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Hydroxylation in endothelial cells is involved in the production of ET-1, a vasoconstrictor that plays a role in the regulation of blood pressure. The production of ET-1 is upregulated under conditions of hypoxia, which is a common feature of tissues that are poorly perfused or have low oxygen levels. This upregulation is mediated by the enzyme endothelial nitric oxide synthase (eNOS), which converts L-arginine to L-citrulline and nitric oxide (NO). NO then reacts with superoxide to form peroxynitrite, which contributes to the production of ET-1. The exact mechanisms by which hypoxia stimulates eNOS activity are not fully understood, but may involve changes in the intracellular levels of calcium, protein kinases, and redox state. The overproduction of ET-1 in hypoxic conditions can lead to oxidative stress and endothelial dysfunction, contributing to the development of various cardiovascular diseases.
ing MITP. Interestingly, homozygotes for the spontaneous mouse mutation is/s as well as the Edn3 knockout mouse also show depigmentation in combination with colonic aganglionosis. In this pedigree the affected children with presumably the same EDN3 mutation show different extents of aganglionosis. Modifying genes and/or environmental factors may account for such a difference.

Mutation screening of the EDN3 gene revealed heterozygous variations in exon 1 in three HSCR patients and in exon 6 in a single patient (results not shown). The variations in exon 1 all proved to be a G— >A transition, resulting in the substitution of a serine for a glycine in codon 57. This variant was also present heterozygously in 4 out of 90 control individuals. Therefore, we consider it a non-causative polymorphism. The exon 6 variant turned out to be a novel mutation, namely a G— >A transition resulting in the substitution of an isoleucine for a methionine in codon 374. As this mutation creates the loss of an Alu site, presence of the mutation could be confirmed by restriction analysis (data not shown). This mutation did not occur in 100 control subjects. Methionine 374 is present in transmembrane domain VII of the EDNRB protein. The mutation might, therefore, affect proper anchoring and functioning of the protein.

Homozygotes for the spontaneous mouse mutations s*/5* and its knock-out counterpart, Edn3 (ref. 12)
show white spotting as a result of the absence of melanocytes. Such depigmentation is also a characteristic of a number of EDNRB mutation carriers.\(^4\)\(^5\) Apparently, a combined WS2–HSCR phenotype might be associated with mutations of either EDNRB or EDN3. However, in the patient with the EDNRB mutation (Met374Ile) no signs of WS2 were found. This was also true for the patient’s father, who turned out to carry the mutation as well, and for the patient’s mother. This might imply that mutations in EDNRB are not necessarily associated with a combined WS2–HSCR phenotype and that modifying genes have a role.

All 40 HSCR patients have also been screened for mutations of \(RET\), with nine presumably causative \(RET\) mutations found (manuscript in preparation). The combined results of our screening for mutations in EDN3, EDNRB and \(RET\) may give some indication of the contributions of these genes to the HSCR phenotype. \(RET\) mutations seem to account for a large part of cases\(^14\)\(^\text{–}\)\(^16\); EDN3 and EDNRB only for a minority. We did not detect patients with mutations in more than one of the genes. The majority of HSCR cases cannot be explained by mutations in any of the genes analysed to date, suggesting that other genes may contribute to the phenotype. A human homologue for the mouse \(dan\) locus\(^7\) and genes coding for the enzymes involved in the cleavage of the preproendothelin are obvious candidates. Moreover, as suggested by the far from rare occurrence of nonpenetrance of known mutations, there may be a role of modifying genes conferring a true polygenic character to the disease.

**Methods**

**Patients.** DNA from 40 unselected HSCR patients was scanned for mutations of EDN3 and EDNRB. Of these 40, 9 were known to be familial. The EDN3 mutation was detected in a male infant. He was the third child of a healthy, phenotypically normal couple of Pakistani ancestry. His parents were first cousins. His elder sister was diagnosed with total colonic aganglionosis, so a rare occurrence of nonpenetrance of known mutations, there may be a role of modifying genes conferring a true polygenic character to the disease.

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