affect translation of the precursor, polypeptide folding or proteolytic processing[13]. 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 CCHS[14]. *EDN3* is expressed in rodent brain and endothelin binding sites are detected throughout the brain, including the brainstem[14–15]. 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 HSCR[16]. 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

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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 α4 subunit of neuronal nicotinic acetylcholine receptor responsible for the disease[1]. The mutation (αS6'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* electroplaques[2], 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 sensitivity[3], we studied the αS6'F mutation in the mouse muscle α1 receptor, and our results suggest that, rather than being a defective receptor, the mutant α4 receptor may be hyper-

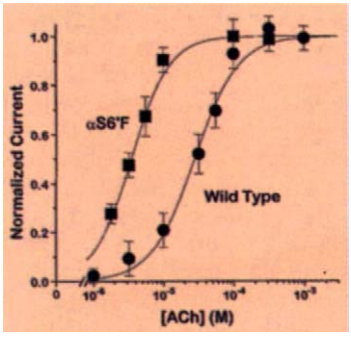

Fig. 1 Normalized ACh dose-response of wild-type and αS6'F mutant mouse muscle nicotinic receptors, expressed in *Xenopus* oocytes. Receptors were expressed by cRNA injection into oocytes for all four receptor subunits, with an RNA ratio of α:β:γ:δ = 2:1:1:1 (though this does not guarantee the normal 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 ± 3 μM for wild type and 3.5 ± 0.4 μM for the αS6'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 αS6'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 region[5–7].

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 isoforms[5-7]. It thus seems likely that the ADNFLE mutation would produce a similar change in the *in vivo* human neuronal α4 isoform.

The hypothesis that the α4-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 physostigmine[1].

Stuart A. Forman[1,2]
Gary Yellen[2]
[1]*Department of Anaesthesia and* [2]*Department 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

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