Human Molecular Genetics, 2003, Vol. 12, Review Issue 1 R9–R13
DOI: 10.1093/hmg/ddg052

Notch signaling and inherited disease syndromes

Thomas Gridley*

The Jackson Laboratory, 600 Main St, Bar Harbor, ME 04609, USA

Received December 2, 2002; Accepted January 2, 2003

The Notch signaling pathway is an evolutionarily conserved, intercellular signaling mechanism essential for proper embryonic development in organisms as diverse as insects, nematodes, echinoderms and mammals. Disruptions in conserved developmental pathways frequently result in inherited congenital anomalies in humans. Mutations in genes encoding Notch pathway components underlie three inherited human diseases: Alagille syndrome, spondylocostal dysostosis, and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Mouse models for these three diseases have been developed, and are leading to novel insights into the pathology of these diseases in humans.

INTRODUCTION

The Notch signaling pathway is an evolutionarily conserved, intercellular signaling mechanism essential for proper embryonic development in all metazoan organisms in the animal kingdom. This review will summarize studies demonstrating that perturbations in the Notch signaling pathway contribute to the pathogenesis of several inherited human diseases. Perturbations in Notch signaling also contribute to the formation of lymphoid malignancies (for a review see 1). Biochemical aspects of Notch signaling have been the subject of numerous excellent reviews (2–8), so only a brief summary of how the Notch pathway functions in mammals will be presented here.

NOTCH SIGNALING PATHWAY

Genes of the Notch family encode large single-pass transmembrane proteins (Fig. 1). In mammals, four Notch family receptors have been described: NOTCH1–NOTCH4. The extracellular domain of Notch family proteins contains a large number of tandemly repeated copies of an epidermal growth factor (EGF)-like motif. A Notch family receptor exists at the cell surface as a proteolytically cleaved product consisting of a large ectodomain and a membrane-tethered intracellular domain. These products remain associated at the cell surface as a heterodimer through non-covalent, calcium-dependent interactions. Notch receptors interact with membrane-bound ligands that, in mammals, are encoded by the Jagged (*JAG1* and *JAG2*) and Delta-like (*DLL1*, *DLL3* and *DLL4*) gene families. The Notch ligands are also single-pass transmembrane proteins that contain multiple EGF-like repeats in their extracellular domains.

The signal induced by ligand binding is transmitted intracellularly by a process involving proteolytic cleavage of the receptor and nuclear translocation of the intracellular domain of the Notch family protein. The receptor/ligand interaction induces two additional proteolytic cleavages that free the intracellular domain of the Notch receptor from the cell membrane. The cleaved fragment translocates to the nucleus due to the presence of nuclear localization signals located in the Notch intracellular domain. Once in the nucleus, the Notch intracellular domain forms a complex with the RBPSUH protein, a sequence-specific DNA binding protein (also known in mammals as CSL, CBF1 and RBP-J). In the absence of Notch signaling, the RBPSUH protein binds to specific DNA sequences in the regulatory elements of various target genes and represses transcription by recruiting histone deacetylases and other components of a corepressor complex. Nuclear translocation of the Notch intracellular domain displaces the histone deacetylase/corepressor complex from the RBPSUH protein, leading to the transcriptional activation of Notch target genes. For a more detailed view of the complexities of the Notch signaling pathway and for references to the primary literature, refer to previous reviews (2–8).

ALAGILLE SYNDROME

Alagille syndrome (AGS; OMIM 118450) is an autosomal dominant disorder characterized by developmental abnormalities of the liver, heart, eye, skeleton and, at lower penetrance, several other organs (9,10). AGS patients typically present with neonatal jaundice and cholestasis resulting from a paucity of intrahepatic bile ducts. Accompanying features of AGS include cardiac defects (including pulmonary artery stenosis and hypoplasia, pulmonic valve stenosis and tetralogy of Fallot), skeletal defects (primarily butterfly vertebrae), ophthalmological

*To whom correspondence should be addressed. Tel: +1 2072886237; Fax: +1 2072886077; Email: gridley@jax.org

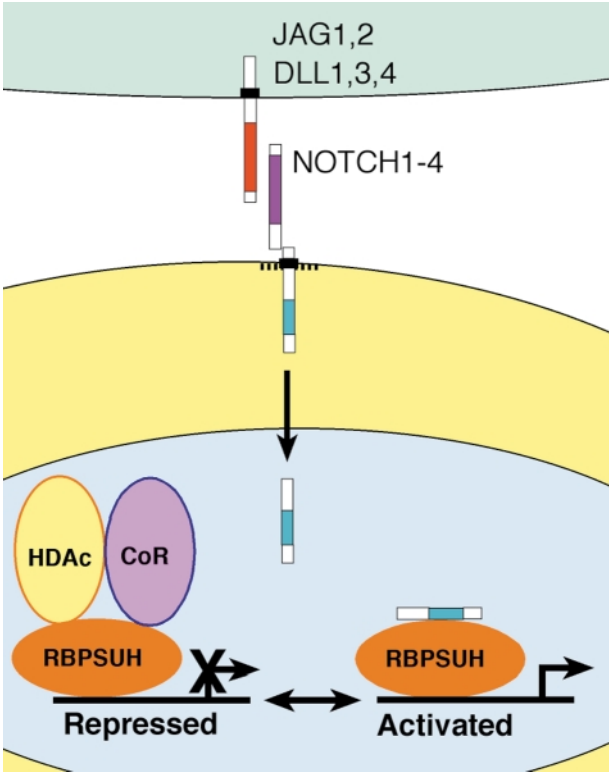

Figure 1. Simplified overview of Notch signaling in mammals. Ligands of the Jagged (JAG1 and JAG2) and Delta-like (DLL1, DLL3, DLL4) families (upper cell, shown in green) interact with Notch family transmembrane receptors (NOTCH1–NOTCH4) on an adjacent cell (lower cell, shown in yellow). The Notch receptor exists at the cell surface as a proteolytically cleaved product consisting of a large ectodomain and a membrane-tethered intracellular domain. The receptor–ligand interaction induces two additional proteolytic cleavages that free the intracellular domain of the Notch receptor from the cell membrane. The cleaved fragment translocates to the nucleus (shown in blue) owing to the presence of nuclear localization signals located in the Notch intracellular domain. Once in the nucleus, the Notch intracellular domain forms a complex with the RBPSUH protein, displacing a histone deacetylase (HDAc)/corepressor (CoR) complex from the RBPSUH protein, leading to the transcriptional activation of Notch target genes.

abnormalities (primarily anterior chamber defects such as posterior embryotoxon), a characteristic facial appearance, renal and pancreatic abnormalities, and intracranial bleeding (9,11–14). Newly reported AGS phenotypes include craniosynostosis (15) and digit abnormalities (16).

Positional cloning studies revealed that AGS is caused by mutations in the Jagged1 (*JAG1*) gene (17,18). An extensive survey of the types and frequency of *JAG1* mutations in AGS patients revealed that 72% led to premature termination codons, 15% were splice site mutations and 13% were missense mutations (19). Three to seven percent of AGS patients have deletions encompassing the entire *JAG1* gene. In a sample of over 300 AGS patients, mutations in the *JAG1* gene have been demonstrated in ~70% of the patients (19).

The phenotypes of patients with *JAG1* deletions were indistinguishable from the phenotypes of patients with intragenic *JAG1* mutations, suggesting *JAG1* haploinsufficiency as at least one cause of AGS (19). *JAG1* missense mutations are nonrandomly distributed across the JAG1 protein, being more frequent near the amino-terminus of the protein. Two missense mutations that were studied in detail resulted in defective intracellular transport and processing of the mutant JAG1 protein. The mutant proteins were abnormally glycosylated and were not present on the cell surface, further supporting functional haploinsufficiency as an AGS disease mechanism (20).

AGS exhibits high penetrance but extremely variable expressivity, even within family members carrying identical *JAG1* mutations (9–11). Possible explanations for this variable expressivity include the existence of either genetic or environmental (i.e. non-genetic) modifiers. Kamath and colleagues recently reported a clear example of non-genetic modification in a case of monozygotic twins with discordant AGS phenotypes (21). One twin had severe pulmonary atresia and mild liver disease, while the other twin had tetralogy of Fallot and severe cholestatic liver disease that required transplantation. The existence of genetic modifiers is supported by studies with mouse models described below.

Since the identification of *JAG1* as the AGS gene, it has become apparent that the majority of *JAG1* mutation carriers within a family do not meet the clinical criteria for diagnosis of AGS (10,22). *JAG1* mutations can cause disease of only one or a few organ systems, without accompanying defects in the liver or most of the other tissues typically affected in AGS patients. More than 90% of individuals with *JAG1* mutations exhibit cardiovascular abnormalities, which may or may not be associated with anomalies in other organ systems (14). Examples include multigenerational pulmonic stenosis due to a *JAG1* missense mutation, and a case of tetralogy of Fallot and hypoplastic pulmonary arteries due to *JAG1* gene deletion (12). In another study, individuals in a large kindred segregating tetralogy of Fallot as an autosomal dominant trait were found to have a *JAG1* missense mutation (13). Another kindred with a *JAG1* missense mutation exhibited hearing loss, inner ear vestibular defects, congenital heart defects and posterior embryotoxon (23). Vestibular defects of the inner ear also have been demonstrated in two independent *Jag1* missense mutant mice identified in large scale mutagenesis screens (24,25).

My laboratory has been characterizing mouse AGS models generated by gene targeting. Mice homozygous for a targeted null mutation of the *Jag1* gene die *in utero* due to vascular defects in the embryo and the yolk sac (26). Mice heterozygous for the *Jag1* mutation, whose genotype mimics that of human AGS patients, proved to be a disappointing animal model for this disease. The *Jag1*/+ heterozygous mice exhibited anterior chamber eye defects, but did not exhibit other phenotypes associated with AGS in humans (26). However, mice doubly heterozygous for the *Jag1* null allele and a *Notch2* hypomorphic allele (27) reproduced most of the clinically relevant phenotypes observed in AGS patients. These mice (designated $J1N2^{+/-}$) exhibited jaundice, growth retardation and bile duct, heart, eye and kidney abnormalities that were similar or identical to the abnormalities observed in AGS patients (28). Mice homozygous for a targeted mutation of the *Hey2* gene, which encodes a basic helix–loop–helix transcription factor that is a downstream Notch target gene (29,30), exhibited cardiac defects similar to those observed in $J1N2^{+/-}$ mice, suggesting that the *Hey2* gene mediates Notch signaling in the developing heart (31,32).

AGS exhibits high penetrance but extremely variable expressivity. A possible explanation for this variable expressivity is the existence of genetic modifiers of the disease phenotype in the human population. Our mouse models demonstrate that the *Notch2* gene acts as a genetic modifier to interact with a *Jag1* mutation to create a more representative model for AGS (28). We hypothesize that similar genetic interactions may occur in human AGS patients, and that particular *NOTCH2* alleles may influence the severity of AGS phenotypes. Preliminary results from my laboratory indicate that the *Jag1* mutant can interact with mouse mutants in additional Notch pathway components to recreate phenotypes similar to those observed in AGS patients. *JAG1* mutations have been found in only 70% of AGS patients (19). It is not clear at present whether the remaining patients have unidentified *JAG1* mutations (perhaps in regulatory regions of the gene), or whether they have mutations in other genes. Our mouse models suggest that genes encoding other components of the Notch signaling pathway are candidates for additional AGS disease genes or modifiers.

SPONDYLOCOSTAL DYSOSTOSIS

In spondylocostal dysostosis (SD), vertebral segmentation defects are associated with rib anomalies. SD patients exhibit short trunk dwarfism due to multiple hemivertebrae accompanied by rib fusions and deletions, and both autosomal dominant and autosomal-recessive modes of inheritance have been reported (OMIM 122600) (33). One form of autosomal-recessive SD was mapped to chromosome 19q13.1–q13.3 (34). Positional cloning studies demonstrated that mutations in the human *DLL3* gene caused this form of autosomal recessive SD (OMIM 277300) (35). Two of the identified mutations were predicted to cause protein truncations, while the third mutation caused a missense mutation in a highly conserved amino acid residue of the DLL3 protein (35). The phenotype exhibited by these patients is very similar to the phenotype exhibited by mice homozygous for the *pudgy* mutation, a spontaneous mutation of the *Dll3* gene (36). The $Dll3^{pu}$ mutant allele has a 4 bp deletion that results in a frameshift and premature protein truncation. Comparison of the phenotypes of embryos homozygous for the $Dll3^{pu}$ mutation with embryos homozygous for a *Dll3* targeted mutation indicated that both were *Dll3* null alleles (37). The similarities between the *Dll3* mutant mice and human SD patients suggest that the human SD mutant alleles also result in loss of *Dll3* function.

The Notch signaling pathway plays a major role in regulating somite formation and in partitioning somites into anterior and posterior compartments (38). Notch pathway components appear to regulate, or constitute essential components of, a cell autonomous oscillator functioning in the presomitic mesoderm. This oscillator has been termed the somite clock (38). Analysis of marker gene expression in *Dll3* mutant mice indicates that progression of the somite clock is disrupted in these mutants (37,39). A similar mechanism probably underlies the vertebral defects observed in SD patients.

Autosomal recessive SD is a genetically heterogeneous condition. For example, one family segregating autosomal-recessive SD did not show evidence of linkage to 19q13, where the *Dll3* gene is located (40). Studies of mouse models have shown that mice homozygous for a null mutation of the Lunatic fringe (*LFNG*) gene, which encodes a glycosyltransferase that modulates Notch signal transduction (41,42), exhibit axial truncation defects and rib fusions very similar to those observed in *Dll3* mutant mice and in SD patients (39,43,44). This suggests that *Lfng* is another candidate gene for autosomal-recessive SD.

CADASIL

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; OMIM 125310) is an autosomal-dominant vascular disorder. Affected individuals exhibit a variety of symptoms, including migraine with aura, mood disorders, recurrent subcortical ischemic strokes, progressive cognitive decline, dementia and premature death (45–47). The vascular lesions underlying CADASIL are a non-atherosclerotic, non-amyloid arteriopathy affecting primarily small cerebral arteries, although the vascular defects are systemic and CADASIL can be diagnosed by ultrastructural or immunohistochemical examination of arterioles in skin biopsies (48–51). Electron microscopic studies revealed the degeneration and loss of vascular smooth muscle cells in CADASIL patients, and the accumulation of granular osmiophilic material within the smooth muscle cell basement membrane and the surrounding extracellular matrix (45–47).

CADASIL is caused by mutations in the *NOTCH3* gene (52). All mutations associated with CADASIL result in a gain or loss of a cysteine residue in one of the 34 EGF-like repeats in the extracellular domain of the NOTCH3 protein. Most mutations are missense mutations that are clustered near the amino terminus of the protein (53). Some splice site mutations have also been described. However, these splicing mutations invariably cause in-frame deletions that result in the loss of cysteine residues (54,55). All EGF repeats contain six conserved cysteine residues that form three intradomain disulfide bonds (56). The types of mutations found in CADASIL patients lead to an odd number of cysteine residues in the affected EGF repeat. This fact, in addition to the absence of any examples of obviously inactivating mutations or deletions of the *NOTCH3* gene of CADASIL patients, strongly suggests that these mutations do not create *NOTCH3* null alleles. Transfection of rat *NOTCH3* cDNA clones encoding CADASIL-like mutant proteins into cell lines demonstrated that the mutations did not affect cell surface expression or ligand binding ability of the mutant proteins (57). However, it is not known whether or how NOTCH3 signaling is altered by the CADASIL mutations.

Expression of the NOTCH3 protein in the vasculature is confined to arterial vascular smooth muscle cells in both humans and rodents (58–61). Joutel and colleagues made the important finding that the ectodomain of the NOTCH3 protein accumulates in the cerebral microvasculature of CADASIL patients (58). The NOTCH3 ectodomain accumulated at the cytoplasmic membrane of vascular smooth muscle cells, in close vicinity to but not within the granular osmiophilic material deposits. These data suggest that the CADASIL mutations impair the clearance of the NOTCH3 ectodomain from the cell surface (58).

Ruchoux and colleagues have developed a mouse CADASIL model by expressing a human NOTCH3 cDNA containing a common CADASIL mutation (Arg90Cys) in vascular smooth muscle cells (62). Transgenic mice expressing the mutant cDNA demonstrated age-dependent accumulation of the NOTCH3 ectodomain and of granular osmiophilic material in both cerebral and peripheral arterioles. Surprisingly, vascular defects were more severe in the tails of the transgenic mice than in the brain. In addition, the transgenic mice did not exhibit any evidence of damage to the brain parenchyma. Further analysis and development of this model should lead to insights into the onset and progression of CADASIL, particularly during its early stages.

PERSPECTIVES

Similar to what has been observed with other developmentally important signaling pathways, mutations in Notch pathway components cause inherited human diseases. Relevant mouse models have been described for each of these diseases, and analysis of these models has led to several potential insights. For example, the segmentation defects observed in *Lfng* mutant mice suggest this gene as a candidate gene for autosomal recessive SD. Similarly, the AGS phenotypes observed in *J1N2*$^{+/-}$ double heterozygous mice suggest that modulation of *Notch2* function may affect the expressivity of the disease in AGS patients. We can anticipate that further studies of Notch pathway mutant mice will lead to additional insights into the pathogenesis of these diseases in humans.

ACKNOWLEDGEMENTS

I would like to thank Anne Joutel for communicating results prior to publication. Work in my laboratory on the Notch pathway is supported by grants from the USA National Institutes of Health and the March of Dimes Birth Defects Foundation.

REFERENCES

1. Aster, J.C. and Pear, W.S. (2001) Notch signaling in leukemia. *Curr. Opin. Hematol.*, **8**, 237–244.
2. 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.
3. Kadesch, T. (2000) Notch signaling: a dance of proteins changing partners. *Exp. Cell Res.*, **260**, 1–8.
4. Mumm, J.S. and Kopan, R. (2000) Notch signaling: from the outside in. *Dev. Biol.*, **228**, 151–165.
5. Weinmaster, G. (2000) Notch signal transduction: a real Rip and more. *Curr. Opin. Genet. Dev.*, **10**, 363–369.
6. Allman, D., Punt, J.A., Izon, D.J., Aster, J.C. and Pear, W.S. (2002) An invitation to T and more: notch signaling in lymphopoiesis. *Cell*, **109**, S1–S11.
7. Baron, M., Aslam, H., Flasza, M., Fostier, M., Higgs, J.E., Mazaleyrat, S.L. and Wilkin, M.B. (2002) Multiple levels of Notch signal regulation. *Mol. Membr. Biol.*, **19**, 27–38.
8. Kopan, R. (2002) Notch: a membrane-bound transcription factor. *J. Cell Sci.*, **115**, 1095–1097.
9. Krantz, I.D., Piccoli, D.A. and Spinner, N.B. (1997) Alagille syndrome. *J. Med. Genet.*, **34**, 152–157.
10. Krantz, I.D. (2002) Alagille syndrome: chipping away at the tip of the iceberg. *Am. J. Med. Genet.*, **112**, 160–162.
11. Emerick, K.M., Rand, E.B., Goldmuntz, E., Krantz, I.D., Spinner, N.B. and Piccoli, D.A. (1999) Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. *Hepatology*, **29**, 822–829.
12. Krantz, I.D., Smith, R., Colliton, R.P., Tinkel, H., Zackai, E.H., Piccoli, D.A., Goldmuntz, E. and Spinner, N.B. (1999) Jagged1 mutations in patients ascertained with isolated congenital heart defects. *Am. J. Med. Genet.*, **84**, 56–60.
13. Eldadah, Z.A., Hamosh, A., Biery, N.J. , Montgomery, R.A., Duke, M., Elkins, R. and Dietz, H.C. (2001) Familial Tetralogy of Fallot caused by mutation in the jagged1 gene. *Hum. Mol. Genet.*, **10**, 163–169.
14. McElhinney, D.B., Krantz, I.D., Bason, L., Piccoli, D.A., Emerick, K.M., Spinner, N.B. and Goldmuntz, E. (2002) Analysis of cardiovascular phenotype and genotype–phenotype correlation in individuals with a JAG1 mutation and/or Alagille syndrome. *Circulation*, **106**, 2567–2574.
15. Kamath, B.M., Stolle, C., Bason, L., Colliton, R.P., Piccoli, D.A., Spinner, N.B. and Krantz, I.D. (2002) Craniosynostosis in Alagille syndrome. *Am. J. Med. Genet.*, **112**, 176–180.
16. Kamath, B.M., Loomes, K.M., Oakey, R.J. and Krantz, I.D. (2002) Supernumerary digital flexion creases: an additional clinical manifestation of Alagille syndrome. *Am. J. Med. Genet.*, **112**, 171–175.
17. Li, L., Krantz, I.D., Deng, Y., Genin, A., Banta, A.B., Collins, C.C., Qi, M., Trask, B.J., Kuo, W.L., Cochran, J. *et al.* (1997) Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat. Genet.*, **16**, 243–251.
18. Oda, T., Elkahloun, A.G., Pike, B.L., Okajima, K., Krantz, I.D., Genin, A., Piccoli, D.A., Meltzer, P.S., Spinner, N.B., Collins, F.S. *et al.* (1997) Mutations in the human Jagged1 gene are responsible for Alagille syndrome. *Nat. Genet.*, **16**, 235–242.
19. Spinner, N.B., Colliton, R.P., Crosnier, C., Krantz, I.D., Hadchouel, M. and Meunier-Rotival, M. (2001) Jagged1 mutations in Alagille syndrome. *Hum. Mutat.*, **17**, 18–33.
20. Morrissette, J.J.D., Colliton, R.P. and Spinner, N.B. (2001) Defective intracellular transport and processing of JAG1 missense mutations in Alagille syndrome. *Hum. Mol. Genet.*, **10**, 405–413.
21. Kamath, B.M., Krantz, I.D., Spinner, N.B., Heubi, J.E. and Piccoli, D.A. (2002) Monozygotic twins with a severe form of Alagille syndrome and phenotypic discordance. *Am. J. Med. Genet.*, **112**, 194–197.
22. Krantz, I.D., Smith, R., Colliton, R.P., Goldmuntz, E., Zackai, E.H., Piccoli, D.A. and Spinner, N.B. (1999) The phenotypic implication of having a Jagged1 mutation: not just Alagille syndrome anymore. *Proc. Greenwood Genet. Center*, **18**, 80–81.
23. Le Caignec, C., Lefevre, M., Schott, J.J., Chaventre, A., Gayet, M., Calais, C. and Moisan, J.P. (2002) Familial deafness, congenital heart defects, and posterior embryotoxon caused by cysteine substitution in the first epidermal-growth-factor-like domain of jagged1. *Am. J. Hum. Genet.*, **71**, 180–186.
24. Tsai, H., Hardisty, R.E., Rhodes, C., Kiernan, A.E., Roby, P., Tymowska-Lalanne, Z., Mburu, P., Rastan, S., Hunter, A.J., Brown, S.D. *et al.* (2001) The mouse slalom mutant demonstrates a role for Jagged1 in neuroepithelial patterning in the organ of Corti. *Hum. Mol. Genet.*, **10**, 507–512.
25. Kiernan, A.E., Ahituv, N., Fuchs, H., Balling, R., Avraham, K.B., Steel, K.P. and Hrabe de Angelis, M. (2001) The Notch ligand Jagged1 is required for inner ear sensory development. *Proc. Natl Acad. Sci. USA*, **98**, 3873–3878.
26. Xue, Y., Gao, X., Lindsell, C.E., Norton, C.R., Chang, B., Hicks, C., Gendron-Maguire, M., Rand, E.B., Weinmaster, G. and Gridley, T. (1999) Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. *Hum. Mol. Genet.*, **8**, 723–730.
27. McCright, B., Gao, X., Shen, L., Lozier, J., Lan, Y., Maguire, M., Herzlinger, D., Weinmaster, G., Jiang, R. and Gridley, T. (2001) Defects in development of the kidney, heart and eye vasculature in mice homozygous for a hypomorphic Notch2 mutation. *Development*, **128**, 491–502.
28. McCright, B., Lozier, J. and Gridley, T. (2002) A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. *Development*, **129**, 1075–1082.
29. Nakagawa, O., McFadden, D.G., Nakagawa, M., Yanagisawa, H., Hu, T., Srivastava, D. and Olson, E.N. (2000) Members of the HRT family of basic helix–loop–helix proteins act as transcriptional repressors downstream of notch signaling. *Proc. Natl Acad. Sci. USA*, **97**, 13655–13660.
30. Iso, T., Chung, G., Hamamori, Y. and Kedes, L. (2002) HERP1 is a cell type-specific primary target of Notch. *J. Biol. Chem.*, **277**, 6598–6607.

31. Gessler, M., Knobeloch, K.P., Helisch, A., Amann, K., Schumacher, N., Rohde, E., Fischer, A. and Leimeister, C. (2002) Mouse gridlock: no aortic coarctation or deficiency, but fatal cardiac defects in Hey2$^{-/-}$ mice. *Curr. Biol.*, **12**, 1601–1604.
32. Donovan, J., Kordylewska, A., Jan, Y.N. and Utset, M.F. (2002) Tetralogy of fallot and other congenital heart defects in Hey2 mutant mice. *Curr. Biol.*, **12**, 1605–1610.
33. Mortier, G.R., Lachman, R.S., Bocian, M. and Rimoin, D.L. (1996) Multiple vertebral segmentation defects: analysis of 26 new patients and review of the literature. *Am. J. Med. Genet.*, **61**, 310–319.
34. Turnpenny, P.D., Bulman, M.P., Frayling, T.M., Abu-Nasra, T.K., Garrett, C., Hattersley, A.T. and Ellard, S. (1999) A gene for autosomal recessive spondylocostal dysostosis maps to 19q13.1–q13.3. *Am. J. Hum. Genet.*, **65**, 175–182.
35. Bulman, M.P., Kusumi, K., Frayling, T.M., McKeown, C., Garrett, C., Lander, E.S., Krumlauf, R., Hattersley, A.T., Ellard, S. and Turnpenny, P.D. (2000) Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. *Nat. Genet.*, **24**, 438–441.
36. Kusumi, K., Sun, E., Kerrebrock, A.W., Bronson, R.T., Chi, D.-C., Bulotsky, M.S., Spencer, J.B., Birren, B.W., Frankel, W.N. and Lander, E.S. (1998) The mouse pudgy mutation disrupts Delta homologue Dll3 and initiation of early somite boundaries. *Nat. Genet.*, **19**, 274–278.
37. Dunwoodie, S.L., Clements, M., Sparrow, D.B., Sa, X., Conlon, R.A. and Beddington, R.S. (2002) Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene Dll3 are associated with disruption of the segmentation clock within the presomitic mesoderm. *Development*, **129**, 1795–1806.
38. Maroto, M. and Pourquié, O. (2001) A molecular clock involved in somite segmentation. *Curr. Top. Dev. Biol.*, **51**, 221–248.
39. Zhang, N., Norton, C.R. and Gridley, T. (2002) Segmentation defects of Notch pathway mutants and absence of a synergistic phenotype in Lunatic fringe/Radical fringe double mutant mice. *Genesis*, **33**, 21–28.
40. Iughetti, P., Alonso, L.G., Wilcox, W., Alonso, N. and Passos-Bueno, M.R. (2000) Mapping of the autosomal recessive (AR) craniometaphyseal dysplasia locus to chromosome region 6q21–22 and confirmation of genetic heterogeneity for mild AR spondylocostal dysplasia. *Am. J. Med. Genet.*, **95**, 482–491.
41. Bruckner, K., Perez, L., Clausen, H. and Cohen, S. (2000) Glycosyltransferase activity of Fringe modulates Notch-Delta interactions. *Nature*, **406**, 411–415.
42. Moloney, D.J., Panin, V.M., Johnston, S.H., Chen, J., Shao, L., Wilson, R., Wang, Y., Stanley, P., Irvine, K.D., Haltiwanger, R.S. *et al.* (2000) Fringe is a glycosyltransferase that modifies Notch. *Nature*, **406**, 369–375.
43. Evrard, Y.A., Lun, Y., Aulehla, A., Gan, L. and Johnson, R.L. (1998) Lunatic fringe is an essential mediator of somite segmentation and patterning. *Nature*, **394**, 377–381.
44. Zhang, N. and Gridley, T. (1998) Defects in somite formation in Lunatic fringe deficient mice. *Nature*, **394**, 374–377.
45. Tournier-Lasserve, E., Joutel, A., Melki, J., Weissenbach, J., Lathrop, G.M., Chabriat, H., Mas, J.L., Cabanis, E.A., Baudrimont, M., Maciazek, J. *et al.* (1993) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12. *Nat. Genet.*, **3**, 256–259.
46. Chabriat, H., Vahedi, K., Iba-Zizen, M.T., Joutel, A., Nibbio, A., Nagy, T.G., Krebs, M.O., Julien, J., Dubois, B., Ducrocq, X. *et al.* (1995) Clinical spectrum of CADASIL: a study of 7 families. *Lancet*, **346**, 934–939.
47. Ruchoux, M.M., Guerouaou, D., Vandenhaute, B., Pruvo, J.P., Vermersch, P. and Leys, D. (1995) Systemic vascular smooth muscle cell impairment in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Acta Neuropathol. (Berl.)*, **89**, 500–512.
48. Ebke, M., Dichgans, M., Bergmann, M., Voelter, H.U., Rieger, P., Gasser, T. and Schwendemann, G. (1997) CADASIL: skin biopsy allows diagnosis in early stages. *Acta Neurol. Scand.*, **95**, 351–357.
49. Mayer, M., Straube, A., Bruening, R., Uttner, I., Pongratz, D., Gasser, T., Dichgans, M. and Muller-Hocker, J. (1999) Muscle and skin biopsies are a sensitive diagnostic tool in the diagnosis of CADASIL. *J. Neurol.*, **246**, 526–532.
50. Ruchoux, M.M., Brulin, P., Leteurtre, E. and Maurage, C.A. (2000) Skin biopsy value and leukoaraiosis. *Ann. NY Acad. Sci.*, **903**, 285–292.
51. Joutel, A., Favrole, P., Labauge, P., Chabriat, H., Lescoat, C., Andreux, F., Domenga, V., Cecillon, M., Vahedi, K., Ducros, A. *et al.* (2001) Skin biopsy immunostaining with a Notch3 monoclonal antibody for CADASIL diagnosis. *Lancet*, **358**, 2049–2051.
52. Joutel, A., Corpechot, C., Ducros, A., Vahedi, K., Chabriat, H., Mouton, P., Alamowitch, S., Domenga, V., Cécillion, M., Maréchal, E. *et al.* (1996) Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*, **383**, 707–710.
53. Joutel, A., Vahedi, K., Corpechot, C., Troesch, A., Chabriat, H., Vayssiere, C., Cruaud, C., Maciazek, J., Weissenbach, J., Bousser, M.G. *et al.* (1997) Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. *Lancet*, **350**, 1511–1515.
54. Joutel, A., Chabriat, H., Vahedi, K., Domenga, V., Vayssiere, C., Ruchoux, M.M., Lucas, C., Leys, D., Bousser, M.G. and Tournier-Lasserve, E. (2000) Splice site mutation causing a seven amino acid Notch3 in-frame deletion in CADASIL. *Neurology*, **54**, 1874–1875.
55. Dichgans, M., Herzog, J. and Gasser, T. (2001) NOTCH3 mutation involving three cysteine residues in a family with typical CADASIL. *Neurology*, **57**, 1714–1717.
56. Campbell, I.D. and Bork, P. (1993) Epidermal growth factor-like module. *Curr. Opin. Struct. Biol.*, **3**, 385–392.
57. Haritunians, T., Boulter, J., Hicks, C., Buhrman, J., DiSibio, G., Shawber, C., Weinmaster, G., Nofziger, D. and Schanen, C. (2002) CADASIL Notch3 mutant proteins localize to the cell surface and bind ligand. *Circ. Res.*, **90**, 506–508.
58. Joutel, A., Andreux, F., Gaulis, S., Domenga, V., Cecillon, M., Battail, N., Piga, N., Chapon, F., Godfrain, C. and Tournier-Lasserve, E. (2000) The ectodomain of the Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients. *J. Clin. Invest.*, **105**, 597–605.
59. Leimeister, C., Schumacher, N., Steidl, C. and Gessler, M. (2000) Analysis of HeyL expression in wild-type and Notch pathway mutant mouse embryos. *Mech. Dev.*, **98**, 175–178.
60. Villa, N., Walker, L., Lindsell, C.E., Gasson, J., Iruela-Arispe, M.L. and Weinmaster, G. (2001) Vascular expression of Notch pathway receptors and ligands is restricted to arterial vessels. *Mech. Dev.*, **108**, 161–164.
61. Prakash, N., Hansson, E., Betsholtz, C., Mitsiadis, T. and Lendahl, U. (2002) Mouse Notch 3 expression in the pre- and postnatal brain: relationship to the stroke and dementia syndrome CADASIL. *Exp. Cell Res.*, **278**, 31–44.
62. Ruchoux, M.M., Domenga, V., Bruin, P., Maciazek, J., Limol, S., Tournier-Lasserve, E. and Joutel, A. (2003) Transgenic mice expressing mutant Notch3 develop vascular alterations characteristic of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Am. J. Pathol.*, **162**, 329–342.