Letters to the Editor

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Mutations of the *RET-GDNF* Signaling Pathway in Ondine's Curse

To the Editor:

The congenital central hypoventilation syndrome (CCHS, or Ondine's curse; MIM 209880; McKusick 1997) is a hitherto unexplained disorder of the ventilatory response to hypoxia and hypercapnia, leading to life-threatening hypoxic episodes starting immediatly after birth (Mellins et al. 1970). The alveolar hypoventilation observed during sleep is regarded as the consequence of a failure of the autonomic control of ventilation, located in the ventral medulla of the brain stem. Although the majority of CCHS cases are sporadic, several familial forms have been reported, and segregation analyses have suggested that the multifactorial and the major-locus models are almost equally likely in CCHS (Weese-Mayer et al. 1993). Interestingly, the CCHS-Hirschsprung disease association has frequently been reported (Haddad syndrome; MIM 209880 [McKusick 1997]; Haddad et al. 1978; Nakahara et al. 1995), and all CCHS families reported, to date, have included at least one sibling with this association.

On the other hand, Hirschsprung disease (HSCR; aganglionic megacolon; MIM 249200; McKusick 1997) is a frequent congenital malformation (1/5,000 live births) that is characterized by the absence of parasympathetic intrinsic ganglion cells of the hindgut and is regarded as a neurocristopathy (Bolande 1973). *RET* mutations account for 50% and 15%–20% of isolated familial and sporadic HSCR, respectively (Attié et al. 1995*a*). Mutations in the endothelin signaling pathway, including the endothelin B receptor gene (*EDNRB*) and the endothelin 3 gene (*EDN3*), account for no more than 5% of HSCR cases (Puffenberger et al. 1994; Attié et al. 1995*b;* Amiel et al. 1996; Edéry et al. 1996). Since CCHS is also regarded as the consequence of an abnormal migration of the neural crest cells toward the central autonomic respiratory system, genes involved in HSCR were considered as candidate genes in CCHS as well, including the *RET* proto-oncogene and its ligand, the glial cell line–derived neurotrophic factor (*GDNF*; Durbec et al. 1996; Salomon et al. 1996), *EDNRB,* and *EDN3*.

Although a heterozygous *EDN3* frameshift mutation has been found in one CCHS patient (Bolk et al. 1996*b*), we failed to detect *EDNRB* or *EDN3* mutations in our series. By contrast, screening the coding sequence of the *RET* and *GDNF* genes in five unrelated cases of isolated CCHS and in two cases of CCHS-HSCR association, we found mutations of the *RET* and the *GDNF* genes in children with isolated CCHS (1/7) and the CCHS-HSCR association (1/7), respectively.

All patients fulfilled the inclusion criteria for diagnosis of CCHS, namely (*i*) hypoventilation, hypoxemia, and hypercapnia during quiet sleep on polygraphic respiratory recording, with (*ii*) no cardiac, pulmonary, neuromuscular, electroencephalographic or cerebral magnetic resonance imaging anomaly. Histopathological criteria for HSCR were (*i*) the absence of enteric plexuses with histological evaluation of the aganglionic tract and (*ii*) increased acetylcholinesterase histochemical staining in nerve fibers. We screened *RET* and *GDNF* genes by SSCP analysis (Attié et al. 1995*a;* Salomon et al. 1996). The PCR products were heated for 10 min at 95°C, loaded onto a Hydrolink mutation detection enhancement gel (Bioprobe), and electrophoresed at 4 W. The gel was then dried and autoradiographed for 48 h. When an abnormal SSCP pattern was observed, direct DNA sequencing was performed by the fluorometric method (DyeDeoxyTerminator cycle sequencing kit, Applied Biosystems).

In a female patient with CCHS and total colonic aganglionosis, we found a hitherto unreported C→T transition at the second nucleotide of codon 1039 in exon 19 of the *RET* gene (C<u>C</u>G→C<u>T</u>G), changing a proline into a leucine in the protein (P1039L; fig. 1). This mutation is located in the terminal end of the intracellular domain of *RET* and is predicted to alter both the long and short carboxy-terminal isoforms of the *RET* protein. The mutation was inherited from the healthy father, and no other deleterious variation in *RET* coding sequence was detected (the A432A and G691S polymorphisms were noted in exons 7 and 11, respectively). In a male patient with CCHS and growth hormone deficiency, we found a C→T transition at the first nucleotide of codon 93 of the *GDNF* gene (<u>C</u>GG→<u>T</u>GG), changing a highly con-

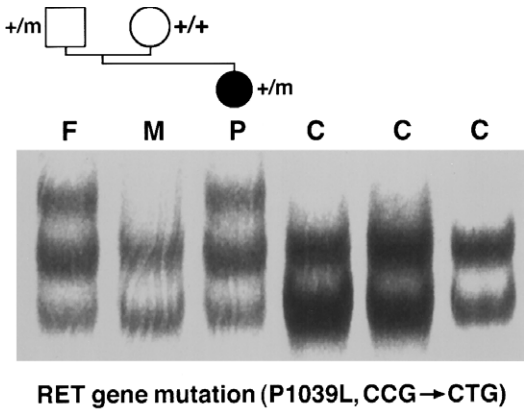

Figure 1 SSCP analysis of *RET* exon 19 in case 1. F = father; M = mother; P = proband; and C = control.

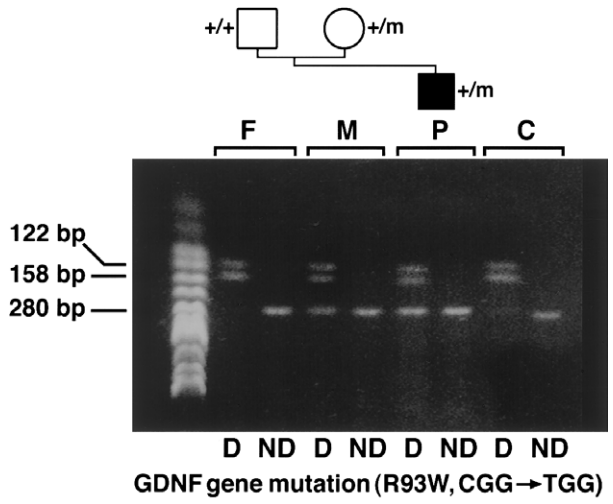

Figure 2 Screening for the R93W mutation by *Hin*fI restriction analysis. The nucleotide change abolishes the *Hin*fI restriction site. Partial digestion is found in the proband (case 2) and his mother (M), showing heterozygosity for the R93W mutation, whereas complete digestion is found in the father (F) and the control (C). D = digested DNA; ND = nondigested DNA.

served arginine into a tryptophan in the protein (R93W; fig. 2). This mutation was inherited from the healthy mother. No other deleterious variation in the *GDNF* coding sequence was detected. The two mutations were absent in 90 normal controls (180 chromosomes). The R93W mutation in the *GDNF* gene has already been reported in sporadic and familial HSCR (Angrist et al. 1996; Salomon et al. 1996). In the latter case, since a *RET* mutation was associated to the *GDNF* mutation, the R93W mutation was regarded as neither necessary nor sufficient to cause isolated HSCR. The lack of penetrance of both *RET* and *GDNF* mutations reported here suggests that a major secondary event, yet to be defined, or the involvement of modifier loci are required for the expression of the CCHS phenotype.

The identification of mutations in the RET-GDNF pathway and the endothelin pathway in Ondine's curse sheds light on the genetic bases of this life-threatening condition and further suggests that CCHS is a neural crest cell disorder. Nevertheless, mutations have been reported in a minority of patients tested thus far (Bolk et al. 1996*a*). Finally, the involvement of at least three genes belonging to distinct signaling pathways, the incomplete penetrance of the mutation in carrier parents, and the variable expression of the respiratory control defect observed in the Ret −/− homozygous mice exposed to hypercapnia (Burton et al. 1997) support the view that an interactive polygenic inheritance is involved in Ondine's curse.

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