

affect translation of the precursor, polypeptide folding or proteolytic processing¹³. However, it is unclear whether our assay is the most appropriate test for this particular sequence change. An assessment of *in vivo* EDN3 expression in the patient is required to conclude definitively whether the mutation affects the function of the EDN3 gene.

The fact that EDN3 and its receptor are expressed in central nervous system structures indicates a potential role for these genes and their signaling pathways in CCHS¹⁴. EDN3 is expressed in rodent brain and endothelin binding sites are detected throughout the brain, including the brainstem¹⁴⁻¹⁵. This is of particular interest as the neural system responsible for autonomic regulation of respiration is located in the medulla and pons, components of the brain stem. Mutations in EDN3, thus far, have been described exclusively in patients with HSCR and the Shah-Waardenburg syndrome, providing evidence that the EDN3/EDNRB

interaction and RET are involved in a common pathway crucial to neural crest cell development, specifically the progenitor cells of the enteric nervous system and epidermal melanocytes. However, our patient has isolated CCHS and no pigmentary anomalies. While our patient has chronic constipation, normal ganglion cells were detected by rectal biopsy, ruling out the possibility of frank HSCR. The only other reported molecular defect in a CCHS patient was found in the RET gene, although this patient also has HSCR¹⁶. The CCHS mutation data indicates that the EDN3/EDNRB interaction and the RET pathway are common to the disease processes leading to HSCR and CCHS.

The discovery of mutations in known HSCR genes in patients with CCHS has finally provided molecular evidence for an aetiological link between the two disorders. With the identification of genes important in downstream events common to the endothelin cascade and the RET signaling pathway, molecular defects causing HSCR

and associated neurocristopathies may be further investigated.

Stacey Bolk
Misha Angrist

Department of Genetics and Center for Human Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio, USA

Jian Xie
Masashi Yanagisawa

Howard Hughes Medical Institute and Department of Molecular Genetics, University of Texas Southwestern Medical Center of Dallas, Dallas, Texas, USA

Jean M. Silvestri
Debra E. Weese-Mayer

Department of Pediatrics, Rush Medical College of Rush University, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois, USA

Aravinda Chakravarti

Department of Genetics and Center for Human Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio, USA

1. Guilleminault, C. *et al. Pediatrics* **70**, 684-694 (1982).
2. Haddad, G. *et al. Medicine* **57**, 517-526 (1978).
3. Weese-Mayer, D. *et al. J. Pediatr.* **120**, 381-387 (1992).
4. Swaminathan, S., Gilsanz, V., Atkinson, J. & Keenes, T. *Chest* **96**, 423-424 (1989).
5. Bolande, R. *Hum. Pathol.* **5**, 409-429 (1974).
6. Bower, R.J. & Adkins, J.C. *Clin. Pediatr.* **19**,

7. Bolk, S. *et al. Am. J. Med. Genet.* (in the press).
8. Romeo, G. *et al. Nature* **367**, 377-378 (1994).
9. Puffenberger, E. *et al. Cell* **79**, 1257-1266 (1994).
10. Ederly, P. *et al. Nature Genet.* **12**, 442-443 (1996).
11. Hofstra, R.M.W. *et al. Nature Genet.* **12**, 445-447 (1996).

12. Chakravarti, A. *Hum. Molec. Genet.* **5**, 303-307 (1996).
13. Xu, D. *et al. Cell* **78**, 473-485 (1994).
14. Koseki, C., Imai, M., Hirata, Y., Yanagisawa, M. & Masaki, T. *Neurosci. Res.* **6**, 581-585 (1989).
15. Krsmanovic, L. *et al. Proc. Natl. Acad. Sci. USA* **88**, 11124-11128 (1991).
16. Arniel, J. *et al. Am. J. Hum. Genet.* **57**, A1189 (1995).

Alternative mechanism for pathogenesis of an inherited epilepsy by a nicotinic AChR mutation

Sir — In a recent article on the genetic basis of autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), Steinlein and colleagues described a missense mutation of the $\alpha 4$ subunit of neuronal nicotinic acetylcholine receptor responsible for the disease¹. The mutation ($\alpha S6'F$) changes a serine to phenylalanine at the 6' position of the M2 transmembrane domain, which is thought to form the ion-conductive pore of the nicotinic receptor. The authors remarked that a similar mutation of this position to tyrosine, studied in the analogous receptor from *Torpedo* electroplaques², produced a small decrease in the conductance

of the pore, and therefore suggested that reduced neuronal nicotinic receptor function was responsible for ADNFLE.

In our recently published study on the role of the M2 pore-forming

region in general anesthetic sensitivity³, we studied the $\alpha S6'F$ mutation in the mouse muscle $\alpha 1$ receptor, and our results suggest that, rather than being a defective receptor, the mutant $\alpha 4$ receptor may be hyper-

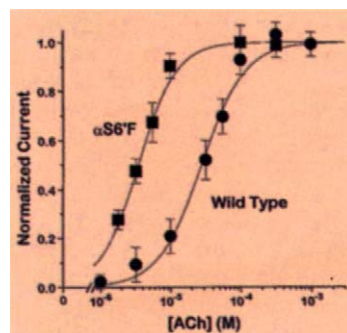


Fig. 1 Normalized ACh dose-response of wild-type and $\alpha S6'F$ mutant mouse muscle nicotinic receptors, expressed in *Xenopus* oocytes. Receptors were expressed by cRNA injection into oocytes for all four receptor subunits, with an RNA ratio of $\alpha:\beta:\gamma:\delta = 2:1:1:1$ (though this does not guarantee the normal stoichiometry of $\alpha_2\beta\gamma\delta$). Within several days of injection, electrical recordings were made from excised outside-out membrane patches, rapidly perfused with various concentrations of ACh. (Methods from ref. 3). The best fit K_{app} for ACh was $28 \pm 3 \mu M$ for wild type and $3.5 \pm 0.4 \mu M$ for the $\alpha S6'F$ mutant.

active and this hyperactivity leads to epileptic activity in ADNFLE.

The impairment of channel conductance seen with mutations at this position is rather a small effect: tyrosine or valine mutations reduce the conductance of *Torpedo* nicotinic receptors by about 20% (ref. 2); an equivalent mutation to alanine in mouse muscle receptor also reduces the sensitivity to charged pore blockers by about 20% (ref. 4).

In our study, we found that the $\alpha 56^F$ mutation in the mouse muscle receptor produces a similar small reduction in single channel conductance (by about 25%). However, this mutation also causes a dramatic increase in sensitivity to acetylcholine (by about 8-fold (Fig. 1)). Thus, in the presence of a low concentration of acetylcholine, this mutant channel will activate to a far greater extent than will wild-type channels. Even though the mutation is located in

the pore-forming region of the receptor protein and not in the ACh receptor site, the effective sensitivity is probably increased because of coupling between ACh binding and the opening of the channel, which does involve the pore region⁵⁻⁷.

Although our results were obtained on the mouse muscle isoform of the nicotinic receptor whereas the studies of Steinlein *et al.* involve a mutation in the human neuronal nicotinic receptor, mutations in the nicotinic receptor pore region have previously been found to have parallel effects on neuronal and muscle isoforms⁵⁻⁷. It thus seems likely that the ADNFLE mutation would produce a similar change in the *in vivo* human neuronal $\alpha 4$ isoform.

The hypothesis that the $\alpha 4$ -S6'F mutation associated with ADNFLE may increase rather than decrease nicotinic receptor activity suggests

in general that inherited epilepsy can result from a gain-of-function in an excitatory receptor system. It also has important implications for the possible treatment of ADNFLE, since it may argue against the use of cholinergic potentiators such as physostigmine¹.

Stuart A. Forman^{1,2}

Gary Yellen²

¹Department of Anaesthesia and

²Department of Neurobiology, Harvard Medical School and the Massachusetts General Hospital, 50 Blossom Street, Boston, Massachusetts 02114, USA

Elizabeth A. Thiele

Department of Neurology, Children's Hospital, Boston, Massachusetts 02115, USA

Correspondence should be addressed to G.Y.

e-mail: yellen@helix.mgh.harvard.edu

- Steinlein, O. K., Mulley, J. C. & Propping, P. *et al.* *Nature Genet.* **11**, 201-203 (1995).
- Imoto, K. *et al.* *FEBS Lett.* **289**, 193-200 (1991).

- Forman, S. A., Miller, K. W. & Yellen, G. *Mol. Pharmacol.* **48**, 574-581 (1995).
- Charnet, P. *et al.* *Neuron* **4**, 87-95 (1990).
- Filatov, G. N. & White, M. M. *Mol.*

Pharmacol. **48**, 379-384 (1995).

- Labarca, C. *et al.* *Nature* **376**, 514-516 (1995).
- Revah, F. *et al.* *Nature* **353**, 846-849 (1991).



545 National Press Building, 529 14th Street, N.W.,
Washington, D.C. 20045
Telephone: 202-626-2513 • Fax: 202-626-0970
e-mail: natgen@naturedc.com

Guide to Authors

Nature Genetics is an international monthly journal publishing exceptional advances in all fields of modern genetic research, with a special emphasis on mammalian genetics and the Genome Project. Manuscripts are selected for publication according to editorial assessment of their general interest and suitability and reports from independent referees. Receipt of all manuscripts will be acknowledged, and those not suitable for review will be returned immediately. Contributors are welcome to suggest potential reviewers as well as informing the Editor of potential conflicts of interest. Authors of papers previously considered by *Nature* but ultimately not accepted are welcome to resubmit to *Nature Genetics*, where they will receive prompt further consideration. Following acceptance of their paper, contributors will receive galley proofs within a few weeks. Contributors will receive a reprint order form with their proofs; reprint orders are processed from our New York office after the manuscript is published and payment received. *Nature Genetics* does not exact page charges.

Format of Articles

Manuscripts should be typed, double-spaced, on one side of the paper only. An original and three copies are required, each accompanied by artwork, together with a computer diskette. Reference lists, figure legends and tables should each be on separate sheets, also double-spaced. Please include four copies of any relevant manuscript in press or submitted for publication. Colour prints will be partly paid for by authors unless otherwise agreed. Articles include Summary, Introduction, Results and Discussion. There is a separate Methods section following the main text and we include full titles of papers in the reference list. The main text should be between 2,000 and 4,000 words in length. There is a maximum of 8 display items.

Titles should be simple and concise. A brief, accessible **Summary** of no more than 100 words should explain the rationale and chief conclusions of the work, without references. **Results** should include short cross-headings to define the main aspects of the study. Authors should deposit sequence data in the databases, and provide an accession number in the paper. The **Methods** section appears at the end of the text, before the references.

References are numbered sequentially as they appear in the text, followed by those in the figure legends and tables. Do not include any annotation. Full titles of papers are required. All authors should be listed unless there are six or more, in which case 'et al.' should be substituted. First and last page numbers must be included in full; references to books should include publisher, place and date. The list should include only papers published or in press; abstracts, papers submitted or in preparation and personal communications should be cited in the text. (Full details are available in our separate 'Style Guide'.) **Figures:** Original artwork should be submitted with the manuscript.

Format of Letters

Nature Genetics also publishes a selection of more concise reports of broad interest to the genetics community. The main **Text** (excluding legends, references and Methods) is limited to 1,200 words and four display items. There is no abstract; in its place, there is a single paragraph of up to 200 words, with references, containing the essential introductory material and culminating in a brief summary of the results. The remainder of the text is devoted to presenting the results and the principal conclusions of the work. There are no cross-headings. **References** should be limited to about 30, but including full titles. Letters also retain a separate **Methods** section.

Progress, Reviews and Commentary. *Nature Genetics* publishes a regular series of articles in these categories. Format is essentially that of regular articles, except for a shorter abstract (up to 75 words) and appropriate cross-headings. The journal does consider unsolicited articles.

Submission

All manuscripts should be sent to the Editor, *Nature Genetics*, 545 National Press Building, 529 14th St. N.W., Washington, D.C. 20045, USA. (Tel: 202-626-2513; Fax: 202-626-0970; e-mail: natgen@naturedc.com). Please provide current fax and phone numbers of the corresponding author on all submissions. DISKETTES. All page proofs are set directly from computer discs provided by the authors. Any common Macintosh or PC word-processing packages (except WordStar or Excel) are compatible and preferable to a text/ASCII file. Text and figures can also be transmitted electronically to our FTP site (please call for details).