HUMAN GENETICS

Notch, stroke and dementia

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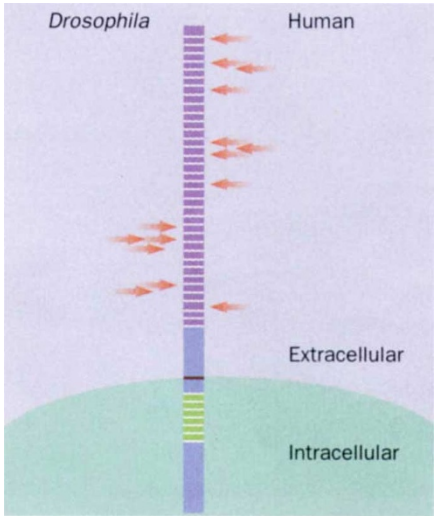
THE Notch intercellular signalling pathway is essential for proper embryonic development in organisms as diverse as insects, nematodes and mammals (reviewed in ref. 1). So it is somewhat surprising that few associations have been described between components of this pathway and human disease. Previously, the only evidence connecting Notch signalling and disease was the involvement of *Notch1* gene translocations in a minority of T-cell lymphoblastic leukaemias[2]. But the paper by Tournier-Lasserve and colleagues on page 707 of this issue[3] now provides important new data linking the Notch signalling pathway to familial causes of stroke in humans.

In developed countries, stroke is the third leading cause of death and the primary cause of acquired physical or cognitive impairment. Recurrent strokes are a feature of a recently described familial syndrome known as CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). Affected individuals exhibit a variety of symptoms, including recurrent subcortical ischaemic strokes, progressive vascular dementia, craniofacial paralysis, migraine, and mood disorders with severe depression[4]. These symptoms usually start to appear at about 45 years of age, and patients typically die by 65. The condition is believed to be largely undiagnosed and, as such, the prevalence is not precisely known.

In previous work, Tournier-Lasserve and colleagues mapped the CADASIL gene to the short arm of chromosome 19, and identified a two-centimorgan critical region containing the CADASIL gene[5]. In the new paper, they describe how they constructed yeast and bacterial artificial chromosome contigs encompassing the critical region, and isolated transcripts from this region by selection of complementary DNA. One of the isolated clones was homologous to the mouse *Notch3* gene[6], and they examined this as a candidate for the CADASIL gene. The analysis showed that there were ten different missense mutations in the *Notch3* genes of 14 unrelated CADASIL patients.

Proteins belonging to the Notch family are transmembrane receptors that contain several conserved peptide motifs (see figure). The extracellular domains contain many tandemly repeated copies of an epidermal growth factor (EGF)-like motif. The intracellular domains contain six copies of another conserved motif, termed the Cdc10/ankyrin repeat. Both the EGF and the ankyrin-repeat motifs are found in many different proteins and, in at least some cases, they have been shown to be involved in protein–protein interactions.

The types and locations of the mutations detected in the *Notch3* gene of CADASIL patients are revealing. All of them were missense mutations, and nine of the ten either added or mutated a cysteine residue in one of the EGF-like repeats. The tenth mutation altered a conserved glycine residue in one of the Cdc10/ankyrin-like repeats. These mutations, particularly those in the EGF-like repeats, would be expected to strongly affect protein conformation. However, an important question is what effect they have on the function of the protein encoded by the *Notch3* mutant allele.



Mutations identified by Tournier-Lasserve and co-workers[3] in the human *Notch3* gene of CADASIL patients, compared with *Abruptex* mutations in the *Drosophila Notch* gene. Arrowheads represent the site of a missense mutation, most of which are found in the extracellular EGF-repeat domain (purple), although one of the CADASIL mutations is in the intracellular ankyrin-repeat domain (green). The *Abruptex* mutations show a more clustered distribution than do the CADASIL mutations. (Sequence data on the *Abruptex* mutations were taken from ref. 11.)

As its name states, CADASIL is inherited in an autosomal dominant pattern. So the alterations in the *Notch3* gene that produce the symptoms of CADASIL must either result from a gain-of-function mutation or they must be due to haploinsufficiency of a *Notch3* null mutation — a haploinsufficiency phenotype results when a single copy of a gene is not sufficient for normal function. At present there are not enough data to decide between these two alternatives. It may be informative, however, to examine mutations in the Notch-family genes of other organisms. For example, in *Drosophila*, the *Abruptex* (*Ax*) mutations are a category of dominant mutant alleles of *Notch*. As in the human *Notch3* gene, the *Ax* mutations are missense mutations in the region that encodes the EGF repeats, but these mutations do not create null alleles of *Notch* — instead, they seem to cause ligand-dependent hyperactivation of the Notch protein[7,8].

Whether the *Notch3* mutations found by Tournier-Lasserve and co-workers[3] in the CADASIL patients cause a loss or gain of *Notch3* function could have important clinical implications. Most of the mutations associated with the CADASIL syndrome cause amino-acid changes in the extracellular domain, so this region of the protein is an obvious target for potential drug development. Studies with transgenic and knockout mice should help to elucidate the effects of these *Notch3* mutations.

The Notch signalling pathway may also be involved in another age-related dementia syndrome — Alzheimer's disease. In a paper published last year[9], Iva Greenwald's group characterized a suppressor of a gain-of-function mutation in the *lin-12* gene, which encodes a Notch-family receptor in the nematode *Caenorhabditis elegans*. Molecular analysis of several alleles of this suppressor revealed that they were mutations in a hitherto unknown gene, *sel-12*, which encodes a transmembrane protein that is highly homologous to the mammalian presenilin genes: the two presenilin genes, *PS-1* and *PS-2*, are responsible for early-onset familial Alzheimer's disease (reviewed in ref. 10).

Over the past year, lights have been burning late in both academic labs and pharmaceutical companies as researchers try to determine whether alterations in the Notch signalling pathway are involved in the pathogenesis of early-onset familial Alzheimer's disease. Now, more work remains to be done to understand how mutations in the *Notch3* gene lead to CADASIL. The results promise to be both scientifically interesting and, at the same time, important for human health. □

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