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Supplemental Data

A Mutation of *COX6A1* Causes a Recessive Axonal or Mixed Form of Charcot-Marie-Tooth Disease

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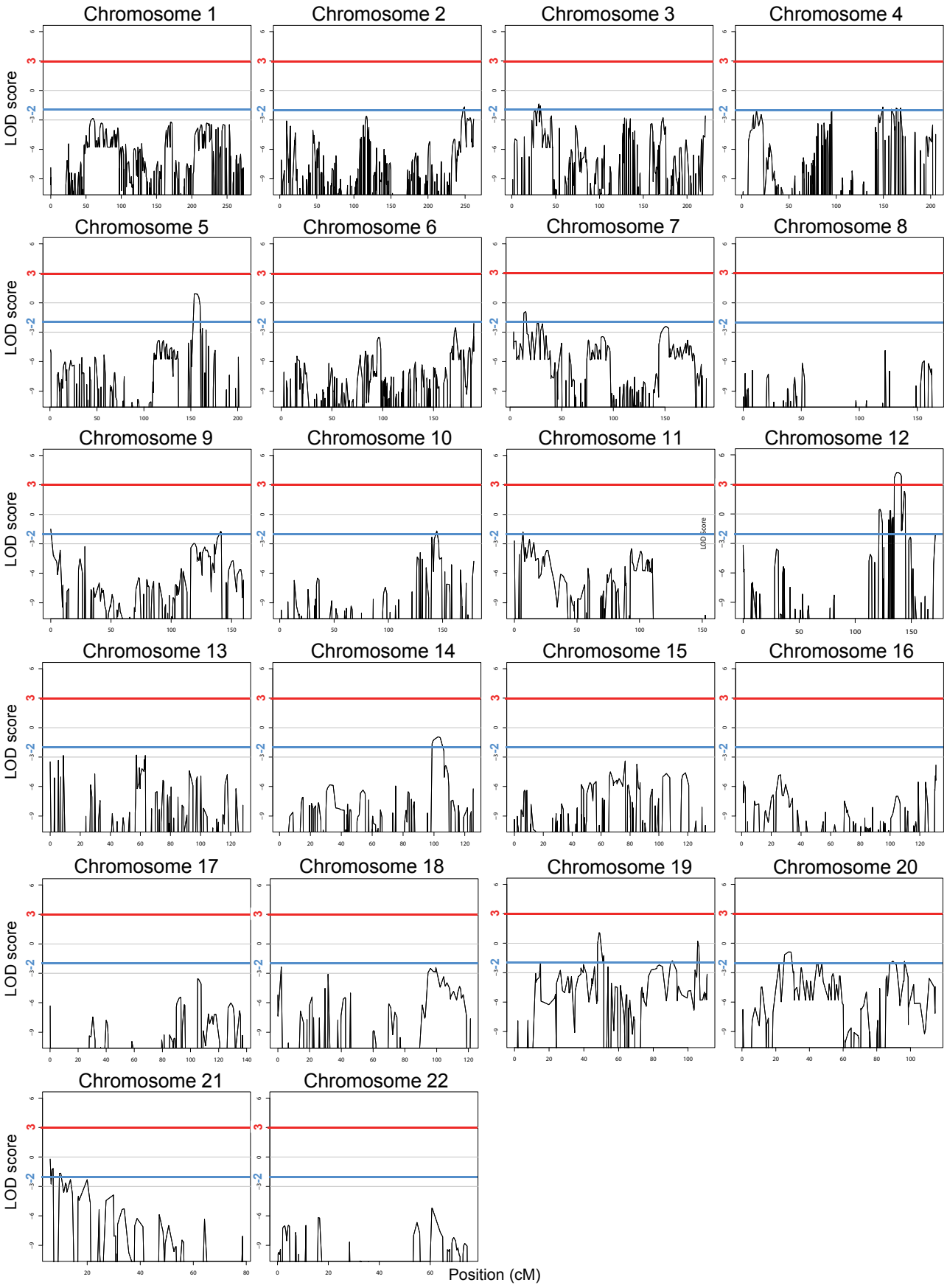

Figure S1. Genome-wide plots of multipoint LOD-scores using the HumanLinkage V SNP panel.

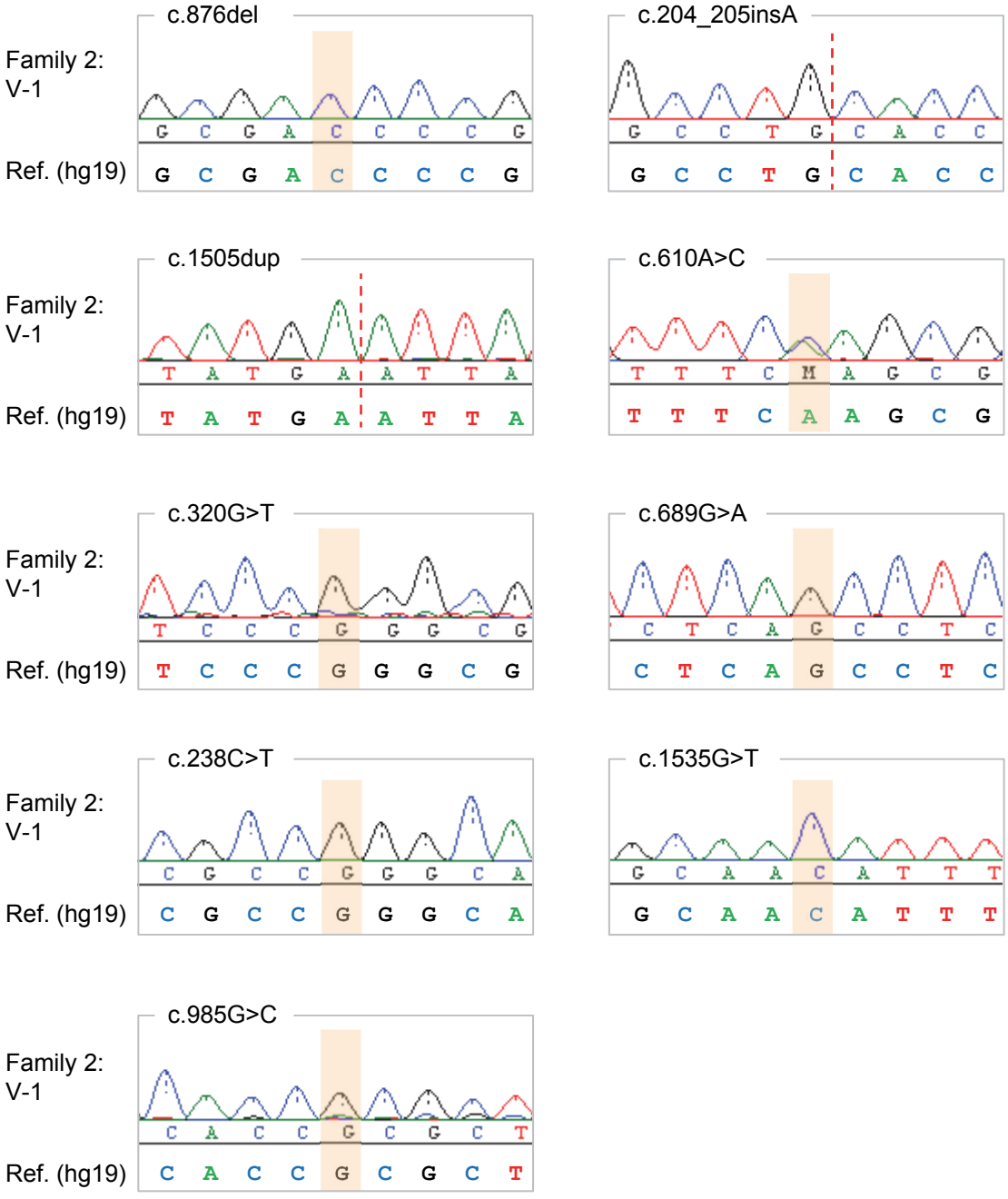

Figure S2. Exclusion of survived variants by genotyping of the proband of family 2.
In the proband of family 2, electropherograms of nine variants listed in Table S8 are shown. We successfully confirmed that these variants except for 5-bp deletion are not carried by the proband of family 2.

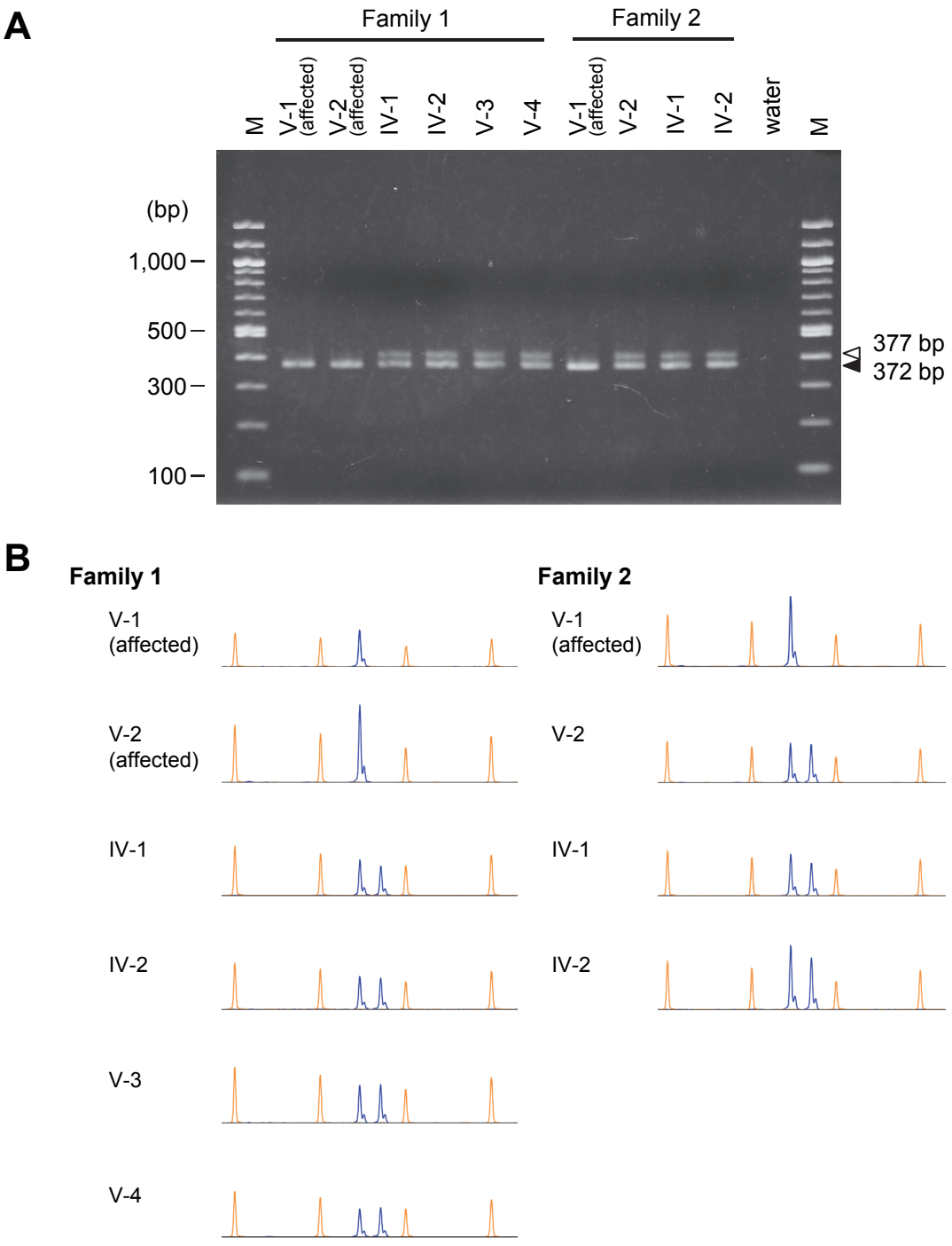

Figure S3. Detection of the 5-bp deletion in *COX6A1*.
The PCR fragments include the 5-bp deletion (372 bp) and wild-type (377 bp) were distinguishable by standard 1.5% agarose gel electrophoresis (A) or GeneScan analysis using 6-FAM labeled primer (B) (see also Figure 1E).

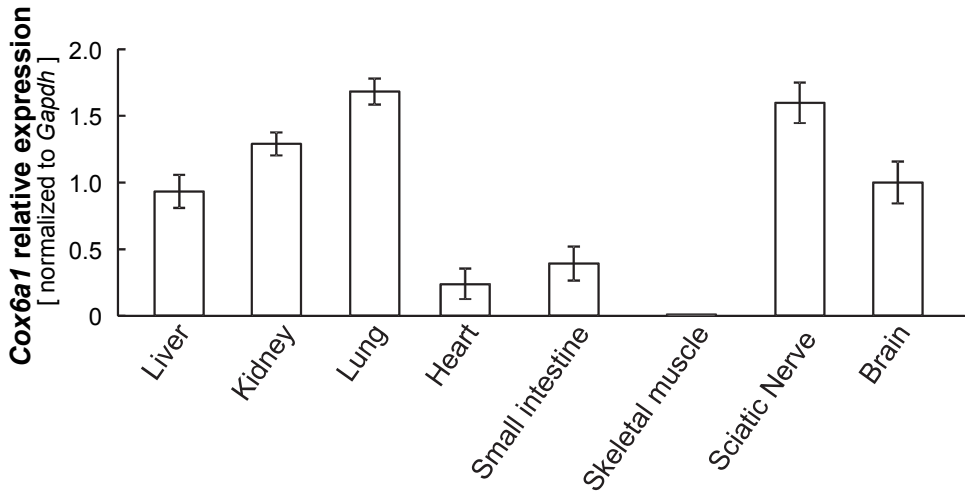

Figure S4. *Cox6a1* expression in mouse tissues.
Cox6a1 expression levels in liver, kidney, lung, heart, small intestine, skeletal muscle, sciatic nerve and brain from three wild-type mice. The error bars represent the standard deviation. Total RNA was extracted using AllPrep DNA/RNA Kit (QIAGEN N.V., The Netherlands) according to the manufacturer's instructions with on-column DNase I treatment. After determining RNA concentrations using Quant-iT RiboGreen RNA Assay Kit (Life Technologies, USA), 50 ng of total RNA per 20 μL reaction was used to synthesize cDNA using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, USA) with random primers. Relative quantification for *Cox6a1* was performed using a pre-developed TaqMan assay (Mm01612194_m1; Life Technologies, USA). Real-time PCR was conducted using TaqMan Universal Master Mix II (Life Technologies, USA) with a 7500 Fast real-time PCR system (Life Technologies, USA). Each reaction was run in triplicate and contained 2 μL of cDNA template in a final reaction volume of 20 μL and data were analyzed following the Delta-Delta Ct method with 7500 Software v2.0.2. All samples were normalized to *Gapdh* as an endogenous control (4352662; Life Technologies, USA). The error bars represent the standard deviation.

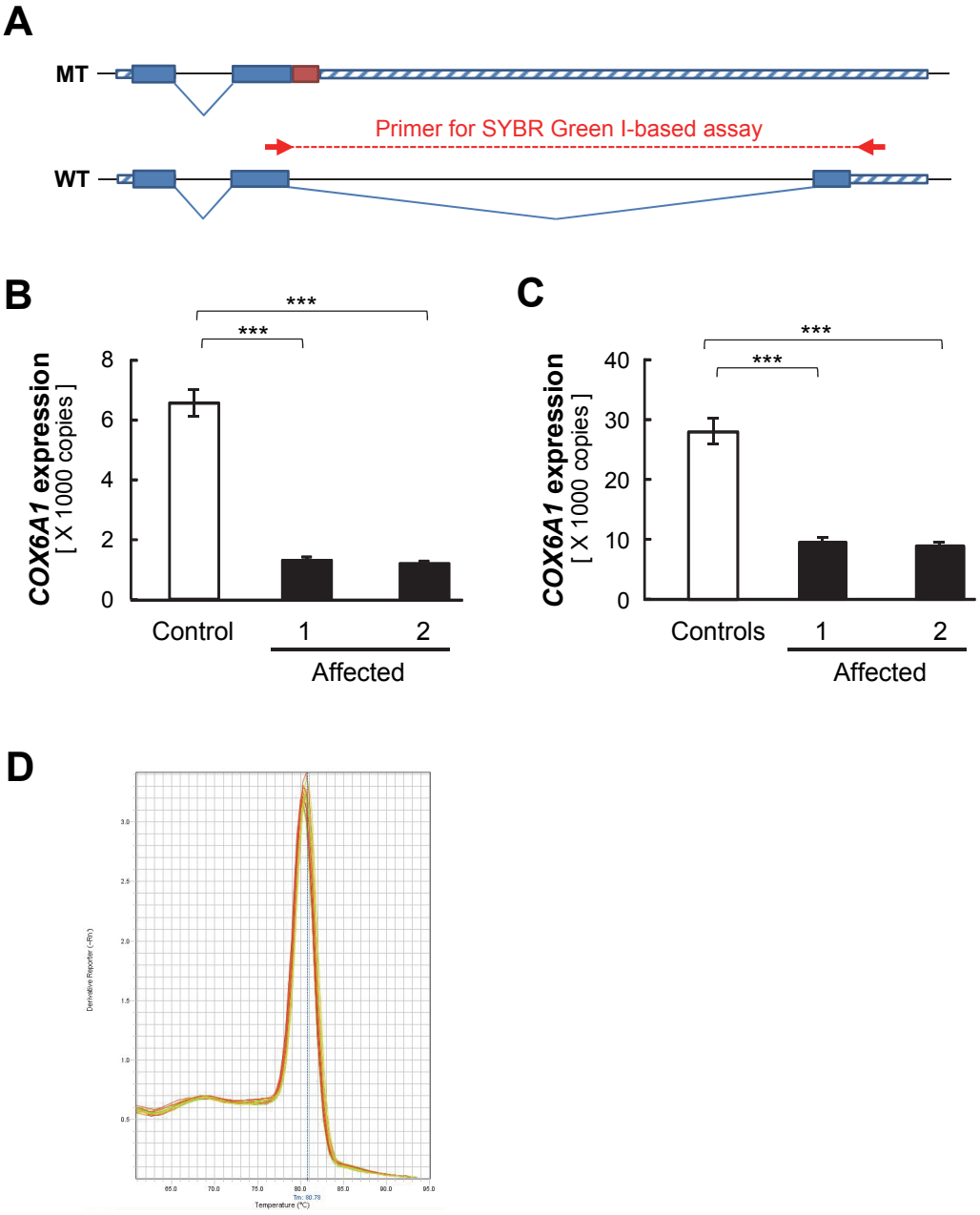

Figure S5. *COX6A1* expression analysis using SYBR Green I-based assay. Primer position (A) and absolute quantification in peripheral white blood cells (B) and EBV transformed B cell lines (C) by real-time RT-PCR. The amplicon reveals a single peak in the melting curve (D). The error bars represent the standard deviation. Three asterisks (***) indicates $p < 0.001$.

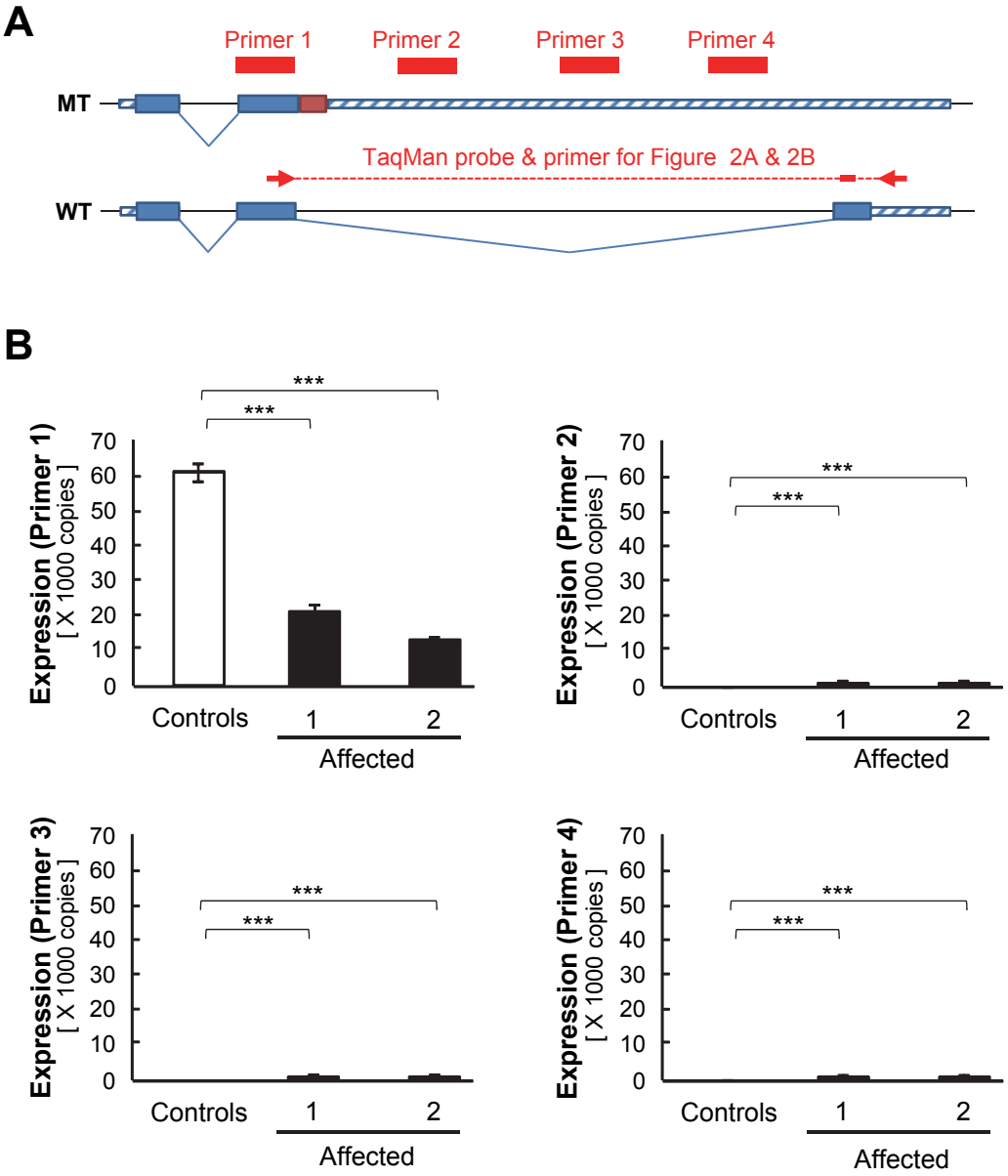

Figure S6. *COX6A1* expression analysis in EBV transformed B cell lines.
Primer position (A) and absolute quantification by real-time RT-PCR (B). To estimate the expression level of both wild-type and mutant-type *COX6A1* in details, the absolute quantification was performed using four primer sets (Table S12) with SYBR Green I fluorescent dye. The standard curves were obtained from PCR products in serial dilution. The error bars represent the standard deviation. Three asterisks (***) indicates $p < 0.001$.

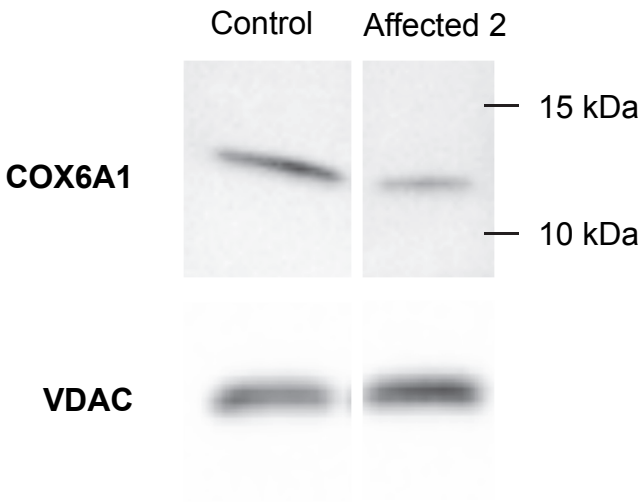

Figure S7. Western blot of COX6A1 and VDAC as control for mitochondria fractions from EBV transformed B cell lines.
The lanes run on different parts of the same blot. The primary antibody for COX6A1 (mouse monoclonal, ab110265; abcam, UK) is diluted 1:1,000. The following abbreviation is used: VDAC, voltage-dependent anion channel.

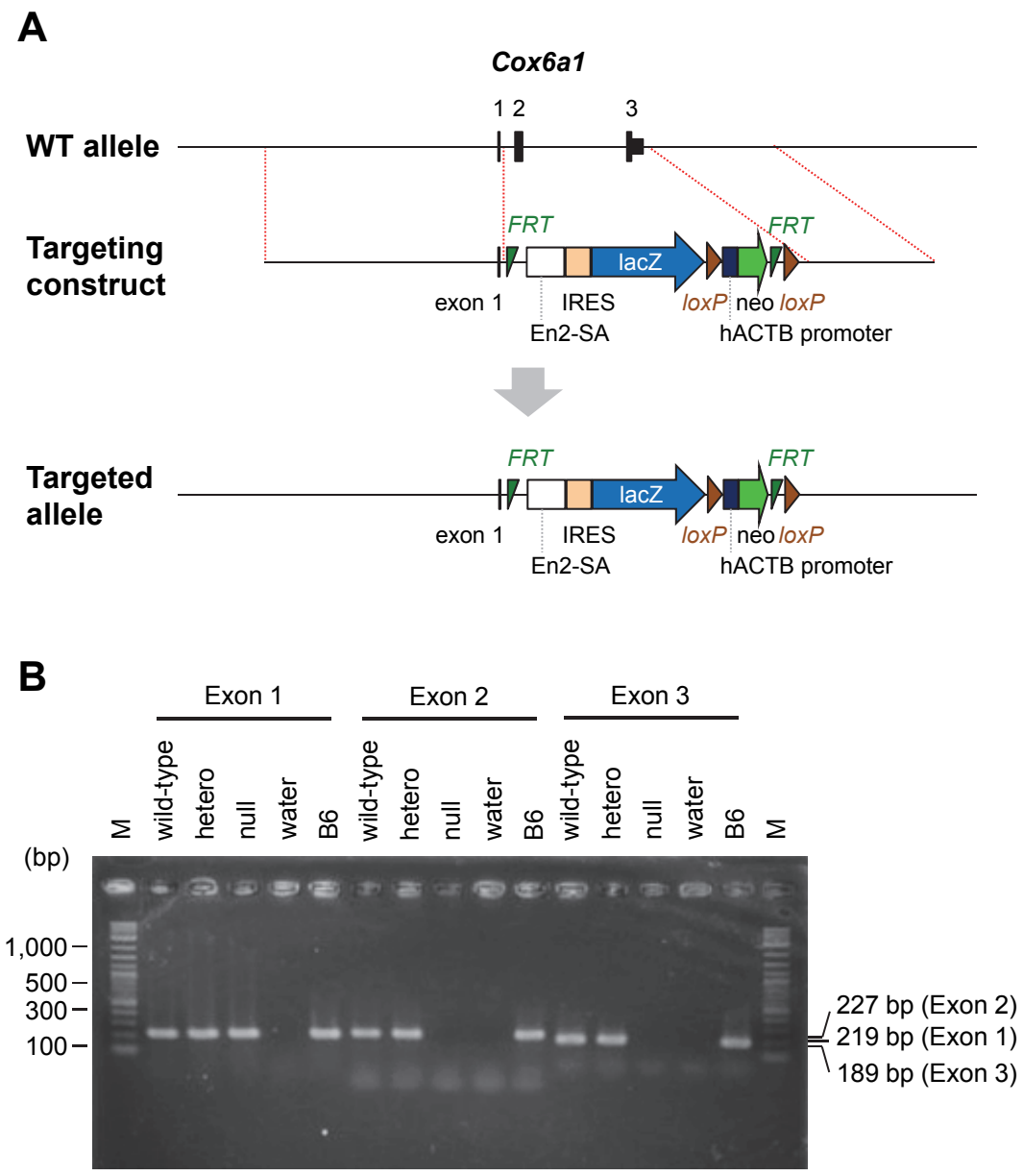

Figure S8. *Cox6a1* knockout mouse.
The knockout construct (A) and PCR genotyping result by agarose gel electrophoresis (B). The following abbreviation is used: B6, mouse background strain C57BL/6. Gene targeting was carried out as part of the International Knockout Mouse Consortium on a C57BL/6N genetic background (Project ID # CSD-74927) [1] and we obtained frozen sperm from Knockout Mouse Project (KOMP) repository, located at the University of California, Davis. Some female C57BL/6JJcl mice (CLEA, Japan) were used as oocyte donors and in vitro fertilization was performed according to the consortium's instruction. To confirm targeted allele, exon-specific PCR were carried out using three different exon-specific primer sets (Table S13). We intercrossed the knockout heterozygous mice to produce homozygous (null) mice.

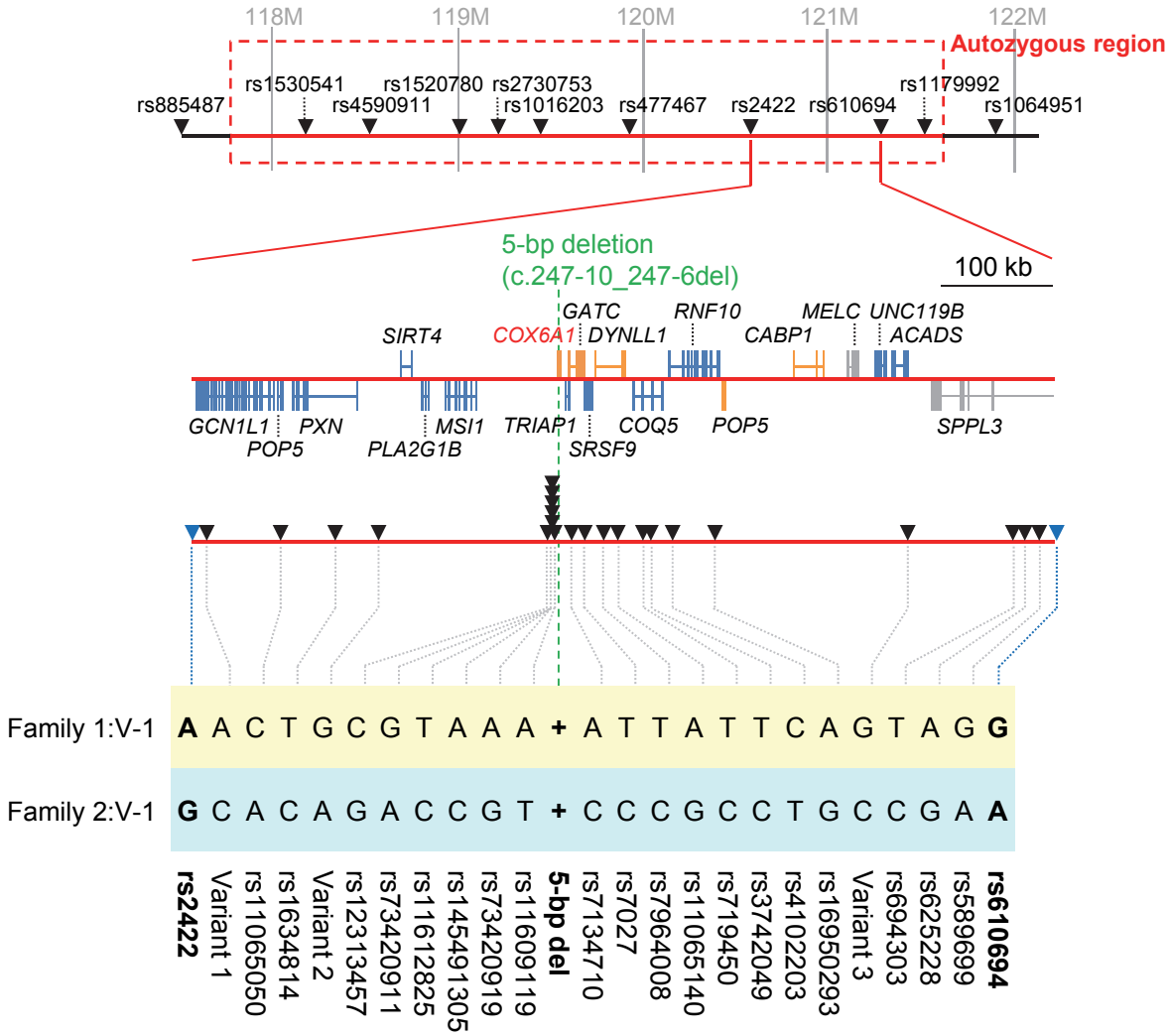

Figure S9. Background haplotype within the interval including the 5-bp deletion. In the 739 kb interval around the 5-bp deletion (between rs2422 and rs610694 from Illumina's linkage panel), 22 SNVs were selected for additional genotyping (see also Table S14). Genotypes were obtained by PCR-direct Sanger sequencing. The PCR fragments were purified by ExoSAP-IT (Affymetrix, USA) and sequenced using the same primers as in the PCR process (Figure S10).

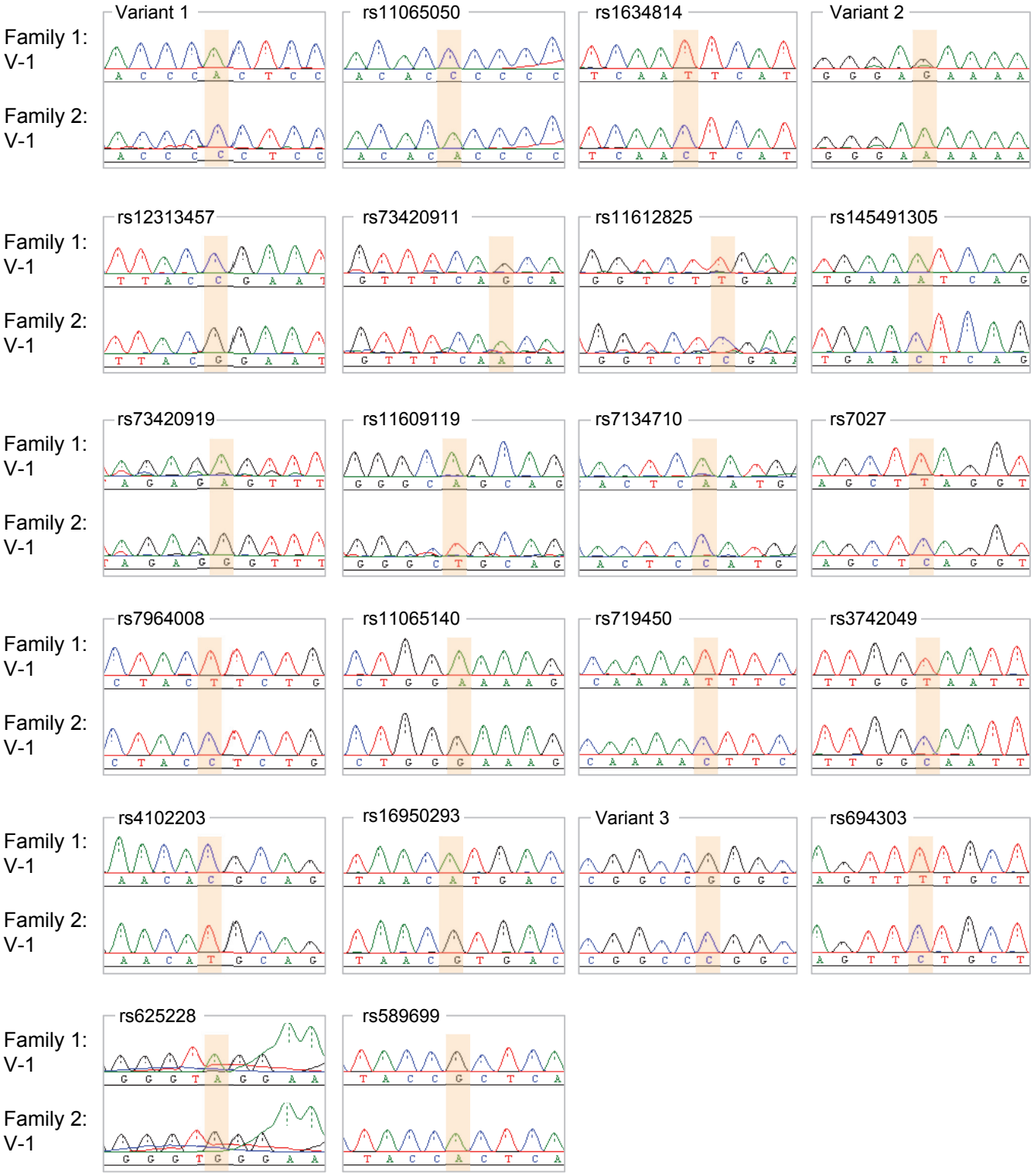

Figure S10. Electropherograms of additionally genotyped single nucleotide variants around the 5-bp deletion.

Around the 5-bp deletion (c.247-10_247-6del), the electropherograms of Sanger-based PCR direct sequencing of 22 SNVs with homozygous alleles are shown.

Table S1. Clinical course of the affected siblings of family 1

Individuals	Parents	Age	Presentation	Examinations			Other Features
				Name	Objects	Results	
Family 1 V-1	Related but healthy parents (second cousins)	Early development	Normal				
		4	Foot drop				
		8		Electrophysiological examination	right median and ulnar nerves	motor NCV 43.4 m/s and 45.0 m/s undetectable sensory nerve action potentials	Evoked action potentials were not detected in the study of motor and sensory tibial nerve.
		9		Biopsy	sural nerve	decreased myelinated fiber density, decrease of myelinated axon caliber and presence of onion bulb formation	
				MRI	brain	mild cerebral atrophy	
		~16	slowly progressed in distal muscle weakness of upper and lower limbs and was wheel-chaired				
		25		Clinical examination		distal muscle wasting of the legs, pes cavus, and clawing of the toes.	Achilles tendon reflexes were absent. Vibration detection, touch and pinprick sensation was reduced in upper and lower distal limbs. He had no cerebral, cerebellar and pyramidal signs and symptoms. He did not have any signs or symptoms of liver and renal diseases with normal laboratory findings including serum albumin, transaminases, electrolytes, urea N and creatinine, and blood lactate and pyruvate.
				Manual muscle strength testing	deltoid and triceps brachii bilaterally	grade 5	
					biceps brachii, and extensors and flexors of wrists bilaterally	grade 4	
					extensor muscles of fingers, iliopsoas, quadriceps femoris and hamstrings bilaterally	grade 2	
					tibialis anterior, gastrocnemius, and extensors and flexors of toes bilaterally	grade 0	
				EMG		proximal and distal lower limb denervation with no evidence of myopathy	
				Electrophysiological examination		delayed motor NCV (35.7 m/s) with low CMAP amplitude and undetectable sensory nerve action potentials (Table S2)	
				Biopsy	sural nerve	decreased myelinated fiber density, decrease of myelinated axon caliber and presence of onion bulb formation	
	V-2 Related but healthy parents (second cousins)			similar clinical manifestation and laboratory findings (Table S2)			

Table S2. Nerve conduction study of the affected siblings of family 1.

Nerve	Motor			F wave		Sensory		
	DL (ms)	CV (m/s)	CMAP (mV)	Lat (ms)	Occur (%)	DL (ms)	CV (m/s)	SNAP (μV)
Affected member V-1								
Rt median	7.6 (N<4.0)	35.7 (N>56)	0.677 (N>3.5)	NE				NE
Rt ulnar	3.3 (N<3.2)	40.7 (N>50)	1.3 (N>2.8)	NE				NE
Rt posterior tibial				NE				NE
Rt Peroneal				NE				NE
Affected member V-2								
Rt median	8.7 (N<4.0)	34.8 (N>56)	0.083 (N>3.5)	NE				NE
Rt ulnar	5.16 (N<3.2)	37.3 (N>50)	0.052 (N>2.8)	NE				NE
Rt posterior tibial				NE				NE
Rt Peroneal				NE				NE

DL: distal latency, CV: conduction velocity, CMAP: compound muscle action potential amplitude, Lat: latency, Occur: occurrence, SNAP: sensory nerve action potential amplitude, Rt: right, N: normal, NE: not evoked

Table S3. Clinical course of the proband of family 2

Individual	Parents	Age	Presentation	Examinations			Other Features
				Name	Objects	Results	
Family 2 V-1	Related parents	Early development	Normal				
		< 5	Unsteady gait				
		5		Clinical examination	upper and lower limbs	steppage gait, pes cavus, and decreased tendon reflexes	She was treated with short leg braces. She had a progression of distal muscle weakness of upper and lower limbs and deterioration of the ankle joint deformities.
				Manual muscle strength testing	distal upper limb with lower limbs	mild decrease	
		8	Tendon achilles lengthening surgery				
		9	Posteromedial release and anterior tibial tendon transfer surgeries				
		39	walked few steps with support	Clinical examination	legs	distal muscle wasting	Deep tendon reflexes were absent in both extremities. Vibration detection, touch and pinprick sensation was reduced in upper and lower distal limbs. She had no cerebral, cerebellar and pyramidal signs and symptoms. She did not have any signs or symptoms of liver and renal diseases.
					toes	clawing	
				Manual muscle strength testing	deltoid, biceps brachii, triceps brachii, iliopsoas and quadriceps femoris bilaterally	grade 5	
					extensors and flexors of wrists, hamstrings and gluteus maximus bilaterally	grade 3	
					extensors and flexors of muscles of fingers, bilaterally	grade 2	
					tibialis anterior, gastrocnemius, peroneus, and extensors and flexors of toes bilaterally	grade 0	
				Electrophysiological examination	right and left median nerves	mildly delayed motor NCV 45.3 and 49.6 m/s, respectively (Table S4)	

Table S4. Nerve conduction study of the proband of family 2.

Nerve	Motor		
	DL (ms)	CV (m/s)	CMAP (mV)
Affected member V-1			
Rt median	3.7 (N<4.0)	45.3 (N>56)	5.0 (N>3.5)
Lt median	3.7	49.6	4.9
Rt ulnar	3.3 (N<3.2)	51.5 (N>50)	4.3 (N>2.8)
Lt ulnar	3.5	41.8	0.8
Rt tibial			NE
Lt tibial			NE

DL: distal latency, CV: conduction velocity, CMAP: compound muscle action potential amplitude, Rt: right, Lt: left, N: normal, NE: not evoked

Table S5. Summary of NGS performance.

Summary	
Reads mapped to genome[a]	1,024,056,995
Data mapped to genome (Gb)	141.8
Average sequenced depth (×)	49.6
Genome covered (%)	96.2

Gb: giga bases

[a]A total of 5 μg genomic DNA sample was subjected to enzymatic fragmentation using the NEB Next dsDNA Fragmentase kit (New England Biolabs, USA) according to the manufacturer's instructions. The resulting size distribution was between 500–700 bp (600 bp peak top) on the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Then, library preparation was performed using Paired-End DNA Sample Preparation Kit (Illumina, USA). After the check of size distribution and concentration by the Agilent Bioanalyzer 2100, total 5 times of cluster generation were performed from same library using a Paired-End Cluster Generation Kit (v4) on Cluster Station and four cBot Paired-End Cluster Generation Kit (v4) on cBot according to the manufacturer's instructions. 101 bp paired-end runs were performed, using TruSeq SBS kits (v5). Basecalling, mapping to a reference human genome (UCSC NCBI37/hg19) and variant detection were performed using the standard Illumina Pipeline (CASAVA 1.7).

Table S6. Gap[a] filling by Sanger-based PCR direct sequencing.

Start	End	Length	Gene Symbols	Exon	PCR Primers	
					Forward	Reverse
118498754	118498900	147	WSB2	exon 1 5' UTR	CCCGATCTTCCCGATCCTGTCG	GTACCGCCTCCCCGATTTTCCATC
120907136	120907142	7	SRSF9	intron	GAGGCGGACAAGGCTCATTTGGAC	CATCCGCGAGATCGAGCTCAAGAAC
121078583	121078598	16	CABP1	exon 1 coding	GTAGAGGCTGCGCTGTCACATGG	CGGCGGCTGGAACGGAGTC
121078674	121078697	24	CABP1	exon 1 coding	GTAGAGGCTGCGCTGTCACATGG	CGGCGGCTGGAACGGAGTC
121078822	121078910	89	CABP1	exon 1 coding	GTAGAGGCTGCGCTGTCACATGG	CGGCGGCTGGAACGGAGTC
121148179	121148186	8	UNC119B	exon 1 5' UTR	CCTCAGGTTGCTGATGGCAGCTCTAG	CCACCAGAGCTACGTGAGCGTGG
121268251	121268289	39	SPPL3	intron	GAGTAAATTTCACTGTTCACGAACTACACC	CTCCCCAACATATGCATGTACACC
121734510	121734607	98	CAMKK2	intron	CTCCGGGCTTTGTGTGCCATG	CCTCTTCGCTACTTTCGTCTTTGCAGG
121904681	121904758	78	KDM2B	intron	GAGGGTCCTGGCACAACTTTGCG	GAGCGCCGAGGACGACGACTATG
121905015	121905015	1	KDM2B	exon 1 5' UTR	AGCGGCAGCAAAACTTTCTCCTCAT	CCTTCCTGGCTCGCCTCATGC
121905364	121905381	18	KDM2B	intron	ATGCATATGCATGAGGCGAGCCAG	GGAGACTGGGCTCTCAGAGCTTATCCAG
121975839	121975878	40	KDM2B	intron	TTTTCCCTCCAACTTGGCGTAACTCC	GTTCAGTTTCAGCGCGGGCATG

[a]All gaps found on the linkage region were filled by Sanger-based PCR direct sequencing.

Table S7. Summary of variants.

Variant categories[a]	counts
Total number of variants	3,716,455
Frame-shift indel	154
In-frame indel	210
Stop gain	92
Stop loss	15
Nonsynonymous	10,556
Splice site	135
5-UTR	27,123
3-UTR	5,223
Synonymous	11,788
Intron	1,267,264
Intergenic	2,266,228
ncRNA	127,667
Homozygous	1,669,570
Heterozygous	2,046,885

[a]We applied ANNOVAR (version May 8, 2013) to appropriately annotate the genetic variants [2]. This included functional annotation by 'refGene' and 'knownGene' annotation databases, dbSNP (build 135 and 137) identifiers, and 1000 Genomes Project (Apr. 2012) allele frequencies. After the annotation process, functional (Frame-shift indel, In-frame indel, Stop-gain SNV, Stop-loss SNV, non-synonymous SNV, and Splice site variant), novel (Not reported in dbSNP 135 and 137) and homozygous variants were included for further analysis.

Table S8. List of homozygous and potentially deleterious variants voted by at least one prediction methods.

Chr	Start	Alleles		Gene Symbols	Types	Accession	Changes		Functional Predictions					Expression in nervous system
		Reference	Variant				Nucleotide	Protein	PolyPhen-2 (HumVar)	Grantham	PROVEAN	SIFT	Mutation Taster[a] (probability)	
12	120878247	CACTC	-	*COX6A1*	splicing	NM_004373.3	c.247-10_247-6del	NA	splicing	splicing	splicing	splicing	splicing	+
2	114257705	C	-	*FOXD4L1*	frameshift	NM_012184.4	c.876del	p.Gly293Alafs	indel	indel	indel	indel	DC (1)	-
14	24470691	-	A	*DHRS4L2*	frameshift	NM_001193637.1	c.204_205insA	p.His69Thrfs	indel	indel	indel	indel	DC (1)	-
19	20807178	-	A	*ZNF626*	frameshift	NM_001076675.2	c.1505dup	p.Ile503Hisfs	indel	indel	indel	indel	DC (0.999)	NA
2	114257443	A	C	*FOXD4L1*	nonsynonymous	NM_012184.4	c.610A>C	p.Lys204Gln	0.062	53	-3.933	0.021	DC (0.999)	-
8	126443464	G	T	*TRIB1*	nonsynonymous	NM_025195.3	c.320G>T	p.Arg107Leu	0.392	102	-4.452	0.002	DC (0.999)	-
2	73151606	G	A	*EMX1*	nonsynonymous	NM_004097.2	c.689G>A	p.Ser230Asn	0.023	46	-2.144	0.341	DC (0.999)	-
2	97637964	G	A	*FAM178B*	nonsynonymous	NM_001122646.2	c.238C>T	p.Arg80Trp	NA	101	-3.127	0	P (0.999)	NA
4	69403401	C	A	*UGT2B17*	nonsynonymous	NM_001077.3	c.1535G>T	p.Cys512Phe	NA	205	-7.344	0.107	P (0.999)	-
9	133556937	G	C	*PRDM12*	nonsynonymous	NM_021619.2	c.985G>C	p.Ala329Pro	0.113	27	-0.66	0.041	DC (0.973)	+

[a]Prediction by Mutation Taster: DC: Disease Causing; P: Polymorphism

Table S9. Autozygosity in the linkage region on 12q24.

	Position			Allelic state	
	start	end	bp	homozygous	heterozygous
5' flanking region	117,539,934	117,882,412	342,479	281	8
Autozygous region	117,882,413	121,605,073	3,722,661	2,891	0
3' flanking region	121,605,074	121,878,659	273,586	110	382

Table S10. PCR primer sequences for validation of variants by Sanger-based PCR direct sequencing.

Chr	Start	Alleles		Gene Symbols	Accession	Changes		PCR Primers		Related Figures and Tables
		Reference	Variant			Nucleotide	Protein	Forward	Reverse	
12	120878247	CACTC	-	*COX6A1*	NM_004373.3	c.247-10_247-6del	NA	TGGAATTAAAACTGTCCTGTAGC	GGTCCATGTGCAGAGTAAC	Figures 2E & S3, Table S11
2	114257705	C	-	*FOXD4L1*	NM_012184.4	c.876del	p.Gly293Alafs	AAGACGAGGTGGAAGACGAG	ATTGTCCGACAGGCTTGAC	Figure S2
14	24470691	-	A	*DHRS4L2*	NM_001193637.1	c.204_205insA	p.His69Thrfs	GAGCACTGCCCTCTATGTCTAG	GCACAATGTGCACATGTACC	Figure S2
19	20807178	-	A	*ZNF626*	NM_001076675.2	c.1505dup	p.Ile503Hisfs	TCATATCAATTCTTAGTTAGAAATTGAGG	AAGCCTTCAAGCGGTCTTC	Figure S2
2	114257443	A	C	*FOXD4L1*	NM_012184.4	c.610A>C	p.Lys204Gln	AGACGAGGTGGAAGACGAG	CGAGAGCAGTAGGTAGCGAG	Figure S2
8	126443464	G	T	*TRIB1*	NM_025195.3	c.320G>T	p.Arg107Leu	AGACCAGTCTGCAAACTCCA	ATCTACTGATCCGCCCTGTG	Figure S2
2	73151606	G	A	*EMX1*	NM_004097.2	c.689G>A	p.Ser230Asn	GCGTGTCAAGGAATGGAGAG	CTTCCTCCAGGGAACCTG	Figure S2
2	97637964	G	A	*FAM178B*	NM_001122646.2	c.238C>T	p.Arg80Trp	GATGGGTAGCCAAGGAGAAG	GTTCTCTCACCCATTTGCTG	Figure S2
4	69403401	C	A	*UGT2B17*	NM_001077.3	c.1535G>T	p.Cys512Phe	GCTCAGTAACTTTTGTGTGGG	CTGGATCGAGCAGTCTTCTG	Figure S2
9	133556937	G	C	*PRDM12*	NM_021619.2	c.985G>C	p.Ala329Pro	CTACAAGTGCCAGGTGTGCCAGAG	CTCTACAGCAGCAGGGAGACTCGG	Figure S2

Table S11. Screening of 5-bp deletion in *COX6A1* among diverse ethnic populations.

Populations	Number of individuals[a]	PCR product size[b]	
		377 bp (wild type)	372 bp (5-bp deletion)
Caucasian	100	100	0
African American	100	100	0
Amerindian	9	9	0
Cambodian	1	1	0
Druze	1	1	0
Melanesian	2	2	0
Pygmy	5	5	0
Japanese	508	508	0
Total	726	726	0

[a]Genomic DNAs of 218 unrelated individuals from diverse ethnic populations were provided by Coriell Cell Repositories. In addition, genomic DNAs of 508 unrelated Japanese individuals were obtained [3].

[b]After amplification (see also Table S10) by standard PCR in 50 μL reaction volume containing 4 ng of genomic DNA, the PCR fragments include the 5-bp deletion (372 bp) or wild-type (377 bp) were produced. They are distinguishable by standard 1.5% agarose gel electrophoresis.

Table S12. Sequences of PCR primers and TaqMan probe used in the expression analysis of *COX6A1*.

Assay type	Target Molecule	Assay Name	PCR Primers		TaqMan Probe	Related Figures
			Forward	Reverse		
TaqMan	Wild-type	-	CACGAGAGACCCGAGTTCATC	TTTCTGGTCCATGTGCAGAGTAAC	(MGB)ACCAAGCCGTTTCC	2A, 2B
SYBR Green I	Wild-type	-	CATCAGGACCAAGCCGTTTC	TTTCTGGTCCATGTGCAGAGTAAC	-	S5
SYBR Green I	both	Primer 1	GGCGGTAGTTGGTGTGTCCTC	CTTGGTCCTGATGCGGAGATG	-	S6
SYBR Green I	Mutant-type	Primer 2	TTCTCTTTTCGTTATGTGTGCCTTA	GCAGGAGGTAGGATGAATCGG	-	S6
SYBR Green I	Mutant-type	Primer 3	GTGAGCCAAGATCCAGCCAT	TGCCGCTCAAAGACTCAACA	-	S6
SYBR Green I	Mutant-type	Primer 4	TTGTTTGAAGTCTGGGATGGTG	CTGGCTACAGGACAGTTTTAATTCC	-	S6

Table S13. PCR primer sequences for genotyping of *Cox6a1* null mice.

Target	PCR Primers		Amplicon size (bp)
	Forward	Reverse	
exon 1	CTAGGCGCTGACAAGCAG	CGCCATCATGGAACTACAC	219
exon 2	GTGAGCATGCTCAACGTG	CTATCTGCACGGACGGAC	227
exon 3	CCATCCCTTCTGCTTGTAAG	TGCTAAGCACAAGAAACCAG	189

Table S14. Additionally genotyped single nucleotide variants around the 5-bp deletion.

Variants	Positions	Distance from 5-bp del (kb)	PCR Primers		Criteria[a]	dbSNP MAF	Genotypes	
			Forward	Reverse			1:V-1	2:V-1
rs2422	**120565188**	**-313.1**	**-**	**-**	**ILP**	**0.495**	**A**	**G**
Variant 1	120578265	-300.0	CTAGGAATTCAGACAAGGATGC	TTGCAGGCTAAGAGCTAAGTG	NGS	-	A	C
rs11065050	120641900	-236.3	GGATGGAAAATAACATGAATGAG	TGTCTTGGTTTTAAACCTGCTAC	HM3,MAF	0.184	C	A
rs1634814	120685747	-192.5	CTGCAGATGTTTCAAGCAAC	CCTGCTCTTCACCTCTCTTG	HM3	0.371	T	C
Variant 2	120724972	-153.3	TGGTAGTGGGTCTCATCTTTG	TACTACAAGGGCTATCTCAGCAG	NGS	-	G	A
rs12313457	120869349	-8.9	AATTTCTTTACAGCAATGCAAG	AAGGTGGTATATTGGAATGTATTG	1KGP,MAF	0.128	C	G
rs73420911	120872479	-5.8	CTGGTGGTATCTTTTGGAAAG	TGTTAGAATTGTCTTGATCTAGTCC	1KGP,MAF	0.128	G	A
rs11612825	120874200	-4.0	CTTCATGAAACTGCATGACC	TATGCCTCAGATCAAGAGCTC	1KGP,MAF	0.127	T	C
rs145491305	120874512	-3.7	ATGGGAGTTCAACTCTTGTTG	TTCCTCTAAAGACACACCTATGTG	1KGP,MAF	0.128	A	C
rs73420919	120874620	-3.6	ATGGTGCAATCTCAGCTCAC	CCCTTTGAGAATGTAATGAGGAC	1KGP,MAF	0.130	A	G
rs11609119	120877821	-0.4	CATCACCTAGCCCATATAGGAC	CAAGCTCAAGTTAGTTACTCATCATC	1KGP,MAF	0.144	A	T
5-bp del	**120878247**	**0.0**	**-**	**-**	**NGS**	**-**	**+**	**+**
rs7134710	120888510	10.3	TGACTGTAGATTTGTGAAAGTGG	GTCAGGGCTTTTATAGCTAAGATG	1KGP,MAF	0.149	A	C
rs7027	120901266	23.0	CTGCTTTCCTACAAGGAAAGAC	CTAGTGCAGTCGGTCTTCATAC	HM3,MAF	0.177	T	C
rs7964008	120918397	40.2	ACCTCAATCTGCATCCAGAG	TGGAACAAAAGGCATAATGG	HM3,MAF	0.150	T	C
rs11065140	120929541	51.3	GGGGTCTTCAATCAACTCTG	AATGTCGCTGGAGACACTAAG	HM3,MAF	0.159	A	G
rs719450	120954033	75.8	CTTTACAGGAGATGAGAGGTCC	AGACACAGGGGAAGTATTTGTATC	HM3,MAF	0.207	T	C
rs3742049	120954490	76.2	TTCAACCTGCCCATCTATAAG	ATCCAGTTAGCTAACACAGGTACTC	HM3,MAF	0.146	T	C
rs4102203	120976371	98.1	GCCATTTGCTTCCTAGCTAC	TACCAGAATAACAGGAAGAATGC	HM3,MAF	0.074	C	T
rs16950293	121015461	137.2	GGAAAGCTGTAACTCACGAAG	TAACAGAAACACTGGTGATTCTG	HM3,MAF	0.158	A	G
Variant 3	121177337	299.1	CATGGGCTACGTGACAGAG	ACCTCAAGGAAAAGACAGACC	NGS	-	G	C
rs694303	121268624	390.4	GGTGAGGAATTCAGTTCTAAGC	GACCGAAGTTGAATGTTTCAC	HM3,MAF	0.201	T	C
rs625228	121278266	400.0	TTTGGGCCATAAAAAGGAG	CATCTGGCCACAATTCTG	HM3	0.469	A	G
rs589699	121288685	410.4	TCCCTTTTTAAATGTGAGTAACAT(	ATTCATTAGGAATGATAACACCTG	HM3,MAF	0.204	G	A
rs610694	**121304826**	**426.6**	**-**	**-**	**ILP**	**0.494**	**G**	**A**

[a]Variants that satisfy the following criteria were selected for the additional genotyping.
ILP: SNP markers nearest the 5-bp deletion in Illumina's Linkage Panel.
NGS: Single nucleotide variants newly found by our Next Generation Sequencing analysis.
HM3: SNP markers tagging each haplotype in probands of family 1 and 2 from HapMap 3 release 2.
1KGP: SNP markers tagging each haplotype in probands of family 1 and 2 from the 1000 Genomes Project (phase 1 version 3).
MAF: SNP markers with MAF $\lesssim$ 0.2 in dbSNP 138.

Supplemental References

1. Skarnes, W. C., Rosen, B., West, A. P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A. O., Thomas, M., Harrow, J., Cox, T., et al. (2011). A conditional knockout resource for the genome-wide study of mouse gene function. Nature *474*, 337-342.
2. Wang, K., Li, M., Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. *38*, e164.
3. Yamagata University Genomic Cohort Consortium. (2014). Pleiotropic Effect of Common Variants at ABO Glycosyltranferase Locus in 9q32 on Plasma Levels of Pancreatic Lipase and Angiotensin Converting Enzyme. PLoS One *9*, e55903.