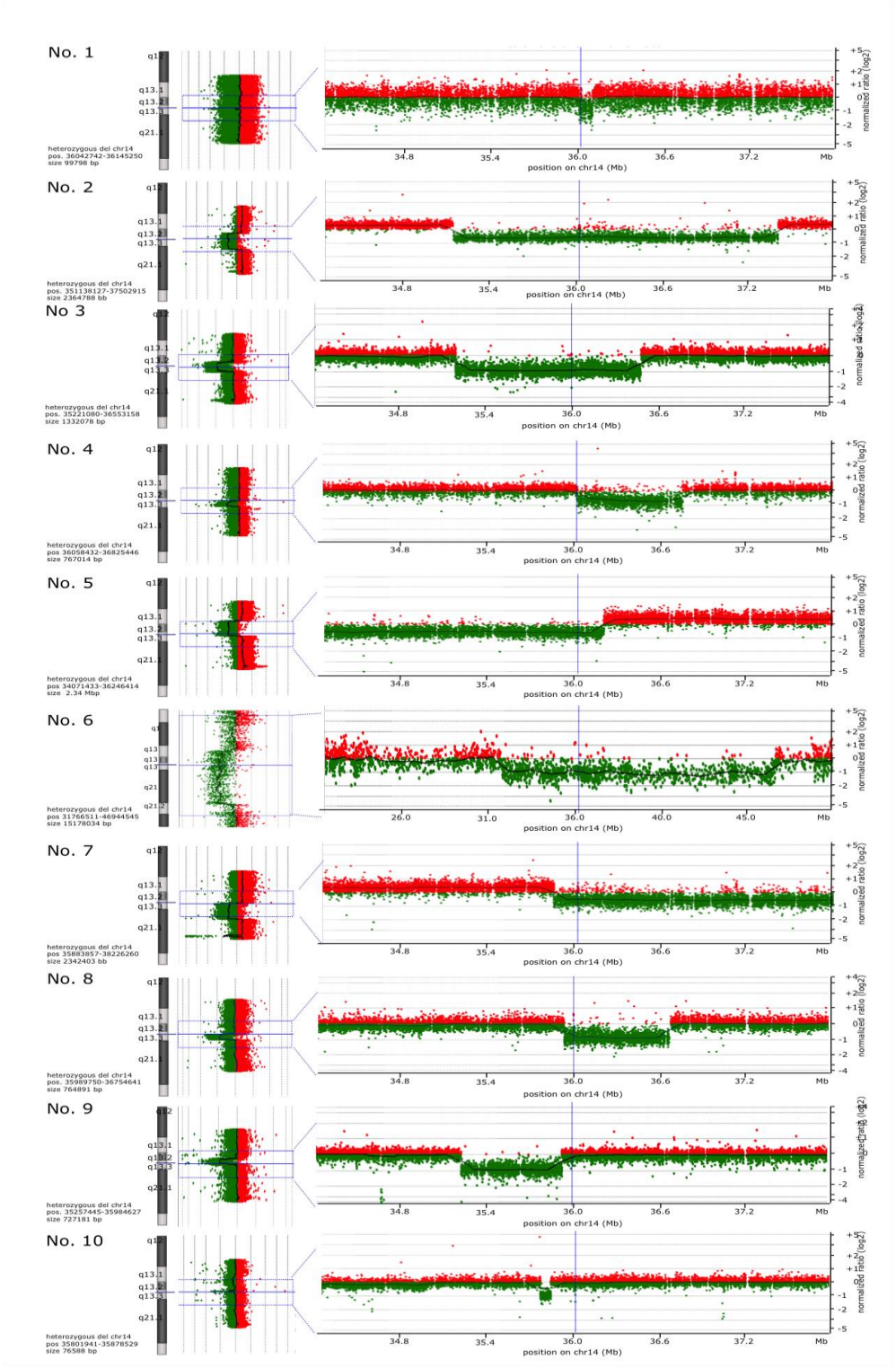
case 26 c.373+4A<G / r.373ins4 / p.Ile155SerfsX126



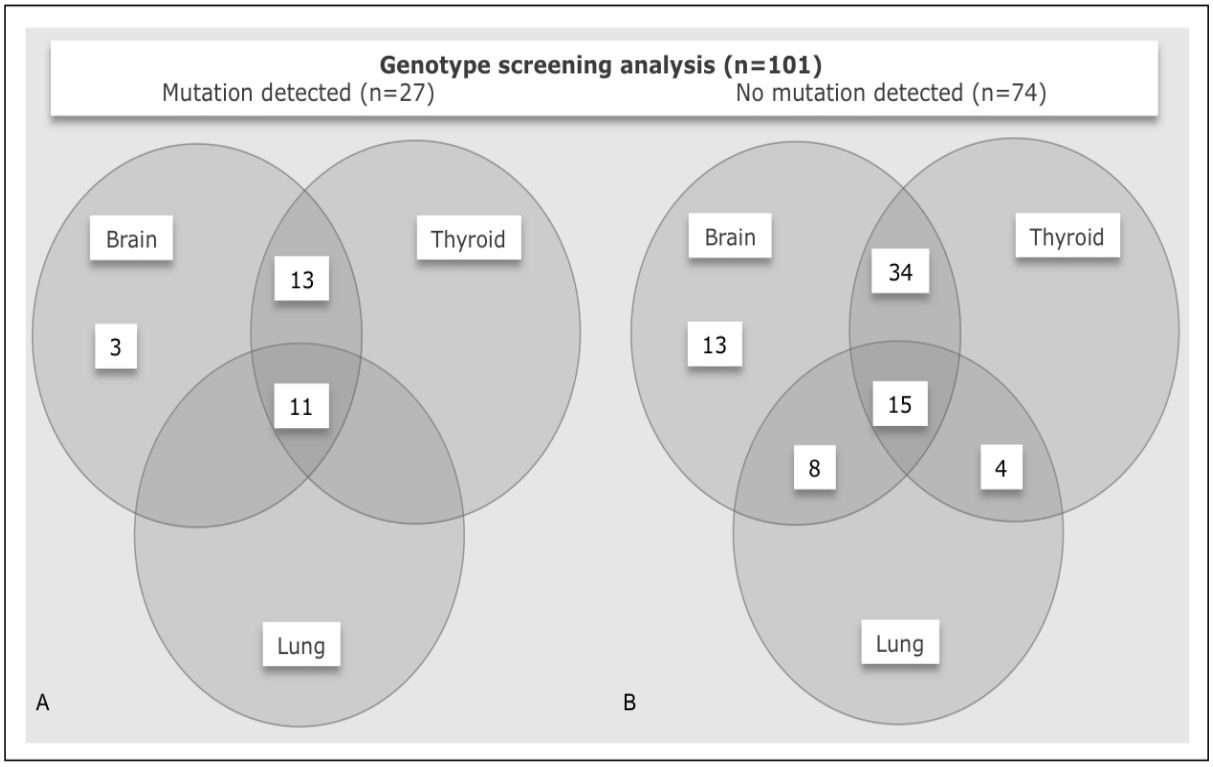
case 27 c.373+5G<C / r.338_373del35 / p.Trp143CysfsX138

Supplementary Figure S2: CNVs detected by high resolution Array-CGH.

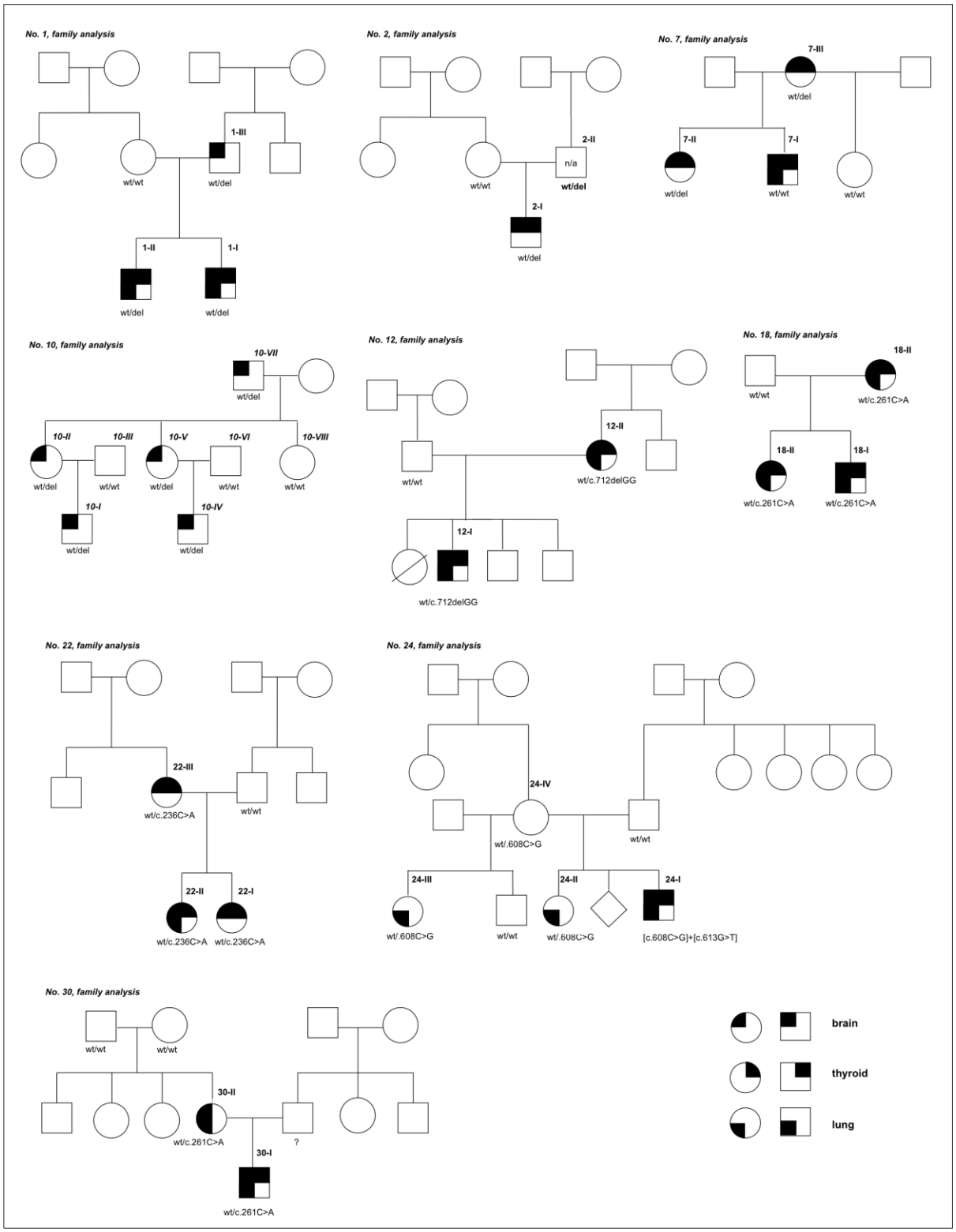
Array CGH results are given as log2 ratio profiles for deletions No. 1-8 for the whole genomic interval covered by our custom oligonucleotide array (left) and as a close-up with focus on *NKX2-1* (right). The blue line, horizontal in the overview and vertical in the zoom-in, pinpoints the position of *NKX2-1*. Size and coordinates of CNVs are given to the left (Hg18).



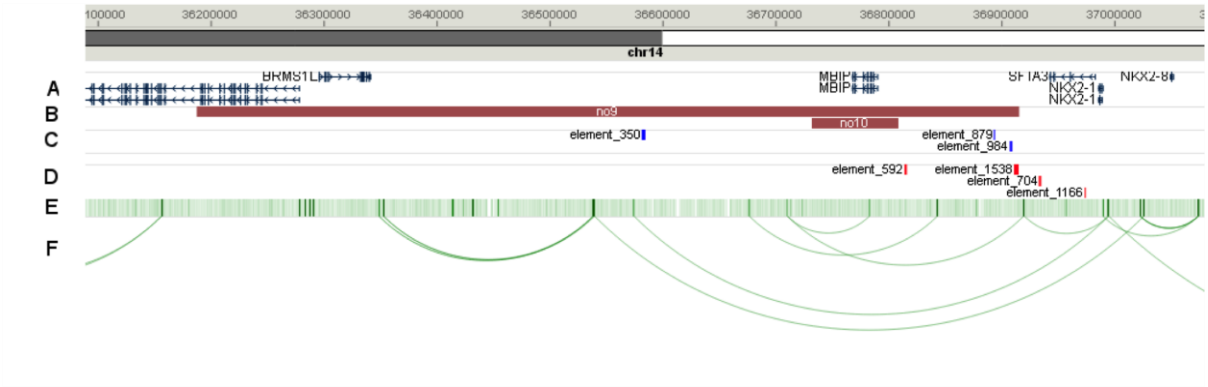
Supplementary Figure S3: Brain, thyroid and lung involvement in index patients and family members Venn diagrams demonstrating that symptom involvement is identical in A index cases (n=32), including also the 5 index patients of our previous study [8] to that of B family members (n=18), except 3 patients with isolated asthma phenotype.



Supplementary Figure S4: Pedigrees demonstrating autosomal dominant mode of inheritance



Supplementary Figure S5: Enhancers and CTCF binding sites near NKX2-1



Supplementary Table 1 Overview of reported *NKX2-1* mutations

DNA sequence		Protein localisation	Protein level		Inheritance	Phenotype of index patient				Reference
as quoted in reference	according to HGNC		as quoted in reference	according to HGNC		Affected family members*	Thyroid dysfunction	Choreo-athetosis/ Muscular hypotonia	Pulmonary malfunction	
del 14q13-21	del 14q13-21	entire protein		p.0	AD	F 1	yes	yes	yes	[6]
del 14q12-13.3	del 14q12-13.3	entire protein		p.0	*de novo*	F 1	yes	yes	yes	[7]
del 14q13.1(1.2 Mb)	del 14q13.1(1.2 Mb)	entire protein		p.0	*de novo*	F 1	n/a	yes	n/a	[10]
del 14q11.2-13.3	del 14q11.2-13.3	entire protein		p.0	*de novo*	M 1	yes	yes	yes	[8]
del 14q12-13.3(3.5 Mb)	del 14q12-13.3(3.5 Mb)	entire protein		p.0	n/a	M 1	n/a	yes	yes	[11]
del 14q13.(0.9 Mb)	del 14q13.(0.9 Mb)	entire protein		p.0	AD	M 3	yes	yes	no	[12]
del 14q13	del 14q13	entire protein		p.0	*de novo*	F 1	yes	yes	yes	[13]
del NKX2.1	del NKX2.1	entire protein		p.0	AD	M 2	yes	yes	yes	[14]
del NKX2.1	del NKX2.1	entire protein		p.0	*de novo*	F 1	yes	yes	no	[14]
14q13.2q22.1 deletion (13.8 Mb)	del 14q13.2-q22.1 (13.8 Mb)	entire protein		p.0	*de novo*	F 1	no	yes	no	[15]
14q13.2q21.2 deletion (6.2 Mb)	del 14q13.2-q21.2 (6.2 Mb)	entire protein		p.0	*de novo*	F 1	yes	yes	no	[15]
14q13.3 deletion (0.3 Mb)	del 14q13.3 (0.3 Mb)	entire protein		p.0	*de novo*	F 1	yes	yes	no	[15]
chr14:g(35519972 2_38886152)del (3.69 Mb)	chr14:g(35519972 2_38886152)del (3.69 Mb)	entire protein		p.0	*de novo*	F 1	yes	yes	no	[16]
chr14:g(36010235_36727597)del (717 kb)	chr14:g(36010235_36727597)del (717 kb)	entire protein		p.0	*de novo*	M 1	yes	yes	yes	[16]
chr14:g(36114849_36219911)del (105 kb)	chr14:g(36114849_36219911)del (105 kb)	entire protein		p.0	*de novo*	M 1	no	n/a	n/a	[16]
chr14:g(34077461_43971142)del (9.89 Mb)	chr14:g(34077461_43971142)del (9.89 Mb)	entire protein		p.0	*de novo*	F 1	yes	yes	yes	[16]
chr14:g(33670261_40648438)del (6.98 Mb)	chr14:g(33670261_40648438)del (6.98 Mb)	entire protein		p.0	n/a	F 1	yes	yes	yes	[16]
chr14:g(24383933_41340975)del (16.96 Mb)	chr14:g(24383933_41340975)del (16.96 Mb)	entire protein		p.0	*de novo*	F 1	yes	n/a	yes	[16]
chr14:g(29701682_45144380)del (15.44 Mb)	chr14:g(29701682_45144380)del (15.44 Mb)	entire protein		p.0	n/a	F 1	yes	yes	yes	[16]
del 14q13.1-q21.1	del 14q13.1-q21.1	entire protein		p.0	n/a	F 1	yes	yes	yes	[32]
del 14q13.3	del 14q13.3	entire protein		p.0	n/a	F 1	yes	yes	yes	[32]
del 14q13.-q21.1	del 14q13.-q21.1	entire protein		p.0	n/a	F 1	yes	yes	yes	[32]
del 14q13.-q21.1	del 14q13.-q21.1	entire protein		p.0	n/a	M 1	yes	yes	yes	[32]
del 14q13.3.	del 14q13.3	entire protein		p.0	n/a	M 1	yes	yes	yes	[32]
2595insGG	c.585nsGG	HD	fs>truncated protein	p.Leu195GlyfsX3	de novo	M 1	yes	yes	yes	[8]
825delC	c.825delC	NK2-STOPP/ C-Term	missense protein	p.Pro275ProfsX76	AD	F 2	yes	yes	no	[18]
c.470_479delinsGCG	c.470_479delinsGCG	TN-HD	p.157fsX196	p.Pro157fsX196	*de novo*	M 1	yes	yes	yes	[19]

c.278_306del29	c.188_216del29	TN-HD	p.Ala93GlyfsX54	p.Ala63GlyfsX54	*de novo*	M 1	yes	n/a	yes	[20]
c.750delTCAGC	c.750delTCAGC	HD-NK2	frameshift	p.Ala250AlafsX165	AD	M 3	yes	yes	yes	[14]
c.399delC	c.399delC	TN-HD	n/a	p.Ser133SerfsX3	*de novo*	F 1	yes	yes	yes	[15]
c.786_787del	c.786_787del	NK2	n/a	p.Ala259GlyfsX149	*de novo*	F 1	yes	yes	yes	[15]
c.493delC	c.493delC	HD	p.Arg165GlyfsX32	p.Arg165GlyfsX32	AD	F 3/M 2	yes	yes	yes	[21]
c.384_391del 8(GCCGTACC)	c.294_301delGCCGTACC	TN-HD	p.Pro129GlyfsX307	p.Pro99GlyfsX307	n/a	F 1	yes	yes	yes	[32]
c.552_556 del (CCCGC)	c. 562_566delCCCGC	TN-HD	p.Pro185AlafsX250	p.Pro155AlafsX250	n/a	F 1	yes	yes	yes	[32]
c.804_812dupCGGCGGGG G	c.714_722dupCGGCGGGG G	HD-NK2	p.Gly269_271dupGlyGly Gly	p.Gly239_241dupGlyGly Gly	n/a	M 2 (unrelated)	no	yes	yes	[32]
c.818_838del21 & c.1034_1047del14	c.728_748del21 & c.944_957del14	NK2-STOPP/ C-Term	p.273_279delGCPQQQQ &p.His346Alafs	p.243_249delGCPQQQQ &p.His316Alafs	n/a	M 1	yes	yes	yes	[32]
c.1044_1045insC	c.954_955insC	NK2-STOPP/ C-Term	p.His349ProfsX90	p.His319ProfsX90	n/a	M 1	yes	yes	yes	[32]
c.1092_1108del17 (CGCCAGCCCCGCGGCGC)	c.1002_1018delCGCCAGCC CCGCGGCGC	NK2-STOPP/ C-Term	p.Ser366GlyfsX67	p.Ser336GlyfsX67	n/a	M 1	yes	no	yes	[32]
c.1157_63 dupACTACGG;	c.1067_63dupACTACGG;	NK2-STOPP/ C-Term	p.Thr389LeufsX52	p.Thr359LeufsX52	n/a	F 1	yes	yes	yes	[32]
C2519A	c.506C>A	HD	SerHDpos9X	p.Ser169X	n/a	M 1	yes	yes	no	[8]
C1302A	c.261C>A	TN-HD	CysHDpos-75X	p.Cys87X	n/a	M 1	yes	yes	yes	[8]
C609A	c.434C>A	TN-HD	S145X	p.Ser145X	*de novo*	F 1/M 2	yes	yes	yes	[26]
c.342C>G	c.342C>G	TN-HD	p.Y114X	p.Tyr114X	n/a	M 1	yes	yes	no	[14]
c.257dupA	c.257dupA	TN-HD	p.Tyr86X	p.Tyr86X	AD	F 3	yes	yes	yes	[15]
c.642C>A	c.642C>A	HD	p.Tyr214X	p.Tyr214X	AD	M 3/F 1	yes	yes	no	[15]
c.G334>T	c.33aG>T	TN-HD	Gly112X	p.Gly112X	AD	M 1	yes	yes	yes	[33]
c.432C>A	c.342C>A	TN-HD	p.Tyr144X	p.Tyr114X	n/a	F 1	yes	yes	yes	[32]
C727A	c.637C>A	HD	R243S	p.Arg213Ser	AD	F 11/M 2	n/a	yes	n/a	[10]
G713T	c623G>T	HD	W238L	p.Trp208Leu	AD	F 6/M 16	n/a	yes	n/a	[10]
G2626T	c.613G>T	HD	ValHDpos45Phe	p.Val205Phe	*de novo*	M 1	yes	yes	yes	[8]
c>g	c.526C>G	HD	L176V	p.Leu176Val	*de novo*	M 1	no	yes	no	[13]
c>t	c.605C>T	HD	P202L	p.Pro202Leu	*de novo*	F 1	yes	yes	no	[13]
a>c	c.629A>C	HD	Q210P	p.Gln210Pro	*de novo*	F 1	yes	yes	no	[13]
ATC_TTC	c.619T>A	HD	I207F	p.Ile207Phe	*de novo*	F 1	yes	n/a	yes	[27]
c.638G<C	c.638G<C	HD	p.Arg213Pro	p.Arg213Pro	AD	M 2	no	yes	no	[15]
	c.581T>G	HD	p.Leu194Arg	p.Leu194Arg	AD	F 3/M 5	yes	yes	yes	[15]
c.526C>G	c.526C>G	HD	p.Leu176Arg	p.Leu176Arg	*de novo*	M 1	yes	yes	yes	[15]
c.553T>G	c.553T>G	HD	p.Tyr185Asp	p.Tyr185Asp	*de novo*	F 1	yes	yes	no	[15]

c.629A>C	c.629A>C	HD	p.Gln210Pro	p.Gln210Pro	*de novo*	F 1	yes	yes	no	[15]
c.621C > G	c.621C > G	HD	p.Ile207Met	p.Ile207Met	n/a	F 1	yes	n/a	yes	[28]
c.583C>T	c.493C>T	HD	p.Arg195Trp	p.Arg165Trp	n/a	M 1	yes	yes	yes	[32]
c.590T>C	c.500T>C	HD	p.Leu197Pro	p.Leu167Pro	n/a	M 1	no	yes	yes	[32]
c.592T>C	c.502T>C	HD	p.Phe198Leu	p.Phe168Leu	AD	F 4	no	no	yes	[32]
c.592T>C	c.502T>C	HD	p.Phe198Leu	p.Phe168Leu	n/a	F 1	no	no	yes	[32]
c.594C>G	c.504C>G	HD	p.Phe198Leu	p.Phe168Leu	n/a	F 1	no	no	yes	[32]
	c.373+1_373+4del	TN-HD (splice acceptor site)		r.spl?	AD	F 3	yes	yes	(yes)	[15]

Nomenclature for mutations according to recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/); Reference sequence REFSEQ Isoform 2 NM_003317.3, P entire protein HD Homeodomain NK2 NK2-specific domain AD autosomal-dominant n/a information not available TN-HD sequence between Tin- and Homeodomain; *including index patient