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Supplemental Data

MECR Mutations Cause Childhood-Onset Dystonia and Optic Atrophy, a Mitochondrial Fatty Acid Synthesis Disorder

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Case descriptions:

Affected individual 1 (figure 1, family A, II:1) is a 48 year old male with marked dystonia, dysarthria, spasticity, optic atrophy and normal intelligence. He is the eldest of two siblings to unrelated parents of Ashkenazi Jewish origin and was born post-date by forceps-assisted delivery. At 2 weeks of age he returned to the hospital severely ill from 'strangulated bowel', which was surgically repaired. His fine and gross motor development were delayed in infancy, which was attributed to neonatal asphyxia. In early childhood, he showed moderate asymmetric spasticity in his lower limbs with up-going planter reflexes, and mild dysarthria. By mid-childhood he was noted to have optic nerve pallor and decreased visual acuity, and he began to develop dystonia and worsening dysarthria. School performance continued normally, however. A bone marrow biopsy at age 8 years showed 'sea blue' histiocytes, and iron-uptake studies suggested a diagnosis of Hallervorden-Spatz syndrome (MIM 234200) in this boy and his sister [1]. The proband had gradual worsening of spasticity, rigidity, dystonia and dysarthria, while his sister never developed significant neurological symptoms. As a young adult, he was diagnosed with keratoconus and found to have slow eye movements in all directions, with limited upward gaze. Performance on WAIS-R was average on the Verbal Scale and superior on the Verbal Memory Index at age 23 years. At the age of 42 years, MRI demonstrated T2 hyperintense signal in the putamen suggestive of striatal degeneration (figure 2A). A follow-up MRI performed 2 years later revealed no significant change or progression. He achieved a master's degree in education, lives semi-independently and ambulates with a walker. His sister has a normal neurological examination as an adult and a normal brain MRI.

Affected individual 2 (Figure 1, family B, II:2) is a 27 month old boy, youngest of 2 children to parents of a mixed Jewish origin (father – Ashkenazi/Moroccan/Indian, mother- Ashkenazi). He was born at term by a normal delivery after an uneventful pregnancy. During infancy he exhibited

failure to thrive, mild motor delay and hypotonia. At 15 months he presented with episodes of paroxysmal dyskinesia and myoclonus. Over a period of two months, coinciding with a prolonged diarrheal illness, his movement disorder evolved into a continuous state of mixed dystonia-chorea involving four limbs and face along with gait ataxia. The movement disorder fluctuated up to a complete loss of ambulation at times; nevertheless there was no apparent impairment of consciousness or cognition. MRI demonstrated T2 pallidal hyperintensity, with milder signal abnormality in the putamen and normal spectroscopy (Fig 2B). Extensive metabolic workup including neurotransmitters and lactate in cerebrospinal fluid (CSF) was normal, except for repeated increased excretion of urinary 3-hydroxy-isovaleric acid. Echocardiogram, electroencephalogram (EEG) and eye exam showed no abnormality. Analysis of mitochondrial DNA revealed neither point mutations nor small deletions and duplications. Despite this, he was put on a mitochondrial cocktail including thiamine, biotin, riboflavin, carnitine, coenzyme Q10 and lipoic acid with apparent stabilization of his symptoms. Currently he exhibits mild limb and facial dystonia, mild dysarthria and mild ataxia, has over 10 words with good understanding and walks with assistance.

Affected individual 3 (Figure 1, family C, II:2) is a 45 year old male, second of 8 children to non-consanguineous parents of a Ashkenazi Jewish origin. He was born at term in a normal delivery following an uneventful pregnancy. His development during the first year of life was reported as normal and from 2 years he showed only mild dysarthric speech and clumsiness. A neurologic examination at the age of 5 years prompted by these symptoms was unimpressive. In subsequent years he gradually developed increasing limb and facial dystonia, had to use a walker at 18 years and by 25 years became wheelchair bound. Visual impairment associated with optic atrophy became apparent at around the age of 12 years. Only a very basic metabolic workup was performed which was reported as normal. Currently he exhibits severe limb, trunk and facial dystonia, with blepharospasm, completely unintelligible speech and swallowing difficulties as well as upper and

lower limb contractures. He suffers from severe visual impairment with intermittent nystagmus, has almost no purposeful hand use and is totally dependent in all daily living activities. Despite his profound disabilities he is cognitively intact.

Affected individual 4 (Figure 1, family C, II:8) is a 27 year old male, the younger brother of affected individual 3. He was also born at term by a normal delivery after an uneventful pregnancy. Early development was reported as normal by the family (he stood at 8 months). At 24 months he was reported to exhibit an unstable gait which became more pronounced at 3 years. A metabolic workup at 3 years was reported as normal, however a muscle biopsy showed possible partial reduction of complex 1 activity. Brain MRI demonstrated bilaterally increased T2 signal in both caudate and putamen, as was also seen in a recent MRI (Figure 2C). Genetic workup for spinocerebellar ataxias (SCA) and *DYT1* was negative. He was put on treatment with coenzyme Q10 at 4 years and a B-complex supplement was added at 6 years, with stabilization and even mild improvement of his motor symptoms according to his parents. Around the age of 8 years he complained of reading difficulties and was found to have a visual decline related to optic atrophy with intact retina. Visual evoked potentials (VEP) were abnormal with delayed conduction. Dysarthric speech appeared around the age of 12 years. At present, he has limb, truncal and facial dystonia, dysarthria with partially intelligible speech, independent ataxic-dystonic walking and pronounced consistent nystagmus with impaired vision. Despite the motor disability his cognition is fully preserved similar to his older brother.

Affected individual 5 (Figure 1, family D, II:1) is the only child born to non-consanguineous Tunisian parents. In the family history, spastic paraplegia was reported in a paternal great-uncle with onset at 39 years of age after an acute myelitis. The proband was delivered at term by caesarean section for breech presentation. Psychomotor development was normal, and she attended

regular school. Her clinical problems began at 6.5 years with cyclic episodes of weakness, headaches and vomiting. She was diagnosed with reduced visual acuity and dysarthria. In this context, VEP and ERG and brain MRI were performed and showed delayed bilateral visual conduction (L>R) and bilateral lesions of the posterior region of putamen (Fig 2D).
At first neurological examination (6 years and 9 months), she manifested dysarthria and oro-facial and left hand dystonia. All laboratory studies of blood, urine and CSF were normal including biotinidase activity. High dose oral biotin (200 mg/d) and thiamine (300mg/d) were started one month later and the cyclic episodes resolved. Dysarthria and visual acuity improved after 3 months of supplementation. Follow-up MRIs 2 and 5 months later confirmed the presence of bilateral putaminal lesions without signs of cavitation and without other associated abnormalities.
At 7 years and 1 month, she weighed 27 kg (+2 SD), her height was 127 cm (+3SD) and her head circumference was 54 cm (+2SD). She had some concentration difficulties but good social interactions. Pale skin color with blond hair and a mild papular erythematous eruption on the upper lip were noticed on general physical examination, and neurologic examination revealed nystagmus on lateral gaze, rhinolalia, dystonic posturing of the left side and difficulties with fine motor skills. There were no cerebellar or pyramidal signs.

Affected individual 6 (Figure 1, family E, II:1) is the eldest of a sibship of 4, delivered at term by caesarean section because of maternal pregnancy-associated hypertension with meconium staining of liquor, birth weight 2.79 kg. Besides feeding difficulties in the first few days of life and torticollis his first year was uneventful. He showed mildly delayed gross motor milestones with sitting at 10m and walking independently at 21m. On his first neurological assessment at 23 months he had age appropriate language skills and normal growth parameters. Examination demonstrated esophoria of the right eye, a wide-based, ataxic gait, mild impairment of fine motor coordination with dystonia and athetosis increased by excitement and low resting muscle tone with hyper-reflexia in the lower

limbs but normal plantar reflexes. Although truncal coordination improved over the next few years, dystonia and choreiform movements were exacerbated during intercurrent illnesses accompanied later by facial and limb myoclonus. At 6y of age he was found to have a new pigmentary retinopathy with progressive subsequent development of optic atrophy and a significant impairment of visual acuity by 10y of age. At the age of 9y there was a documented deterioration in language skills and executive functions with a decline in verbal comprehension index on the Wechsler Intelligence Scales for Children version IV (WISC IV®) from the low average to the extremely low range.

In his last assessment at 16 years of age, he was able to walk independently for short distances only, had mild dysarthria, was unable to read or write and showed mild to moderate dystonia and dyskinesia, with the myoclonus improved to a modest degree by levetiracetam. His height and weight had declined to the 5th and 10th percentiles respectively.

Serial cranial MRI scans demonstrated bilateral areas of hyperintense T2 signal in the globi pallidi at 4y, with a subsequent cavitation in the left pallidum by 6 years (Figure 2E,F), and no significant change at 10y. Spectroscopy performed at the age of 4y was normal but at the age of 10y a lactate peak was visible (Figure 2H).

Urine, blood and CSF metabolic studies were normal. A muscle biopsy at 3 years of age demonstrated normal histology but borderline low complex IV and II activities (26% and 31% respectively of control means relative to citrate synthase) with elevated activity of the other enzymes (137-382%). Complex IV in fibroblasts was 68% relative to protein and 53% relative to citrate synthase.

Affected individual 7 (Figure 1, family E, II:3), the younger brother of affected individual 6, is a dizygotic twin born by elective caesarean section at 35 weeks gestation, birth weight 2.3 kg. He had hypotonia with mildly delayed gross motor development, started walking independently at 18

months, however his gait was unsteady and mild dystonia and dyskinesia were evident from 3 years of age. He developed a stutter, mild dysarthria and dysphagia. Dystonia and dyskinesia progressively increased, with modest symptomatic benefit from levetiracetam. In his last evaluation at the age of 12, he was walking independently but fatigued easily and used a wheelchair for longer distances. Weight was on the 10^{th} and height on the 5^{th} centile. He also developed optic atrophy with visual acuity 6/24 at 5 years of age deteriorating to 6/60 at the age of 12y. Cognitive assessment at the age of 12 using the WISC IV demonstrated low average abilities on the verbal comprehension index with extremely low abilities on the other indices.

Serial cranial MRI and MRS studies at 3 and 6 years of age demonstrated bilateral globus pallidus lesions and high lactate peak similar to his brother's (Figure 2G,I), although without development of cavitation. Urine organic acid tandem mass spectrometry at age 3 showed a trace of 3-methylgluconate.

Supplemental figures and tables:

Figure S1) cDNA analysis of the c.830+2_830+3insT *MECR* splice site variant.

Only one band is seen in the father (family B, I:1) and a control, while 3 bands are seen in the affected individual 2 (family B, II:2) and the mother (family B, I:2) carrying the splice site variant, suggestive of two mutant transcripts. On the right – a prediction of the mutant transcripts sizes based on the aberrant bands. M(100kb) = marker; A = affected individual ; M = mother; F = father; C = control.

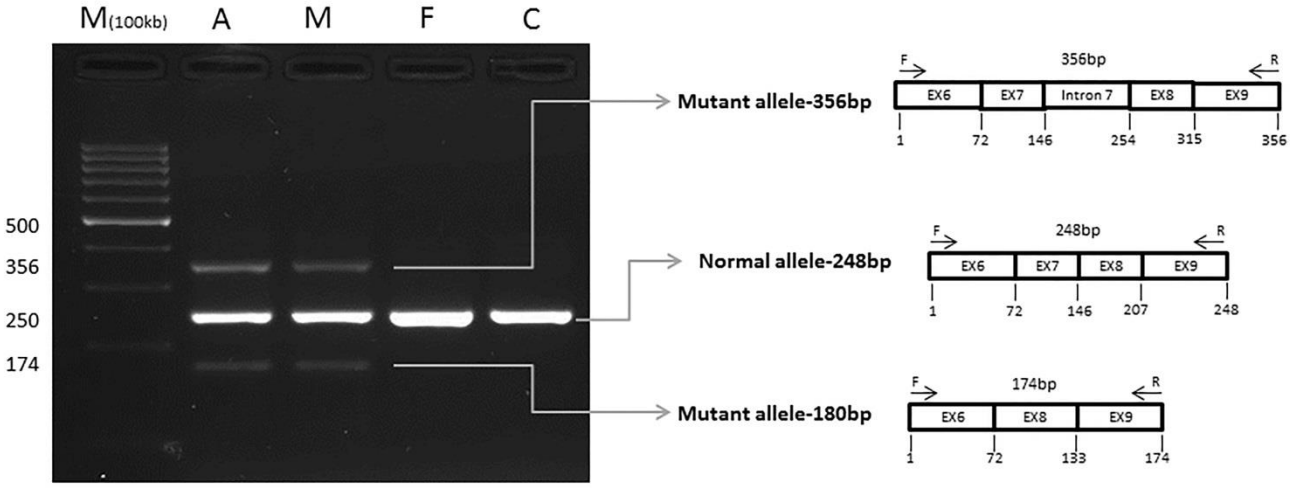

Figure S2) Modeling of the c.695G>A and c.855T>G mutated *MECR* alleles in the respiratory deficient yeast W1536 8B *etr1*Δ mtFAS defective strain**.**

(A,B) Analysis of rescue of respiratory growth of the *etr1*Δ mutant by the *MECR* c.695G>A and c.855T>G allele variants, respectively. Yeast cells transformed with plasmids carrying the indicated constructs were grown on liquid media, normalized according to cell density and serial diluted (1x, 1/10x, 1/100X and 1/1000). For each mutation two independent clones transformed with mutation plasmids were tested. Equal volumes of cell suspensions were spotted on synthetic media containing only a non-fermentable carbon source (glycerol or lactate) or plates containing the fermentable carbon source glucose as growth control, and grown for several days. Growth on lactate or glycerol indicates respiratory competence.

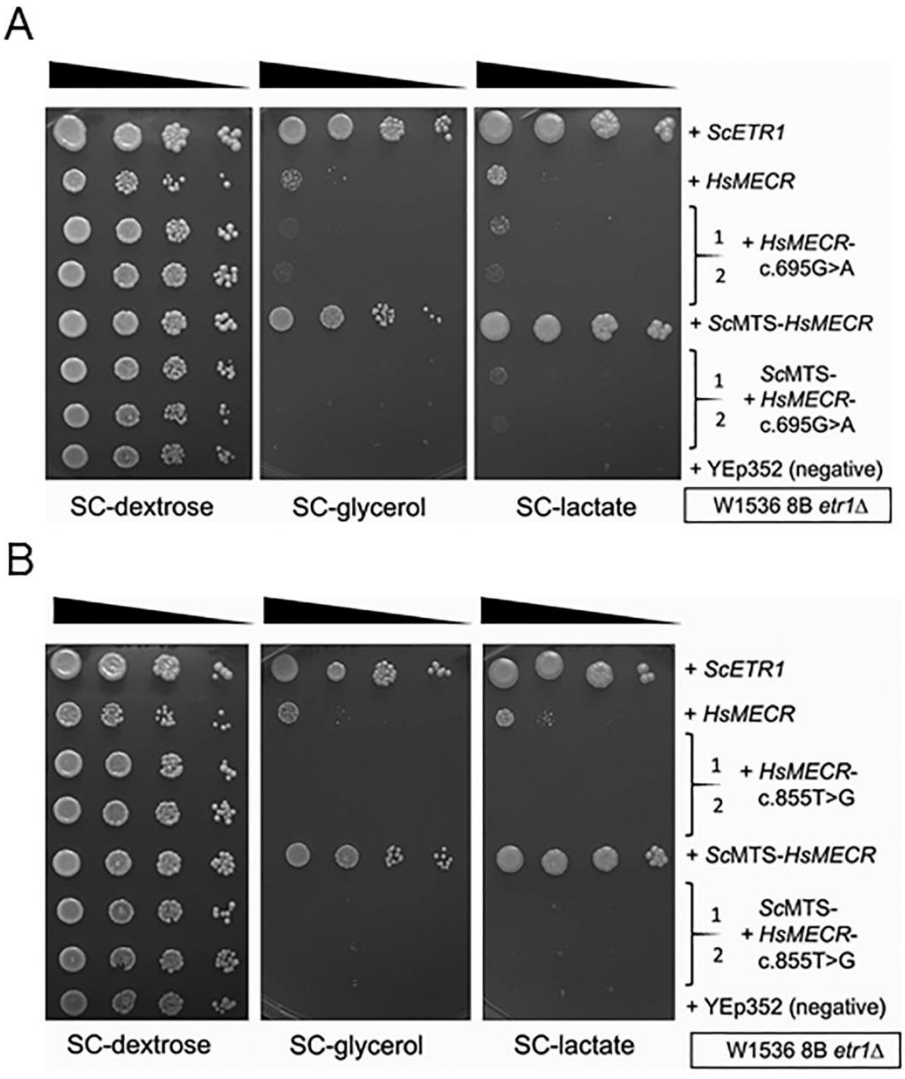

Figure S3: Assessment of oxidative phosphorylation enzyme production activity in cell line fibroblasts of affected individual 6.

(A) Western blotting of complexes I-V show no clear reduction the amount of any of the OXPHOS complexes in affected individual 6 (family E, II:1) compared to controls. (B) Dipstick assay measurements in his fibroblasts show that the activity of Complex I and IV was only modestly reduced to 65% and 75% of controls respectively. Dipstick assay results from independent experiments were pooled and analyzed with SPSS 22 software using an independent samples Mann-Whitney U test. Results are presented as the mean ± SEM (error bars), n = 6 for each group.

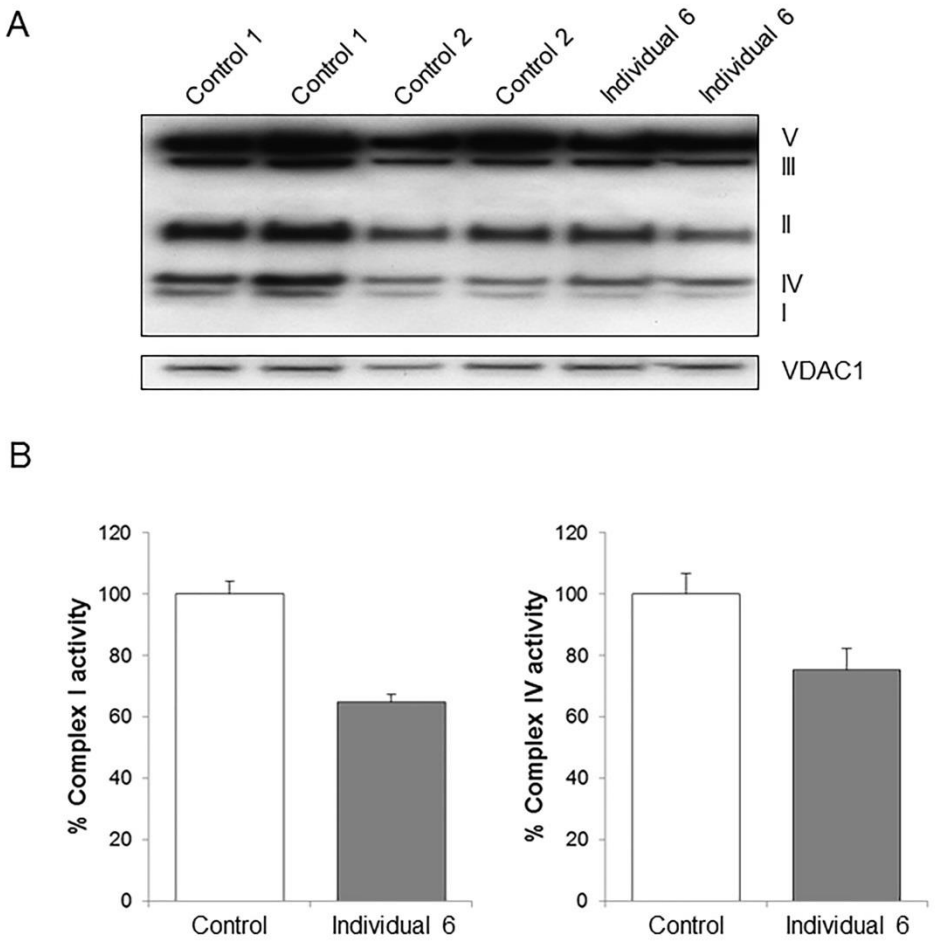

Table S1) Prediction of the mutation consequence for the three missense variants detected in this study.

Tool	**g.chr1:29528516C>T p.Gly232Glu**	**g.chr1:29522747T>C p.Tyr285Cys**	**g.chr1:29527086G>A p.Arg258Trp**	**Prediction type**
cupsat	+1.85 Kcal/mol (destabilize)	+0.84 Kcal/mol (destabilize)	+0.84 Kcal/mol (destabilize)	Thermo stability
duet	+1.033 Kcal/mol (destabilize)	+1.109 Kcal/mol (destabilize)	+0.88584 Kcal/mol (destabilize)	Thermo stability
mCSM	+0.97 Kcal/mol (destabilize)	+1.26 Kcal/mol (destabilize)	+0.861 Kcal/mol (destabilize)	Thermo stability
MAESTRO	+0.028 Kcal/mol (destabilize)	+0.220 Kcal/mol (destabilize)	+0.040 Kcal/mol (destabilize)	Thermo stability
SDM	+2.73 Kcal/mol (destabilize)	+3.5 Kcal/mol (stabilizing)	-1.38 Kcal/mol (stabilizing)	Thermo stability
I-Mutant2	Destabilizing(RI score5)	Destabilizing (RI score3)	Destabilizing (RI score7)	Thermo stability
Fold-X	+5.59 Kcal/mol (destabilize)	+3.45 Kcal/mol (destabilize)	+1.68 Kcal/mol (destabilize)	Thermo stability
Sift	Damaging (0.0)	Damaging (0.0)	Damaging (0.0)	Function
Polyphen2	Damaging (0.99)	Damaging (0.99)	Damaging (1.0)	Function
LRT	Damaging (0.0)	Damaging (0.0)	Damaging (0.0)	Function
MutationTaster	Disease causing (1)	Disease causing (1)	Disease causing (0.99)	
MutationAssessor	High impact (3.56)	Medium impact (2.56)	Medium impact (3.02)	Function
PROVEAN	Damaging (-8)	Damaging (-8.1)	Damaging (-5.2)	Function

Supplemental references:

1. Swaiman KF, Smith SA, Trock GL, Siddiqui AR (1983). Sea-blue histiocytes, lymphocytic cytosomes, movement disorder and 59Fe-uptake in basal ganglia: Hallervorden-Spatz disease or ceroid storage disease with abnormal isotope scan? *Neurology*, 33(3):301-305.