

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Strauss KA, Puffenberger EG, Huentelman MJ, et al. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. *N Engl J Med* 2006;354:1370-7.

SUPPLEMENTAL MATERIAL FOR NEJM MS 05-2773:

Symptomatic focal epilepsy caused by a mutation in contactin associated protein-like 2

K.A. Strauss, et al., 2005

METHODS

Patients and clinical studies

Routine clinical investigations included 24-hour digital video-electroencephalography (EEG) with sphenoidal electrodes added during pre-operative evaluations, cerebral magnetic resonance imaging (MRI) at 1.5T that included 3 mm coronal T2 slices perpendicular to the long axis of the temporal lobe, and timed-injection^{1,2} single photon emission computed tomography (SPECT) studies using ^{99m}Tc ECD (Neurolite). Three patients underwent surgery for intractable and disabling focal seizures that could be localized to one brain region based on consensus among pre-operative studies. Surgical techniques used (i.e. temporal lobectomy, selective amygdalohippocampectomy, limited cortical resection) were determined in the operating room based on provocative electrocortigraphy-guided seizure mapping with remifentanyl.³

Genetic mapping

Total genomic DNA isolation from whole blood using the PUREGENE DNA Isolation Kit (Gentra Systems, Minneapolis, MN) and SNP genotyping using the GeneChip Human Mapping 10K assay kit (Affymetrix, Santa Clara, CA) were performed according to manufacturers' instructions.⁴ SNP positions came from dbSNP build 115 and NCBI build 33 of the human genome. Normal, untransmitted chromosomes from this study and others were used to estimate population-specific SNP allele frequencies.⁴ Two-point LOD scores were calculated for each genotyped SNP using an approach similar to Broman and Weber.⁵ Cumulative two-point LOD scores for a block of homozygous SNPs were considered the "location score" for the region. These location scores provide a relative measure of the likelihood that a particular genomic region harbors the disease gene. Results similar to those obtained in VARIA were found using customized spreadsheets to assess shared SNP haplotype length, two-point LOD scores, location scores, and allelic associations.

Mutation identification

DNA sequence analysis was performed for the *CENTG3* and *CNTNAP2* genes from the linked region on chromosome 7q36. The *CENTG3* gene contains 19 exons spanning 58 kb on chromosome 7q36 (GenBank NM_031946). The *CNTNAP2* gene contains 24 exons spanning 2.3 Mb (GenBank NM_014141). The coding sequence of each target gene was amplified using specific oligonucleotide primers and 30-50 ng of genomic DNA from affected and unaffected family members. Primer sequences for PCR amplification and sequencing were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). All coding sequences, as well as intron-exon boundaries and putative branch sites, were investigated. PCR products were purified using QiaQuick columns (Qiagen, Valencia, CA) and sequenced using the BigDye Terminator cycle sequencing protocol (Applied Biosystems, Foster City, CA). Extension products were size-fractionated on an ABI 310 Genetic Analyzer. Sequences were compared to normal mRNA and genomic sequence for each gene using GenBank and BLAST (<http://www.ncbi.nlm.nih.gov>) in order to identify sequence variants. Population-based control samples were sequenced in an identical fashion.

Histology

For staining of hippocampal and temporal neocortical sections (8 μ m) with primary antibodies against CASPR2 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-K_v1.1 alpha subunit, and anti-Na_v1.2 alpha subunit (both purchased from Upstate Cell Signaling Solutions, Charlottesville, VA) antigen retrieval was performed, and endogenous peroxidase activity was blocked in 3% hydrogen peroxide. Sections were equilibrated in TBST (50mM Tris, 138mM NaCl, 2.7mM KCl, pH 8.0), blocked with 10% normal serum and then incubated with primary antibody (1:100 dilution of anti-K_v1.1 alpha subunit and anti-Na_v1.2 alpha subunit, 1:10 dilution of anti-CASPR2). Vectastain ABC (Vector Laboratories, Burlingame, CA) technology was used to detect primary antibodies. Finally, sections were incubated for 5 seconds in undiluted hematoxylin counter stain and mounted for imaging. Control samples were treated with similar methodology in parallel fashion (Sun Health Research Institute, Sun City, AZ).

REFERENCES

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Table S1: Griffiths Mental Development Scales for three CDFE patients

Chronological Age	PATIENT AGE¶		
	32 months	40 months	73 months
Developmental Age (months)			
• Locomotor	22.7	24	21.2
• Eye-hand coordination	20.2	19.6	8.0
• Non-verbal reasoning	20.5	18.4	20.4
• Personal-social	18.9	14.4	12.4
• Language	17.9	15.6	10.2
Global mental age	20.5	17.2	13.1
Developmental quotient*	0.64	0.43	0.18

NOTES

¶ Patients were evaluated prior to epilepsy surgery.

* Developmental quotient = developmental age/chronological age

Figure S1: Cortical dysplasia and focal epilepsy (CDFE) in the Old Order Amish of Pennsylvania. The four patients used for genome-wide SNP mapping are denoted with asterisks. Six other affected individuals indicated on the pedigree were identified by targeted sequencing of the 3709delG mutation in *CNTNAP2* exon 22. Patient IX-5 is currently asymptomatic at 4 months of age and is not included in the clinical Results section or Table 1.

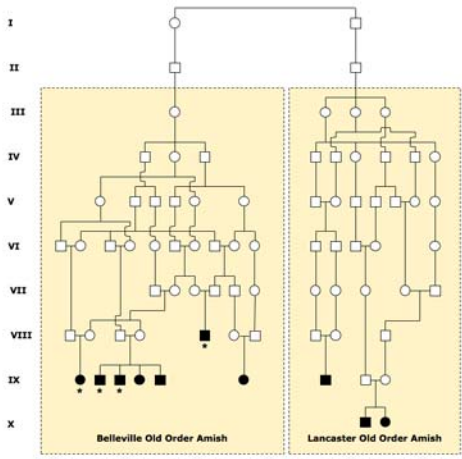


Figure S2. Malformation of the left striatum in 6 year-old boy with CDFE syndrome. A) T2 coronal MRI shows serpiginous signal hyperintensity of left anterior limb of the internal capsule, extending into the caudate head (arrow); gadolinium and gradient echo studies showed this was not a vascular structure. B) Apparent diffusion coefficient was high in the dysplastic region (arrow). C) Injection of ^{99m}Tc ECD 70 seconds following the onset of a brief right frontal seizure discharge (post-ictal) revealed a complex disturbance of perfusion, with reduced blood flow to the right frontolateral cortex (arrow), both temporal lobes (arrowheads), and the malformed striatum (arrow).

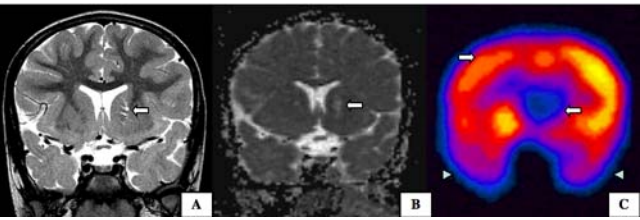
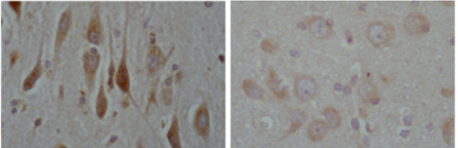


Figure S3. Immunohistochemistry, resected hippocampus. Comparable hippocampal sections from a 7 year-old CDFE patient and a normal adult brain (columns) processed in parallel as slide pairs and stained with primary antibodies against CASPR2, K_v1.1 alpha subunit, and Na_v1.2 alpha subunit (rows). CASPR2 expression is reduced in the CDFE specimen. In control hippocampus, K_v1.1 staining is seen along efferent projection fibers from CA region to perirhinal/entorhinal cortex and on terminal axons within the CA sector neuropil (white arrowheads). In CDFE specimens, anti-K_v1.1 heavily stains the cell body of some (black arrow), but not all (white arrow), CA sector neurons. In contrast, K_v1.1 expression on axons is sparse, suggesting abnormal localization of the protein. Anti-Na_v1.2 stains the nuclear membrane of neurons, endothelia, and glial cells throughout the normal brain. In CDFE hippocampus, Na_v1.2 is completely absent within the hyperplastic dentate granule cell layer. Due to limited tissue availability, this staining could only be performed on tissue from a single CDFE patient. The observations should be interpreted with caution.

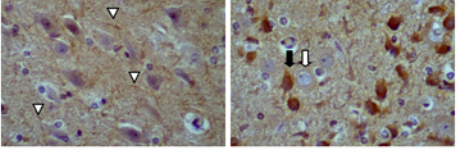
CONTROL

CDFE

Anti-Caspr2



Anti-K_1,1



Anti-Na_1,2

