HUMAN GENETICS

Hirschsprung — a model of complexity

Hirschsprung disease (HSCR) — the most common hereditary cause of intestinal obstruction — is in itself an obstructive disease to study. It is clinically variable, shows non-Mendelian inheritance and is associated with several genes, all of which, including *RET*, *EDNRB* and *SOX10*, are involved in neural-crest development. Mutations in these genes explain the transmission of the long-segment form of HSCR (L-HSCR), but generally not that of the more common, non-syndromic, short-segment form of the disease (S-HSCR). The two forms of HSCR differ in the extent to which agangliosis — caused by the failure of neural-crest-derived ganglionic cells to migrate into the intestinal tract — occurs in the intestine. Aravinda Chakravarti, Stanislas Lyonnet and colleagues now report the genetic analysis of S-HSCR in an approach that could be a model for studying other complex diseases.

The study began with a genome scan of 49 S-HSCR families, including 67 distinct affected sibpairs (ASPs), in which the authors tested both genome-wide polymorphic markers and L-HSCR-associated genes for linkage to S-HSCR. They detected statistically significant allele sharing among ASPs (by identity by descent — IBD) at three loci: 10q11, 19q12 and 3p21. Neither 19q12 nor 3p21 has been previously associated with HSCR. Because *RET* maps to 10q11, the authors screened the studied families for *RET* mutations and found 17 of them, but only in 40% of *RET*-linked families, indicating that non-coding *RET* mutations might contribute significantly to S-HSCR. Moreover, nearly all the *RET* mutations mapped to the protein's extracellular domain, in contrast to the gene-wide *RET* mutations seen in multi-generational HSCR (S-HSCR has a lower familial incidence than L-HSCR). In 27 of the families, one IBD *RET* allele was shared, which, in 21 of these families, was maternally transmitted — a transmission bias that might explain why HSCR shows a greater than expected inheritance through the maternal line.

Further analyses of the linkage data revealed that segregation at all three loci is sufficient and necessary to explain S-HSCR. Moreover, the expected frequency of individuals heterozygous at each loci — the most common at-risk genotype — closely matches the observed incidence of the disease.

So, how might these loci interact to cause S-HSCR? The authors tested four possible models — additive, multiplicative, mixed multiplicative and epistatic — and found that the multiplicative model, in which the combined effects of all three loci cause the disease, provides the simplest explanation of disease clustering. As S-HSCR usually does not segregate in the absence of *RET*, the other two loci are probably *RET* modifiers.

Although several questions remain to be answered — such as the identity of the genes at 19q12 and 3p21 — this comprehensive study sheds new light on the genetic architecture of S-HSCR. It also importantly provides new solutions to the long-standing problem of identifying the contribution of specific genes to a complex disease.

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References and links

ORIGINAL RESEARCH PAPER Bolk Gabriel, S. *et al.* Segregation at three loci explains familial and population risk in Hirschsprung disease. *Nature Genet.* 15 April 2002 (DOI 10.1038/ng868)

FURTHER READING Passarge, E. Deconstructing Hirschsprung. *Nature Genet.* 15 April 2002 (DOI 10.1038/ng878)

TECHNOLOGY

A super tool for RNAi

As most worm geneticists will tell you, RNA interference (RNAi) is a rapid, easy and specific way in which to inactivate gene function. However, RNAi — which is mediated by double-stranded RNAs that trigger sequence-specific mRNA degradation — is cytotoxic to most mammalian cells, a problem that was overcome last year by using synthetic, short interfering RNAs (siRNAs, see NRG Highlights July 2001). But siRNAs have a shortcoming themselves as a tool for investigating gene function — their effects are transient. Now Thijn Brummelkamp and colleagues have created a new vector, pSUPER, that generates siRNAs in mammalian cells and brings about sustained gene inactivation without cytotoxicity, allowing *in vitro* loss-of-function phenotypes to be assayed over longer periods of time.

pSUPER has several key features that equip it for the job. It contains an RNA-polymerase-III promoter, a well-defined transcription start site and a termination signal that consists of five Ts. It encodes a small RNA transcript that is cleaved after the second U of the termination signal to generate a transcript that has two overhanging 3′ U nucleotides, as found in synthetic siRNAs. Finally, the gene-specific insert comprises two complementary, target-derived 19-nucleotide (nt) sequences that are separated by a short spacer — an arrangement that is predicted to generate a 19-nt stem–loop structure similar to that of *let-7*, the *Caenorhabditis elegans* gene that controls the timing of developmental events by an RNA-mediated mechanism.

Brummelkamp *et al.* have used pSUPER to knock down more than ten genes in several mammalian cell lines. Their pSUPER-CDH1 vector worked as well as a synthetic *CDH1* siRNA and most effectively when the 19-nt stem structure contained 9 nts of loop sequence. A single-nucleotide mismatch introduced into this sequence resulted in siRNAs that failed to suppress *CDH1* expression, illustrating the specificity of siRNA activity. A pSUPER-p53 vector suppressed the endogenous *TP53* transcript by ~90%, and transfected cells failed to undergo p53-mediated G1 arrest following their exposure to ionizing radiation. Moreover, *TP53* siRNA was still present in cells two months after transfection, and the cells showed more than a 90% reduction in wild-type p53 levels and no obvious signs of cytotoxicity.

So how can this vector be put to good use? The authors' finding that a single-nucleotide mismatch in the targeting sequence can abrogate siRNA activity points to its potential application in gene therapy. SiRNA-generating vectors could be designed to inactivate disease-associated transcripts that contain point mutations to leave unaffected the expression of the remaining wild-type transcript. These vectors could also be used in high-throughput *in vitro* screens for loss-of-function phenotypes. How they will be used *in vivo*, however, remains to be seen.

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References and links

ORIGINAL RESEARCH PAPER Brummelkamp, T. *et al.* A system for stable expression of short interfering RNAs in mammalian cells. *Science* 21 March 2002 (DOI 10.1126/science1068999)