CADASIL: Notch signaling defect or protein accumulation problem?

Commentary

See related article, pages 597–605.

Nancy B. Spinner

Divisions of Human Genetics and Clinical Laboratories, and Department of Pediatrics, The Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, Pennsylvania 19104, USA.
Phone: (215) 590-4177; Fax: (215) 590-3850; E-mail: spinner@mail.med.upenn.edu.

When studies of the genetic etiology of human disease intersect with those of basic mechanisms in developmental biology, our understanding of normal and abnormal development is increased, and the knowledge gained enriches the work of both human geneticists and basic scientists. Such is the case with the work of A. Joutel et al. (1), who have previously demonstrated that mutations in the *Notch3* gene are the cause of the autosomal dominant disorder CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). CADASIL is an adult-onset neurologic disorder (average age of onset is 45 years) characterized by recurrent strokes and dementia. Through positional cloning, *Notch3* was found to be the gene responsible for the disorder (1), and mutations have been demonstrated in more than 90% of CADASIL patients (1, 2). The highly conserved Notch signaling pathway was originally identified and studied in the fruit fly *Drosophila melanogaster*. The name "Notch" derives from the characteristic notched wing found in flies carrying only 1 functioning copy of the gene. Homozygous *Notch* mutations are lethal, and affected embyros have severe abnormalities, including an excess of neural cells (3). A large body of work in the fruit fly, the nematode *Caenorhabditis elegans,* and more recently in vertebrates has revealed that this pathway plays a prominent role in development by contributing to the determination of cell fate in many different tissues (3).

The study of the effects of mutations in members of this pathway and their roles in human disease is in its infancy. Of the 4 human Notch genes (*Notch1*, *2*, *3*, and *4*), only *Notch1* and *Notch3* have been implicated in human disease, and only *Notch3* has been associated with germline mutations that cause inherited disease. *Notch1* (previously called *TAN-1*, for translocation-associated *Notch1*) has been shown to be involved in 3 translocations in patients with T-cell neoplasms (4). At least 4 ligands for Notch are known in humans (Jagged1, Jagged2, Delta-like1, and Delta-like3); to date, only the *Jagged1* gene has been associated with human disease. In the mouse, mutations in *Delta-like3* cause the Pudgy phenotype, which is characterized by severe vertebral and rib deformities (5). Mice homozygous for mutations in *Jagged2* die in utero, secondary to severe defects in craniofacial morphogenesis, and also display defects of the limbs and thymus (6). Mutations in human *Jagged1* underlie Alagille syndrome, a dominant congenital disorder associated with abnormalities of the liver, heart, skeleton, eye, and face (7, 8). In general, the nature of the mutations in this disorder suggests that haploinsufficiency for *Jagged1* causes disease, because most of the mutations truncate the *Jagged1* open reading frame or delete the gene entirely (9, 10).

The adult-onset character of CADASIL contrasts with the developmental abnormalities associated with mutations in the other Notch signaling pathway genes studied to date. The *Notch3* mutations seen in patients with CADASIL are transmitted in a dominant fashion, but unlike Alagille syndrome, CADASIL does not seem to arise from haploinsufficiency. Most CADASIL patients have a missense mutation leading to addition or loss of a cysteine residue within 1 of the 34 EGF-like repeats (2) in the Notch3 protein. The EGF-like repeats consist of 40–50 amino acids, and are found in a large number of extracellular proteins with diverse functions. These invariably contain 6 conserved cysteine residues that form 3 disulfide bonds, which are believed to be important for protein stabilization and protein-protein interaction (11). *Notch3* mutations in CADASIL patients all lead to an odd number of cysteine residues in the affected EGF domain, which would be predicted to disrupt the canonical disulfide pairing.

In this issue of the *JCI*, A. Joutel et al. present studies on the expression and subcellular localization of Notch3 in normal brains and in the brains of individuals with CADASIL. In healthy individuals as well as in CADASIL patients, Notch3 is present in the smooth muscle cells in the cerebral vasculature, but in the CADASIL patients, the extracellular domain of the receptor accumulates abnormally (12). Evidence from *Drosophila* has demonstrated that Notch1 reaches the cell surface in 2 parts. The nascent Notch1 peptide is cleaved into 2 domains in the trans-Golgi network, and appears on the cell surface as an extracellular protein complexed with an integral membrane protein that consists of the original transmembrane segment and the intracellular domain (3). On stimulation of the receptor by ligand, the intracellular domain translocates into the nucleus, where it mediates downstream effects in conjunction with intercellular regulatory proteins (13). Levels of the intracellular domain of Notch3 are not elevated in CADASIL tissue (12). Presumably, during or after normal Notch signaling, the extracellular proteins must be cleared from the cell membrane. Little is known about this process in vertebrates, but in *Drosophila,* normal Notch signaling requires the functioning of the protein dynamin, which is required for endocytosis of the receptor (14).

Perhaps mutant Notch3 molecules sop up ligand without transmitting a signal, dominantly inhibiting the normal pathway.

Is CADASIL caused by a defect in Notch signaling, or by an inability to

effectively remove the extracellular domain from the membrane? Do the missense mutations seen in CADASIL patients block endocytosis of the Notch receptor? The common features of the CADASIL mutations and the fact that none of the patients studied to date carries an obvious null allele of *Notch3* suggest that this disease does not arise simply from loss of Notch signaling. Further experiments are needed to determine if the mutant Notch3 molecule can respond to ligand and transmit a signal. This will require analysis of these mutants in an in vitro assay designed to measure downstream signaling of stimulated Notch3, which is not currently available. Even if the mutant molecules prove inactive in such an assay, the abnormal protein may be pathogenic because it fails to be cleared from the membrane, rather than because of a simple 2-fold reduction in Notch signaling. Thus, perhaps the mutant Notch3 molecules that accumulate on the cell surface sop up ligand without transmitting a signal, dominantly inhibiting the normal pathway. Direct evidence for such a model awaits development of reagents with which to study ligand-receptor interactions with respect to binding, clearance, and signal transmission.

Excess Notch3 extracellular domain may also prove toxic for other reasons that are still poorly understood, as suggested by studies of other neurologic conditions. The abnormal accumulation of protein in progressive neurologic disease is becoming a recurrent theme. Alzheimer's, Parkinson's, and Huntington's diseases, which are all late-onset, neurodegenerative disorders, are associated with abnormal protein deposition (15). In 1 form of Alzheimer's disease (AD1), missense mutations in the amyloid precursor protein lead to its abnormal accumulation (16). Furthermore, in some families, Parkinson's disease is associated with missense mutations in α-synuclein and the presence of proteinaceous cytoplasmic inclusions known as Lewy bodies (17). Huntington's disease arises from an expansion of a nucleotide triplet repeat at the DNA level, which results in synthesis of huntingtin protein containing an abnormal polyglutamine tract. The mutant huntingtin molecule also forms protein aggregates (18), although the role of these aggregates in pathogenesis is hotly debated.

Multiple lines of evidence suggest that Notch signaling is important in the normal development of vascular structures. Mice homozygous for a defective *Jagged1* gene die in utero, with defects in vascular remodeling in both the embryo and the yolk sac (19). Individuals with Alagille syndrome demonstrate abnormal vessels, primarily in the pulmonary vasculature (9). Studies to increase understanding of the molecular pathology caused by the *Notch3* missense mutations in CADASIL patients will elucidate the role of this pathway in the normal and abnormal functioning of the cerebral vasculature, and will deepen our fundamental understanding of this key developmental pathway.

1. Joutel, A., et al. 1996. Notch3 mutations in CADASIL, a hereditary adult onset condition causing stroke and dementia. *Nature.* **383**:707–710.
2. Joutel, A., et al. 1997. Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. *Lancet.* **350**:1511–1515.
3. Artavanis-Tsakonas, S., Rand, M.D., and Lake, R.J. 1999. Notch signaling: cell fate control and signal integration in development. *Science.* **284**:770–776.
4. Ellison, L.W., et al. 1991. TAN-1, the human homolog of the *Drosophila Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell.* **66**:649–661.
5. Kusumi, K., et al. 1998. The mouse pudgy mutation disrupts Delta homologue *Dll3* and initiation of early somite boundaries. *Nat. Genet.* **19**:274–278.
6. Jiang, R., et al. 1999. Defects in limb, craniofacial, and thymic development in Jagged2 mutant mice. *Genes Dev.* **12**:1046–1057.
7. Li, L., et al. 1997. Alagille syndrome is caused by mutations in human *Jagged1*, which encodes a ligand for Notch1. *Nat. Genet.* **16**:243–251.
8. Oda, T., et al. 1997. Mutations in the human *Jagged1* gene are responsible for Alagille syndrome. *Nat. Genet.* **16**:235–242.
9. Krantz, I.D., et al. 1998. Spectrum and frequency of Jagged1 (JAG1) mutations in Alagille syndrome patients and their families. *Am. J. Hum. Genet.* **62**:1361–1369.
10. Crosnier, C., et al. 1999. Analysis of mutations of the *Jagged1* gene in patients with Alagille syndrome: evidence for most cases being sporadic. *Gastroenterology.* **116**:1141–1148.
11. Campbell, I.D., and Bork, P. 1993. Epidermal growth factor-like module. *Curr. Opin. Struct. Biol.* **3**:385–392.
12. Joutel, A., et al. 2000. The ectodomain of the Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients. *J. Clin. Invest.* **105**:597–605 (2000).
13. Schroeter, E.H., Kisslinger, J.A., and Kopan, R. 1998. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature.* **393**:382–386.
14. Seugnet, L., Simpson, P., and Haenlin, M. 1997. Requirement for dynamin during Notch signaling in Drosophila neurogenesis. *Dev. Biol.* **192**:585–598.
15. Heintz, N., and Zoghbi, H. 1997. α-Synuclein—a link between Parkinson and Alzheimer diseases? *Nat. Genet.* **16**:325–327.
16. Glenner, G.G., and Wong, C.W. 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120**:885–890.
17. Polymeropoulos, et al. 1997. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science.* **276**:2045–2047.
18. Scherzinger, E., et al. 1999. Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implications for Huntington's disease pathology. *Proc. Natl. Acad. Sci. USA.* **96**:4604–4609.
19. Xue, Y., et al. 1999. Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. *Hum. Mol. Genet.* **8**:723–730.