STATISTICAL GENETICS

Flagging non-paternity

The use of discordant — one affected, one unaffected — sibling (sib) pairs in linkage study designs is currently in vogue in human genetics research because they are believed to afford greater statistical power to genome scans in search of markers linked to disease loci. In such study designs, discordant sib pairs are expected to share fewer alleles at disease-associated loci (or at loci linked to them) and to share alleles more frequently at unlinked loci. Lack of allele sharing is therefore the hallmark of linkage to the disease.

But there are problems associated with the use of discordant sib pairs, one of which is the focus of the study by Neale *et al.* — that of non-paternity. Their mathematical simulation shows that selecting for extreme phenotypic discordance between sib pairs can enrich for cases of non-paternity in a study sample, resulting in a sample that contains half, as well as full, sib pairs. Such a sampling bias can lead to false-positive reports of linkage because allele sharing is reduced in half sibs relative to full sibs.

In their study, Neale *et al.* first calculated the proportion of half sibs that are likely to be in a study sample that has been selected on the basis of extreme phenotypic discordance. They then plotted this proportion against the heritability of the trait under investigation. Strikingly, this analysis showed that the setting of extreme phenotypic thresholds for the selection of discordant sib pairs will probably result in nearly all the sibs in the sample being half sibs, rather than full sibs, when the trait being selected for is highly heritable. However, when lower selection thresholds are used and the trait is less heritable, smaller increases in the proportion of half sibs in a study sample can be expected.

Although, in practice, researchers rarely set such extreme phenotype selection thresholds — partly owing to the rarity of extremely discordant sib pairs — Neale *et al.* still predict that there is a risk that half sibs could be present in a study sample selected from the upper 10% of a phenotype's distribution. Together with the fact that estimates of non-paternity in the general population range from 1–20%, this study flags the need for researchers to be aware of this risk and to carry out tests on genome-wide marker information to check for relationship errors in sib-pair samples.

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References and links

ORIGINAL RESEARCH PAPER Neale, M. C. *et al*. Nonpaternity in linkage studies of extremely discordant sib pairs. *Am. J. Hum. Genet.* **70**, 526–529 (2002)

FURTHER READING Boehnke, M. & Cox, N. J. Accurate inference of relationships in sib-pair linkage studies. *Am. J. Hum. Genet*. **61**, 423–429 (1997)

GENE REGULATION

Downward progress

RNA interference (RNAi), or post-transcriptional gene silencing, is proving to be a powerful method for reducing the expression of specific genes in many organisms — particularly in plants and invertebrates. If RNAi could be applied with equal facility to mammals, this could open the floodgates to studying gene function in cell lines and whole organisms, and could even give rise to therapeutic applications. Two recent papers report progress in both of these areas.

RNAi is a naturally occurring phenomenon and is mediated by double-stranded (ds) RNA. If the dsRNA matches the sequence of a transcript within a cell, it will direct the degradation of that transcript by endogenous nucleases. Any gene can therefore be downregulated by supplying dsRNA of the same sequence as the target transcript. This can be done by transfecting cells with dsRNA itself or with a construct that expresses a version of the target transcript containing an inverted repeat.

Although this method works extremely well in invertebrates, such as *Caenorhabditis elegans*, its use in mammalian cells has been less successful. Partly, this is because the presence of dsRNA in mammals activates a protein kinase, PKR, which is involved in a defensive response to viruses and leads to a generalized reduction of gene expression. Last year, it was shown that PKR can be circumvented by using shorter dsRNAs, of ~21 nucleotides in length, which allowed the transient silencing of target genes in several mammalian cell lines.

Paddison *et al.* have extended this observation by showing that another way to get round PKR is to express an inhibitor of PKR function. Using this approach, they successfully carried out RNAi in somatic mouse cells, using longer dsRNA. In addition, they showed that stable silencing of target genes could be achieved in a mouse embryonal carcinoma cell line by expressing a transcript containing an inverted repeat. This work could pave the way towards alternative strategies for knocking out gene function in mice, as well as in somatic mouse cell lines.

The aim of the study by Caplen *et al.* was to see whether RNAi could be used to downregulate the expression of a known disease-causing gene. Specifically, they were studying the mutant form of the androgen receptor that causes spinal and bulbar muscular atrophy (SBMA). This disease — like Huntington disease and several other neurodegenerative disorders — is caused by the expansion of a sequence that encodes a stretch of glutamines. Caplen *et al.* showed that RNAi could be used to reduce the expression of the expanded repeat in a tissue culture model of SBMA in both *Drosophila* cells and human cells. In the human cells, RNAi also reduced the cytotoxic effects that are associated with the expression of these polyglutamine repeats.

Although these new studies extend the uses of RNAi in mammalian cells, some important technical issues remain to be addressed, such as cell-type specificity. For example, if we could understand why RNAi works better in some cell types than others, it might be possible to target RNAi to the cells that are affected by a given disease. Such a finding would herald some exciting therapeutic opportunities.

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WEB SITES

Richard Morgan's lab: http://www.nhgri.nih.gov/Intramural_research/People/morgan.html

Greg Hannon's lab: http://www.cshl.org/public/SCIENCE/hannon.html

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