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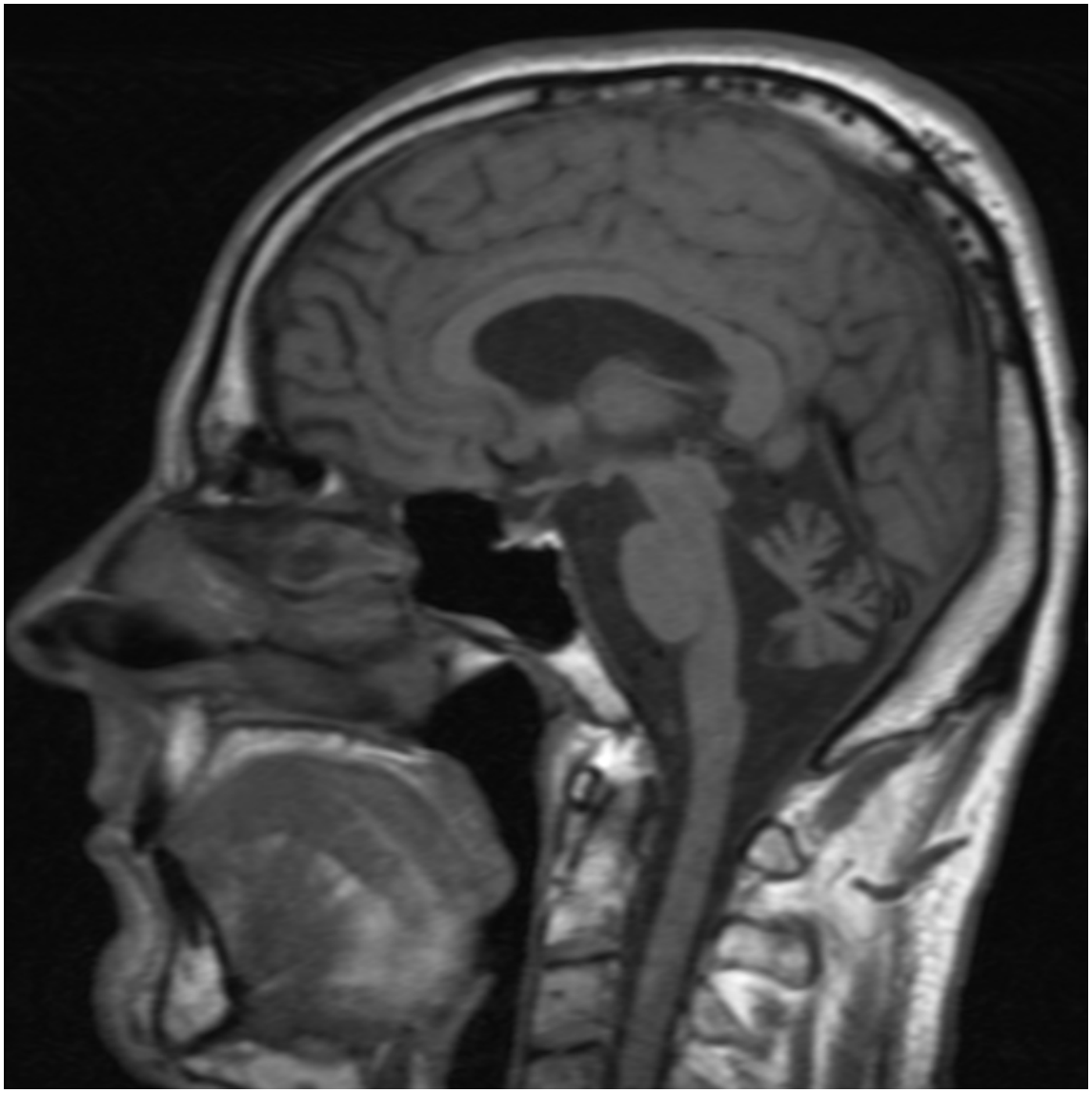
Supplemental Data

Targeted Next-Generation Sequencing of a 12.5 Mb Homozygous Region Reveals *ANO10* Mutations in Patients with Autosomal-Recessive Cerebellar Ataxia

Sascha Vermeer, Alexander Hoischen, Rowdy PP Meijer, Christian Gilissen, Kornelia Neveling, Nienke Wieskamp, Arjan de Brouwer, Michel Koenig, Mathieu Anheim, Mirna Assoum, Nathalie Drouot, Slobodanka Todorovic, Vedrana Milic-Rasic, Hanns Lochmüller, Giovanni Stevanin, Cyril Goizet, Albert David, Alexandra Durr, Alexis Brice, Berry Kremer, Bart P.C. van de Warrenburg, Mascha M.V.A.P. Schijvenaars, Angelien Heister, Michael Kwint, Peer Arts, Jenny van der Wijst, Joris Veltman, Erik-Jan Kamsteeg, Hans Scheffer, and Nine Knoers

Figure S1. Magnetic Resonance Imaging of One of the Affected Patients Displaying Marked Diffuse Cerebellar Atrophy on Both Saggital (Upper Picture) and Axial (Lower Picture) Images



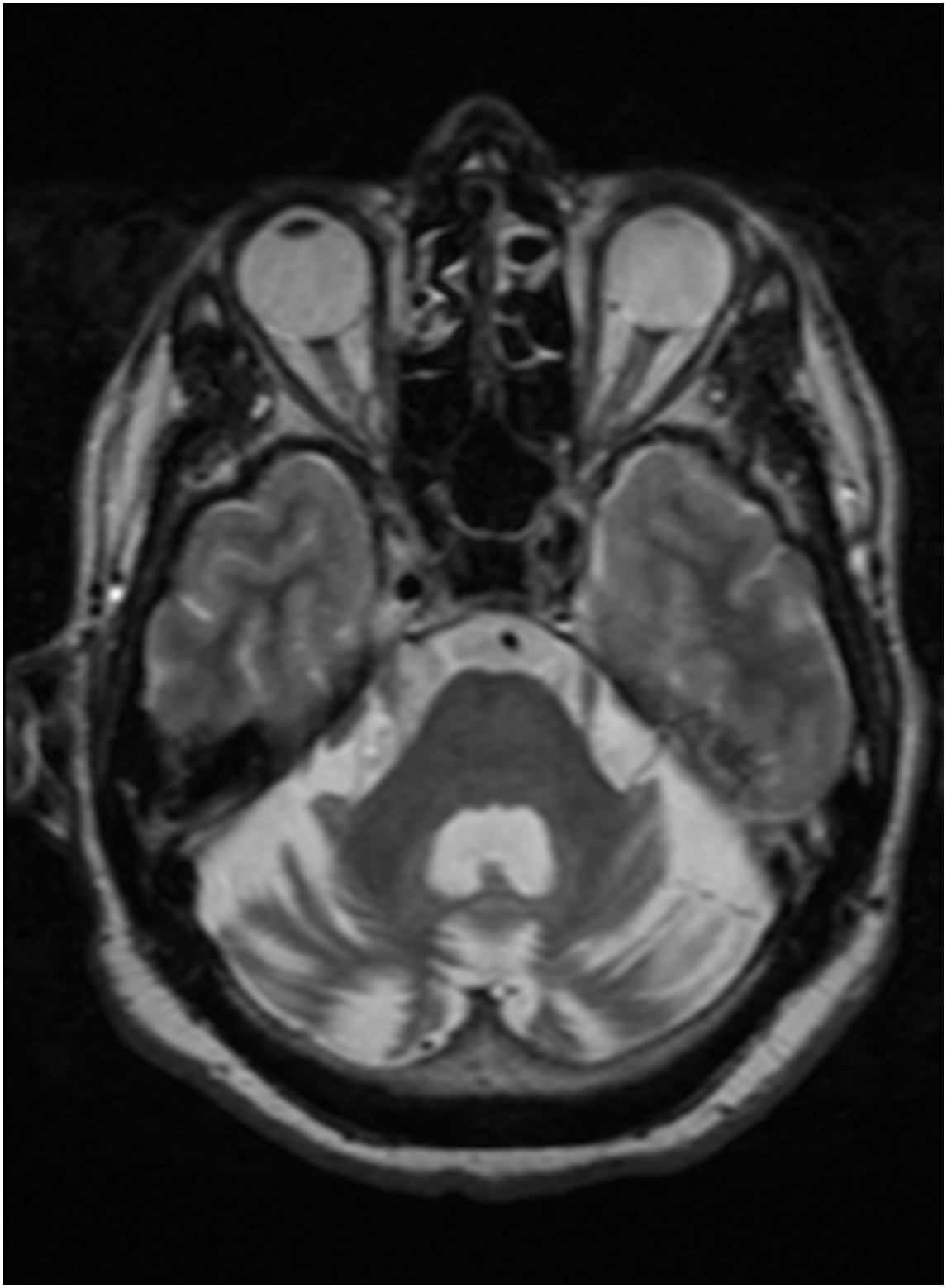


Table S1. Targeted Regions of the Array Analysis

1a. Targeted regions.

1b. Base pairs targeted.

1c. Total base pairs targeted after probe selection

Table S1a

Chromosome	start	end	size
Chr.1	77,105,773	78,116,669	1,010,896
Chr.1	91,918,489	93,199,672	1,281,183
Chr.2	140,705,465	142,605,740	1,900,275
Chr.3	17,173,658	17,869,885	696,227
Chr.3	32,123,105	33,009,854	886,749
Chr.3	34,355,326	44,599,977	10,244,651
Chr.3	78,729,077	80,456,380	1,727,303
Chr.3	126,284,169	127,489,262	1,205,093
Chr.3	131,245,012	131,313,041	68,029
Chr.4	147,394,586	147,692,007	297,421
Chr.14	59,132,446	59,624,078	491,632
Sum			19,809,459

Table S1b

Total bp targeted
1905376

Table S1c

Total bp tiled
2178492

Table S2. General Statistics of the Sequencing Run

Coverage statistics		Variant statistics	
Total number of mapped bases	109,790,683	Number of variants detected	3917
% bases mapped to or near (500bp) targets	93.50%	Known SNPs (dbSNP130)	3521
Average coverage of the targets	32.28x	Novel variants of those:	396
% targets covered at least 1x	99.01%	Splice site (SS) or coding variants	10
% targets covered at least 5x	98.48%	Non synonymous (NS) or SS	8
% targets covered at least 10x	95.80%	Homozygous SS or NS	4
% targets covered at least 15x	89.77%		
% targets covered at least 20x	79.66%		

Left side: statistics on the sequence coverage. Right side: statistics on the detected variants by the Roche 454 software. Known SNPs are only variants with the exact nucleotide change as described in dbSNP

Table S3. Overview of Variants after Initial Prioritization

Chr	Position	Ref	Var	% Var	Gene	Gene Id	Ref AA	Mut AA	PhyloP	Grantham
chr2	1.41E+08	A	-	89	LRP1B	NM_018557	SS		0.40	0
chr3	36754018	C	G	98	DCLK3	NM_033403	R	S	0.24	0
chr3	43571913	A	C	94	ANO10	NM_018075	L	R	5.26	145
chr3	43718918	G	T	100	ABHD5	NM_016006	R	L	2.65	102

Columns are from left to right: chromosome (Chr), position of the variant (Position), reference allele on the plus strand (Ref), variant allele on the plus strand (Var), percentage of reads with the variant allele (% Var), gene name in which the variant is located (Gene), RefSeq gene id (Gene Id), reference amino acid, where “SS” indicates a canonical splice site (Ref AA), variant amino acid (Mut AA), PhyloP log odds ratio for evolutionary conservation (PhyloP) (Pollard et al. 110-21) and Grantam score for the amino acid substitution (Grantham). The lightly shaded variant was reported in our internal variant database. The dark shading indicates the causative mutation.

Table S4. Primers Used for Amplification and Sequence Analysis of Human ANO10

Exon	Forward (5' > 3')	Reverse (5' > 3')	Amplicon Length (bp)
1	TACGATCGCCCAGTGAGG	GCTCTGTGGCTGCAAGG	340
2	TGCTTTTATCTTGGAAGCCAG	GGGAGGCTGAGCATACAGTG	327
3	AAAGAACTGCCATCCCTAATG	AAAAAGTTTGCTGATCCCTGA	457
4	CATACTGCTTTCTGCTCATTGG	ATTTTCATGTACAATGTTAGGGC	744
5	TGAAGCGTATCATGCACAATC	ATATCTGCCCAAGGGAGCTG	275
6-1	AGGGTTGAATGATCCCCAC	AAATGCGCAACTGTCTCTTG	459
6-2	AACATGACCTACAGGTGGGG	TGTGAATCCCATGATCTAGGC	435
7	GACTGAGGCTCTGATGTTGG	TCAATCCTTGCCTATTTGCAC	304
8	AGGCTGGGAGCTGTAGACTG	GCATAATACACAATGCCATTCC	399
9	GAAACACCATTCTAACACCTAGC	AGCAGTGCTTCAATGCAAAG	569
10	AGAGGCCACAGCTTTGATTAG	TTTCCCTGTCATAACACCTCG	341
11	AGGATGAGGAAATATGGAAGC	TTGCTCAATTGTCAGTCATGG	290
12	GGCCTGCTTGGTCTTTGATAC	TCCTGAACTGGAGTCCTCTG	404
13-1	GCTTCCACAGAGAGCAGAGG	ACTGCTATGAGGGGAACGTG	490
13-2	CCTTCTCAGTTTCGCAGTGG	ATCTCACCGCTCCACCTTC	499