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Supplemental Data

Biallelic Mutations in *DNAJC12* Cause Hyperphenylalaninemia, Dystonia, and Intellectual Disability

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SUPPLEMENTAL CASE REPORTS

A-IV-2 (Family A, **Figure 1A**) was a newborn male with HPA identified by NBS, born from consanguineous parents of Moroccan origin by caesarean section at 36 weeks of gestation due to oligohydramnios. The anthropometric measurements were at the 5th percentile, and he appeared normal apart from axial hypotonia attributed to mild prematurity. Despite rapid normalization of the Phe level after performing the BH_4 loading test, a BH_4 defect was ruled out due to a normal urinary pterin profile and normal dihydropteridine reductase (DHPR) activity. He was diagnosed with BH_4-responsive PKU and was discharged home with 10 mg/kg/day BH_4, and his Phe level was maintained below 340 μmol/l (**Table S1** in the Supplemental Data). At 10 months, he developed severe axial hypotonia with dystonia of the upper limbs and multidirectional nystagmus. Cerebrospinal fluid (CSF) biogenic amine analyses confirmed a neurotransmitter defect with dopamine and serotonin metabolite deficiencies (**Table S1**). Treatment with neurotransmitter precursors (a dopamine precursor, L-Dopa/carbidopa, and a serotonin precursor, 5-hydroxytryptophan) and folinic acid was added to the current BH_4 regimen, resulting in normalization of the neurological status within a few weeks. Brain MRI was normal (not shown). He is now 9.5 years old and has a low-normal IQ (WISC-IV: verbal comprehension=81, perceptual reasoning=73, and processing speed=88), has no extrapyramidal signs and does not require special education services.

A-IV-4, the younger sister of A-IV-2 (Family A, **Figure 1A**) was born at term after an uneventful pregnancy and delivery. NBS revealed that she had HPA. Because of her brother's history, she underwent CSF analysis at the age of 3 months, which indicated that her neurotransmitter profile was similar to that of her brother (**Table S1**). She was immediately treated with neurotransmitter precursors, BH_4 and folinic acid. The results of clinical examinations were consistently normal and remained so at the age of 22 months.

B-IV-1 (Family B, **Figure 1A**), born to consanguineous parents of Arab-Muslim descent, was diagnosed with HPA following NBS and was subsequently treated with a Phe-restricted diet. Her early development was normal, but mild global developmental delay was noticed at 2 years. At 3.5 years, she developed left lower limb dystonia, and developmental assessment revealed a DQ of 62. Electroencephalogram was normal. At the age of 13 years, clinical examination demonstrated obesity and cold, cyanotic hands. Neurologic examinations revealed Parkinsonism, including hypomimia with no habituation on glabellar tap, rigidity, and bradykinesia; no tremor was apparent. Cognitive assessment results were consistent with moderate intellectual disability (ID). Brain CT was normal. CSF neurotransmitter analysis showed low HVA and 5-HIAA levels (**Table S1**). Subsequently, treatment with L-Dopa/carbidopa and selegiline was initiated, which improved the Parkinsonism signs but not the cognitive status. Empirical treatment with BH_4 (10 mg/kg/day) led to improvements in both her motor and neurocognitive statuses.

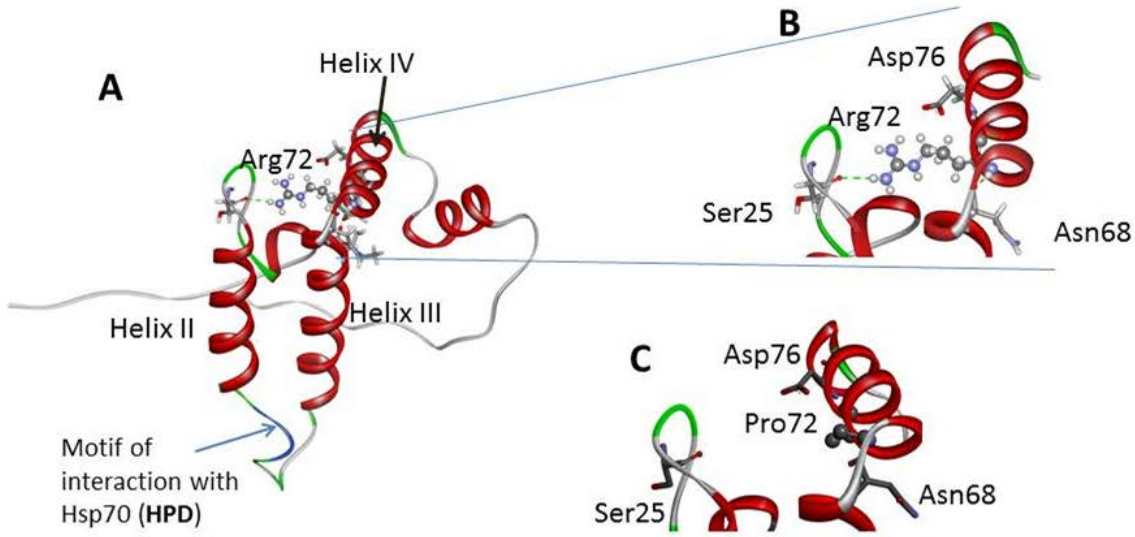
B-IV-2 (younger brother of B-IV-1 in Family B, **Figure 1A**) was born at term after an uneventful pregnancy and delivery. NBS, performed using bacterial inhibition assay and not tandem mass spectrometry, showed a normal Phe level. At 10 years, he displayed attention deficit and easy fatigability, and his school performance showed deterioration. A repeat measurement revealed that the serum Phe level was elevated (**Table S1**). Clinical examination showed cold, cyanotic hands and mild hypertonia of the lower limbs, and brain MRI and MR spectroscopy were normal (not shown). CSF neurotransmitter analysis showed low HVA and 5-HIAA levels (**Table S1**). Treatment with L-Dopa/carbidopa and selegiline caused dizziness and did not improve memory or school abilities and was subsequently halted. Later, empirical treatment with BH_4 led to substantial improvement in school performance, reduced fatigue and increased exercise tolerance.

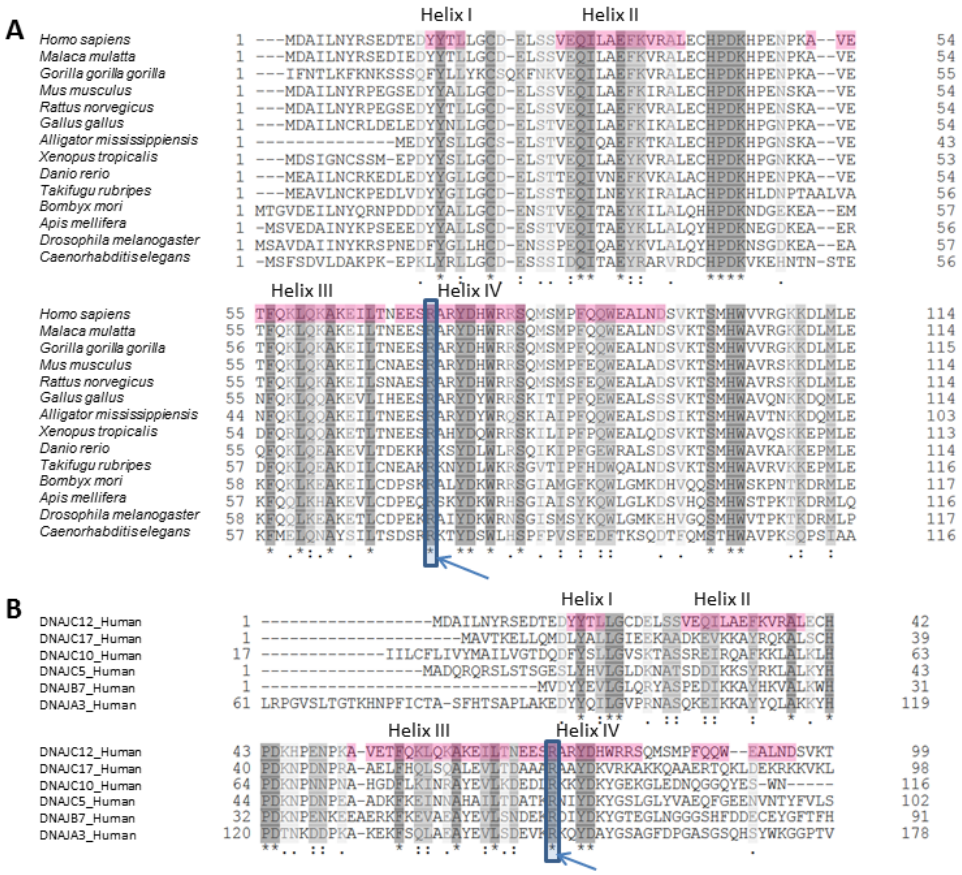
C-II-4 (Family C, **Figure 1A**), the fourth child of consanguineous parents of Turkish origin, was born at term via caesarean section after an uneventful pregnancy. NBS revealed an elevated Phe level. At 6 months, he had oculogyric crises and extrapyramidal signs. BH_4 disorder was ruled out due to normal urinary pterin profile and normal DHPR activity. He subsequently showed global developmental and speech delays, and at 2 years, his plasma Phe level was elevated. Brain MRI was normal (not shown). CSF analysis revealed low HVA and 5-HIAA levels (**Table S1**). At 2.5 years, treatment with L-Dopa/carbidopa was initiated, resulting in striking improvements in the neurological symptoms and in development. The oculogyric crises ceased, but nonverbal neuropsychological assessment (Snijders-Oomen nonverbal test; SON-R) revealed an IQ of 74. Because of the persistent but moderate elevation in the Phe level, BH_4 treatment was initiated. The L-dopa/carbidopa dose was increased in a stepwise manner, with continuous improvement of his neurological situation. At 7 years, detailed neurodevelopmental clinical investigation still showed minor gross motor function deficits.

D-V-1 (Family D, **Figure 1A**) was the first child of consanguineous Moroccan parents. The pregnancy, birth and neonatal period were normal. No NBS had been performed. At the age of one year, D-V-1 exhibited early autistic features. Motor development was delayed. At 6 years, she had febrile seizures. The Phe level was found to be elevated (503 µmol/l), while urinary pterin metabolites and DHPR activity were normal. Dietary management was initiated (reduction of natural protein intake to 1.1 g/kg/day; Phe-free amino acid mixture), and the Phe level was maintained between 200 and 800 µmol/l. At 7 years, BH_4 (10 mg/kg/day) treatment was initiated, which further decreased the affected individual's Phe level to 100-400 µmol/l; however, her autistic features showed no improvement at 10 years. Currently, she has ID with no expressive language but some nonverbal communication. In addition, she has muscle hypotonia, a broad-based gait, bradykinesia, and dystonia. CSF neurotransmitter deficiencies were documented at the age of 10.5 years (**Table S1**).

SUPPLEMENTAL FIGURES

Anikster et al. Supp Fig. S1





SUPPLEMENTAL FIGURE AND TABLE LEGENDS

Supplemental Figure S1

Arg72 is essential for maintaining the 3D structure of the J-domain of DNAJC12

NMR structure of the J-domain of DNAJC12 (PDB ID 2CTQ, chain A) (A), with amplification of the area around Arg72 in the wild-type (B) and Arg72Pro mutant (C). Arg72 is located at the start of helix IV, and its side chain forms an H-bond with the backbone carbonyl of Ser25 at the start of helix II. This interaction stabilizes the structure and determines the positioning of the canonical HPD motif that interacts with Hsp70 (in a loop located between helices II and III). Arg72 is also kept in position through an electrostatic interaction with Asp76 (in helix IV) and additional backbone hydrogen H-bonds with Asp76 and Asn68 (between helices III and IV).

Supplemental Figure S2

Sequence alignments, showing conservation of the Arg72 residue

The Arg72 residue of DNAJC12 (arrows) is highly conserved among the same J-domain subfamily in other species (A) and among J-domains in human DNAJ/HSP40 proteins (B).

Supplemental Table S1
Biochemical characteristics of DNAJC12-defective patients
Neurotransmitter profiles in the cerebrospinal fluid (CSF) and additional biochemical results for the patients with the *DNAJC12*-associated phenotype.
Age-adjusted normal values are presented in square brackets. The bold numbers signify abnormal values. 5-HIAA=5-hydroxyindoleacetic acid. HVA=homovanillic acid. 5-MTHF=5-methyltetrahydrofolate. Neo=neopterin. Bio=biopterin. DHPR=dihydropteridine reductase activity. DBS=dried blood spot. NBS=newborn screening. n.d.=not done. U.Pt=urinary pterins.

Supplemental Table S2
List of all the candidate regions resulting of the homozygosity mapping analysis performed by SNP array on all of the members of family B. Only loci larger than 0.5Mb were selected. In red, the *DNAJC12* locus.

Supplemental Table S3
Whole-exome sequencing quality metrics and statistics data

Supplemental Table S1

Family	Ind. (Age)	Sex	NBS Phe	Pterins/ DHPR	BH_4 Loading	5HIAA	HVA	HVA/ HIAA	5MTHF	Neo	Bio	BH4	Phe	Phe	Prolactin	Treatment at spinal tap
			DBS	Urine/DBS		CSF								Blood		
			(µM)			(nM)	(nM)		(nM)	(nM)	(nM)	(nM)	(µM)	(µM)	(ng/ml)	
													[5-18]	[23-94]	[<25]	
A del6943 *	A-IV-2	m	**410**	Normal	Rapid decrease (6 hours) of blood Phe to values below 120 µM after a 20 mg/kg BH4 loading oral dose	**28**	**192**	**6.9**	162	**35****	20**	n.d.	**59**	**202****	**33**	BH_4 (10 mg/kg/d)
						[114-336]	[295-932]	[1.5-3.5]	[64-182]	[12-30]	[15-40]					
	A-IV-2					**477****	515**	**1.1**	133**	n.d.	n.d.		n.d	**290****	20**	BH_4 (10 mg/kg/d) L-Dopa/carbidopa (10 mg/kg/d) 5-OHTrp (10mg/kg/d) Folinic acid (5 mg/kg/d)
						[114-336]	[295-932]	[1.5-3.5]	[64-182]							
	A-IV-2					**395****	570**	**1.4**	141**	15	32			n.d.	11.5**	
						[105-299]	[211-871]	[1.5-3.5]	[64-182]	[8-43]	[8-54]					
	A-IV-2					197**	418**	2.1	89**	21	20				9**	
						[105-299]	[211-871]	[1.5-3.5]	[64-111]	[7-55]	[10-52]					
	A-IV-2					149**	267**	1.8	89**	17	21			**300****	16**	
						[105-299]	[211-871]	[1.5-3.5]	[64-111]	[7-55]	[10-52]					
	A-IV-2					134**	179**	**1.3**	75**	17	19			**340****	23.4**	
						[88-178]	[144-801]	[1.5-3.5]	[64-111]	[7-55]	[10-52]					

	A-IV-4	f	**275**	Normal	n.d.	**76** [150-800]	390 [310-1100]	**5.1** [1.5-3.5]	163 [83-176]	26 [9-34]	**90** [12-44]	n.d.	**85**	**320**	**38**	none
B c.158-2A>T	B-IV-1 (13y)	f	**460**	Normal	n.d.	**5** [74-163]	**44** [133-551]	**9.5** [1.5-4]	n.d.	**51** [9-30]	**49** [10-30]	n.d.	n.d.	**332**	13.2	none
	B-IV-1 (20y)					**12** [66-141]	**98** [115-488]	**8** [1.5-4]	57 [41-90]	**31** [9-30]	29** [10-30]		**26.6****	**133****	**25.4**	BH_4 (10 mg/kg/d)
	B-IV-2 (12y)	m	84	Normal	n.d.	**2.2** [74-163]	**47** [133-551]	**21** [1.5-4]	n.d.	**42** [9-30]	**47** [10-30]	n.d.	n.d.	**339**	n.d.	none
	B-IV-2 (19y)					**6** [66-141]	**44** [115-488]	**7** [1.5-4]	43 [41-90]	20 [9-20]	27** [10-30]		**19****	**115.5****	10.8	BH_4 (10 mg/kg/d)
C p.R72P	C-II-4 (2.5y)	m	**145**	Normal	n.d.	**28** [155-359]	**80** [364-870]	2.9 [1.6-3.9]	142 [38-177]	20 [5-53]	n.d.	54 [20-79]	**112.5**	**491**	n.d.	none
	C-II-4 (3.5y)					**88** [130-362]	**207**** [313-824]	2.4 [1.5-4.1]	150 [32-148]	20 [5-53]		**165**** [20-61]	**87.9****	n.d.	22.1**	L-Dopa/carbidopa (7,5 mg/kg/d) BH_4 (10 mg/kg/d)
D del6943*	D-V-1(10.5)	f	n.d.	Normal	n.d.	148 [90-237]	**27** [220-560]	**5.5** [1.7-3.7]	94 [33-138]	**39**** [7-27]	n.d.	**68**** [20-49]	**30.2****	**240****	5.3	BH_4 (10 mg/kg/d)

* c.298-968_503-2603del

**under treatment/supplementation

Supplemental Table S2. Areas of homozygosity in Family B

Chromosome	Start (bp)	End (bp)	Size (Mb)
chr1	49100000	53400000	4.3
	156250000	159950000	3.7
chr2	88800000	96600000	7.8
	51550000	65650000	14.1
	109600000	113700000	4.1
	237840000	241570000	3.73
chr3	182700000	187500000	4.8
chr5	125760000	133460000	7.7
chr6	0	11930000	11.93
chr7	44940000	50710000	5.77
chr8	130600000	139500000	8.9
chr9	38650000	70175000	31.525
chr10	6390000	7730000	1.34
	11150000	30050000	18.9
	68040000	71200000	3.16
	64300000	65000000	0.7
	103430000	112550000	9.12
chr16	18150000	23960000	5.81
	83510000	90350000	6.84
chr18	1060000	14750000	13.69
	28360000	37760000	9.4
chr19	19720000	21025000	1.305
	39680000	40300000	0.62
	41550000	42750000	1.2
	46700000	48280000	1.58
Total			182.02

Supplemental Table S3

Individuals	Family	Enrichment kit	Number of reads sequenced	Number of reads mapped	Avg cov (exome)	Uncovered	Cov 1x	Cov 8x	Cov 20x	Number of detected SNV
A-IV-2	A	SureSelect50Mbv5	62239893	61992788	72.94	0.16	99.84	98.92	94.26	66434
B-IV-1	B	TruSeqV1	53254851	44340236	38.4	12.5	NA	NA	NA	43090
C-II-4	C	SureSelect50Mbv4	102059102	101614649	106.11	0.26	99.74	98.27	91.82	65401
D-V-1	D	SureSelect50Mbv5	98891265	98686524	127.54	0.28	99.72	98.86	96.43	68084