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Supplemental Data

Exome Sequence Reveals Mutations in CoA Synthase as a Cause of Neurodegeneration with Brain Iron Accumulation

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Table S1. Next generation Sequencing Statistics

Id	Type	Reads	Mapped	Percent	Seq (Gb)	on bait	Avg cov	Cov 1x	Cov 4x	Cov 8x	Cov 20x
#55633	SureSelect50Mb	87259289	80916002	92.73	8.81	73.85	93.96	99.61	98.79	97.3	92.4

Table S2. Variants Identified by Exome Sequencing

Variants filtering	
Synonymous variants	11846
NSV	11601
NSV with frequency <0.1% in ‘in-house and public databases	340
≥ 2NSV / gene	22
Genes carrying homozygous rare NSV	12

NSV = missense, nonsense, stop/loss, splice site disruption, insertions, deletions

Table S3: detailed list of the 12 genes carrying homozygous rare NSV

gene #	gene symbol	OMIM	chromosomal position of identified variant	UCSC transcript, predicted mutation at nucleotide and protein level	dbSNP (rs number)	prefunction	pph2	Sift	Occurence of predicted recessive-type variants in 3,159 samples with unrelated phenotypes	Other arguments against a causal role
1	GUCA2A	*139392	chr1:42629092-42629092	uc001chd.1, c.265G>T,p.Glu89*	rs147564899	nonsense		0.39	-	uc001chd.1, c.265G>T,p.Glu89* also found in healthy subjects I-2 and II-4 of family 1
2	HRNR	*114085	chr1:152189999-152189999	uc001ezt.1, c.4106G>T, p.Ser1369Phe		missense	benign	0.05	>1000 other patients with predicted recessive type mutations and unrelated phenotypes	
			chr1:152190002-152190002	uc001ezt.1, c.4103G>A, p.Gly1368Asp		missense	benign	0.3		
3	ADAM8	*602267	chr10:135082994-135082994	uc021qbe.1, c.1903T>G, p.Trp635Gly		missense	probably damaging		16 other patients with predicted recessive type mutations and unrelated phenotypes	
4	FBXO47	*609498	chr17:37118301-37118301	uc002hrc.2, c.182-1G>A, p.?		splice			absence of mutations in 56 NBIA subjects	Suggested role as a tumor supressor.
5	CACNB1	*114207	chr17:37353687-37353687	uc002hrm.2, c.62A>G, p.Glu21Gly		missense	benign	0.17	1 other patient with predicted compound heterozygous mutations and mental retardation.	
6	**COASY**	***609855**	**chr17:40717686-40717686**	**uc010cyj.3, c.1582C>T, p.Arg528Cys**	**rs140709867**	**missense**	**probably damaging**	**0**	-	
7	BZRAP1	*610764	chr17:56385086-56385086	uc002ivx.4, c.4869G->C, p.:Gln1623His	rs141226909	missense	possibly damaging	0.12	52 other patients with predicted recessive type mutations and unrelated phenotypes	
8	C17orf47	not available	chr17:56621291-56621291	uc002iwq.2, c.257C>T, p.Arg86Gln		missense	benign	0.28	2 other patients with predicted recessive type mutations and unrelated phenotypes	
9	LRP1B	*608766	chr2:141641491-141641491	uc002tvj.1, c.4064A>G, p.Asn1355Ser		missense	probably damaging	0.56	151 other patients with predicted recessive type mutations and unrelated phenotypes	
10	EVC2	*607261	chr4:5624616-5624616	uc003gjj.3, p.2149C>G, p.His717Asp		missense	possibly damaging	0.32	25 other patients with predicted recessive type mutations and unrelated phenotypes	Associated with Ellis-van Creveld syndrome (MIM *607261)
			chr4:5667370-5667370	uc003gjj.3, c.877C>T, p.Pro293Ser		missense	probably damaging	0.14		
11	KIAA1797	*614606	chr9:20885216-20885216	uc003zog.1, c.2612C>T, p.Ala871Val	rs187654100	missense	benign	0.29	16 other patients with predicted recessive type mutations and unrelated phenotypes	
12	IFNW1	*147553	chr9:21141080-21141080	uc003zol.1, c.490A>G, p.Trp164Arg	rs147009804	missense	probably damaging	0	-	uc003zol.1, c.490A->G, p.Trp164Arg also found in healthy subjects I-2, II-4 and II-5 of family 1

Table S4. Primer sequences and PCR conditions to amplify the 9 exons of *COASY* gene.

Exons	Forward primer	Reverse primer	DNA polymerase	Annealing Temp
1a	GATTTTTGGATCCCCAGCCC	CAAGAACCTCAAACGTGGCC	*AmpliTaq Gold*®	*58°C*
1b	TCCCATACAGGCCTCTGCC	GGCCTTAGCACCAATTGAGTG	*AmpliTaq Gold*®	*60°C*
2	TGCTTGCCTGTTTCTTCACCT	CGGGCAAACTGAGAACCAGTT	GoTaq® *Flexi*	59°C
3	GGAGCCTGGGTAGGAAGGG	CTCAGCCTCATGCCTGGG	GoTaq® *Flexi*	60°C
4	CCTGGCAATGCTGGAGAGTAG	GGCCTGGCTAGCTCCTCACT	GoTaq® *Flexi*	60°C
5	GGCCTCTGTGCTCAGTTGTCT	GCAAGGTGGTGTTGGGAACA	GoTaq® *Flexi*	60°C
6	CCCAGAATGCCATTTCCATT	GACGTTCTCCCAGGCAGAAC	GoTaq® *Flexi*	59°C
7	GAGGATGGCAACAGCTAAGGG	TTTGTCAGGAAGAATTCCAGCTC	GoTaq® *Flexi*	59°C
8	CCCCCCACACCATCCCTAC	GATGAGTCTTGGGAATGCGCT	GoTaq® *Flexi*	60°C
9	TACTGCCTGACCCTGCCCT	AAAGCTCGCCTCTGGCTAGC	*AmpliTaq Gold*®	*60°C*

GoTaq® *Flexi* DNA Polymerase (Promega); *AmpliTaq Gold*® (Life Technologies) were used. PCR conditions were: 94°C, 1min (1 cycle); 94°C 30sec, annealing time was 30sec at the indicated temperatures, 72°C 1min (30 cycles).