Supplemental Notes

1. Detailed description of mutation-carrying patients

2. Patient 47 with a homozygous NNT mutation, p.Gln40Lysfs*9

   The proband was the first child to healthy unrelated Japanese parents. He was born at 39 weeks of
gestation after an uneventful pregnancy. His birth weight and length were 2.9 kg and 48.4 cm, respectively. At
nine months of age, he was referred to us for progressive hyperpigmentation. He had normal growth (length
1.8 SD, weight 0.6 SD) and normal male genitalia. Endocrinological evaluation revealed a normal serum
cortisol level (4.4 µg/dL, reference range 5-23 µg/dL), a high plasma ACTH level (>1500 pg/mL, reference
range 25-100 pg/mL) and a normal serum 17α-hydroxyprogesterone level (<0.1 ng/mL). The levels of serum
electrolytes (Na 136 mEq/L, K 4.7 mEq/L) and aldosterone (139.0 pg/mL, reference range 50-900 pg/mL)
were normal, but the plasma renin activity (PRA 22.2 ng/mL/h, age-specific reference ≤15) was high. His
adrenal glands appeared normal-sized on computed tomography. He was clinically diagnosed as having
adrenal hypoplasia congenita because his adrenal glands were not enlarged despite the high plasma ACTH
level, and he was initially put on hydrocortisone replacement therapy. While his plasma ACTH level
decreased by this therapy, his PRA levels remained high (8.6-45.4 ng/mL/h, median 25.8 ng/mL/h, N=6) with
normal serum electrolyte levels (Na 136.8-140.2 mEq/L, median 138.6 mEq/L, K 4.28-4.98 mEq/L, median
4.56 mEq/L), suggesting mild mineralocorticoid deficiency. Fludrocortisone was subsequently introduced.
Siblings (patient 46-a and 46-b) with compound heterozygous AAAS mutations, p. [Trp84*];[Arg342*]

The proband (patient 46-a) was the first child born to healthy unrelated Japanese parents. She was born at 42 weeks of gestation after an uneventful pregnancy. Her birth weight and length were 2.7 kg and 49.0 cm, respectively. At six years of age, she experienced afebrile seizures with hypoglycemia (serum glucose level 39 mg/dL). At that time, skin hyperpigmentation was noted. Endocrinological evaluation revealed a low serum cortisol level (1.4 µg/dL) with high plasma ACTH (2740 pg/mL) and normal serum 17α-hydroxyprogesterone levels (data not shown). The levels of serum electrolytes (Na 140 mEq/L, K 4.6 mEq/L) and aldosterone (183 pg/mL), and the PRA (7.7 ng/mL/h) were normal. Her adrenal glands were not visualized on magnetic resonance imaging (MRI). She was diagnosed as having ACTH resistance and was placed on hydrocortisone replacement therapy. At the time of diagnosis, she had no clinical signs suggestive of triple A syndrome (achalasia, alacrima, and neurologic disabilities). At age 14 years, she still did not have any clinical symptoms of triple A syndrome. However, following the detection of AAAS mutations, she was evaluated for achalasia, alacrima, and neurologic disabilities. While gastrografin swallow did not reveal abnormal findings, esophagography revealed mild dilatation of a hypokinetic esophagus, suggesting achalasia. Ophthalmologic examination revealed mild alacrima. The Schirmer test revealed mildly decreased tear production (4 mm after 5 min on both eyes, reference range >5mm), while her tear film breakup times were normal (5-6 seconds on both eyes, reference range >5 seconds). Brain MRI showed no abnormalities, and her nerve conduction studies showed normal.

After the proband’s clinical diagnosis of ACTH resistance, we evaluated the adrenal function of her
two-year-old brother (46-b), who did not have any symptoms other than mild pigmentation of his gingiva.

Endocrinological evaluation revealed a normal serum cortisol level (5.4 µg/dL) with a high plasma ACTH level (2110 pg/mL). The levels of serum electrolytes (Na 141 mEq/L, K 4.1 mEq/L) were normal, and he was thus also diagnosed as having ACTH resistance. After the proband’s genetic diagnosis, it was revealed that her brother also carried the same compound heterozygous AAAS gene mutations, and he was also evaluated for achalasia, alacrima, and neurologic disabilities at 10 years of age. Gastrografin swallow did not show abnormal findings, while esophagography could not be performed well. Ophthalmologic examination suggested mild alacrima. The Schirmer test revealed mildly decreased tear production (5 mm and 4 mm after 5 min on the right and left eyes, respectively), and his tear film breakup times were short (3-4 seconds on both eyes, normal range >5 seconds). Brain MRI showed no abnormalities, and his nerve conduction studies showed normal.