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Supplemental Data

Mitochondrial Carbonic Anhydrase VA Deficiency Resulting from *CA5A* Alterations Presents with Hyperammonemia in Early Childhood

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SUPPLEMENTAL APPENDIX

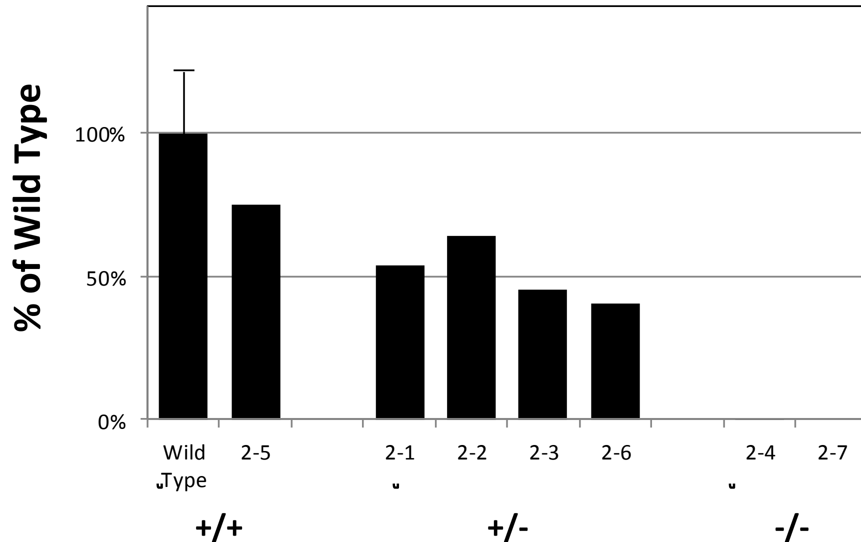

Figure S1. Quantitative PCR of genomic DNA from Family 3.

qPCR was conducted using genomic DNA as a template and primers specific to sequences within (exon 6) and outside (exon 4 or 7) the 4078-bp deletion region. Quantitation was normalized to the values of exon 4 or 7 amplifications and presented as the percentage of wild-type amplification product. The wild-type control reaction was performed three times, and the mean and standard error of the mean are shown. Sample numbering corresponds to the pedigree in Figure 3.

Methods for Long PCR and qPCR (Family 3)

Long PCR was used to amplify the deletion region encompassing exon 6 with the following primers: 5′-TCCCATTCTAGCTGCCTGAGGA and 5′AGACGAATGCCCTGTTCCTGTACC using Elongase® Enzyme Mix (Life Technologies) according to manufacturer's protocol. Briefly, 60 ng of genomic DNA was added to a PCR reaction containing 60 mM Tris-SO_4, pH 9.1; 18 mM $(NH_4)_2SO_4$; 1.1 mM MgSO4; and 200 nM primers in a final volume of 50 µL.. PCR cycling conditions were as follows: 94°C for 5 minutes; followed by 10 cycles of 94°C (90 seconds), a decreasing annealing temperature from 68°C to 63°C (0.5°C decrease per cycle; 30 seconds), and 68°C (6.5 minutes); followed by 25 cycles of 94°C (30 seconds), 64°C (30 seconds), and 68°C (6.5 minutes); with a final extension at 68°C for 10 minutes. PCR products were analyzed by 0.8% agarose gel electrophoresis. Quantitative PCR (qPCR) was used to measure the copy number of specific regions of the CA5A gene from genomic DNA samples. Primers (5′-TAACTCCACAACGCTCTCATGC and 5′-TGGGATTACTGGACCTACGC) were designed to amplify a 201-bp region of exon 6 at chr16:87925306–87925506 (putative deletion). CA5A exons 4 and 7 were similarly amplified by qPCR as quantitation references. qPCR reactions comprised 10 ng genomic DNA, SYBR Green I PCR Master Mix (Applied Biosystems), and 200 nM primers in a 20 mL reaction. Amplification and detection were performed with an ABI7500 fast real-time PCR system with 7500 software, version 2.0.6. qPCR cycling conditions were as follows: 95°C for 10 minutes, followed by 40 cycles of 95°C (15 seconds) and 60°C (1 minute). Amplification integrity was confirmed by melting curve analysis and direct Sanger sequencing of the qPCR products. The relative quantity (RQ) of amplified DNA in individual samples was calculated using the ΔΔCt method.

Bowtie + BWA	Reads processed	Percentage of reads aligned *(with good quality)*	Median of coverage *(exons)*	Total variants	Coding variants *(not synonymous, not in dbSNP)*
Affected girl	49397074	79.90%	30.1	113054	680
Affected boy	61280340	80.40%	36.8	113736	658
Mother	53949713	78.50%	32.1	114628	669
Father	46810250	79.40%	28.1	126991	605

GSNAP	Reads processed	Percentage of reads aligned *(with good quality)*	Median of coverage *(exons)*	Total variants	Coding variants *(not synonymous, not in dbSNP)*
Affected girl	54885198	88.70%	32.86	183341	1237
Affected boy	67456880	88.50%	39.8	177191	1298
Mother	61101170	88.90%	35.64	219357	1262
Father	52262234	88.70%	30.79	202381	1166

Table S1. Coverage and variants of the exome dataset.

A combination of Bowtie, BWA, and GSNAP were used to map the reads to the hg19 reference genome, and Samtools was used to identify variants. SNPEff was used to assign annotations to the variations, with respect to the hg19 database. Allele frequency was assessed in dbSNP (version 137; downloaded from UCSC Table Browser "All SNPs(137") on Feb 19, 2013. ESP data was downloaded from the NHLBI ESP server on June 2, 2013. The total number of starting reads for each individual is listed in the second column, and only reads with mapping quality of ≥20 are kept (percentage shown in third column). Coverage is shown in the fourth column, and is based on all known human exons compiled from Ensembl BIomart. The total number of variations, including indels, is listed in the fifth column. The sixth column lists the number of variations that remain after filtering against intergenic or intronic variations, polymorphisms, and synonymous mutations.