Int. J. Dev. Biol. 46: 365-374 (2002)

Diverse requirements for Notch signalling in mammals

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ABSTRACT The Notch signalling pathway has a central role in a wide variety of developmental processes and it is not therefore surprising that mutations in components of this pathway can cause dramatic human genetic disorders. One developmental process in which the Notch pathway is involved at multiple levels is somitogenesis, the mechanism by which the embryo is divided into segments that ultimately form structures such as the axial skeleton and skeletal muscle of the trunk. We are investigating the human genetic disorder spondylocostal dysplasia (SCD), which is a group of malsegmentation syndromes that occur when this process is disrupted. Mutations in the Notch ligand *DELTA-LIKE 3 (DLL3)* are responsible for cases of autosomal recessive SCD type I (SCDO1), and we are using information derived from these mutations to study the structure of the DLL3 protein. To aid in elucidation of the underlying developmental defect in SCDO1, we have generated a mouse model by targeted deletion of the *Dll3* gene (Dunwoodie *et al.*, 2002). These mice show segmentation defects similar to those seen in SCDO1. In addition, these mice have a distinct set of neural defects that may be useful in future neurological assessment of affected individuals. Finally, since not all cases of SCD are due to mutation of *DLL3*, we are investigating various genes to find other candidates involved in this genetic disease.

KEY WORDS: *Spondylocostal dysplasia, Dll3, somitogenesis, Notch signalling pathway*

Introduction

The Notch signalling pathway is a highly evolutionarily conserved signal transduction mechanism that has key roles in the regulation of cell fate decisions during embryonic development of metazoans, from sea urchins to humans (reviewed in Artavanis-Tsakonas *et al.*, 1999). Notch proteins are large transmembrane receptors that are expressed in a wide range of different cell types in temporally and spatially restricted patterns. These receptors interact with membrane-bound ligands belonging to both the Serrate/Jagged and the Delta-like families. Ligands tend to be expressed in more highly restricted patterns than the receptors within the developing embryo. In the mammalian system there are four Notch homologues (Notch 1-4; Weinmaster *et al.*, 1991; Weinmaster *et al.*, 1992; Lardelli *et al.*, 1994; Uyttendaele *et al.*, 1996;), three Delta-like proteins Dll 1, 3 and 4; Bettenhausen *et al.*, 1995; Dunwoodie *et al.*, 1997; Shutter *et al.*, 2000) and two Serrate/Jagged proteins (Jagged 1 and 2; Lindsell *et al.*, 1995; Shawber *et al.*, 1996). Structurally the mammalian receptors consist of several distinct conserved domains including EGF, Notch/lin-12 and ankyrin repeats; and RAM23, PEST and transmembrane domains (depicted schematically in Fig. 1; reviewed in Fleming, 1998). The mammalian ligands bind to EGF repeats 11 and 12 of the receptor via the Delta/Serrate/Lag-2 domain (DSL). In addition, both families of ligands contain EGF repeats and a transmembrane domain, and the Jagged proteins also contain a cysteine-rich domain (Fig. 1). As a consequence of both receptor and ligand being membrane-bound, activation of the Notch pathway requires intimate cell-cell interaction. Ligand binding results in proteolytic cleavage of the receptor close to the transmem-

Abbreviations used in this paper: AD, Alzheimers disease; AGS, Alagille syndrome; CADASIL, Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy; cp, choroid plexus; CSL, CBF1/Supressor of Hairless/Lag-1; Dll1, Delta-like 1; Dll3, Delta-like 3; dpc, days post coitum; DSL, Delta/Serrate/Lag-2; EGF repeat, epidermal growth factor-like repeats; MuLV, murine leukaemia virus; NICD, Notch intracellular domain; PSM, presomitic mesoderm; *pu*, pudgy; SCD, spondylocostal dysplasia; SCDO1, spondylocostal dysplasia, autosomal recessive type I; SNP, single nucleotide polymorphism; VER, ventral ectodermal ridge.

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† This article is dedicated to the memory of Rosa Beddington (March 23, 1956 to May 18, 2001).

0214-6282/2002/$25.00

Printed in Spain
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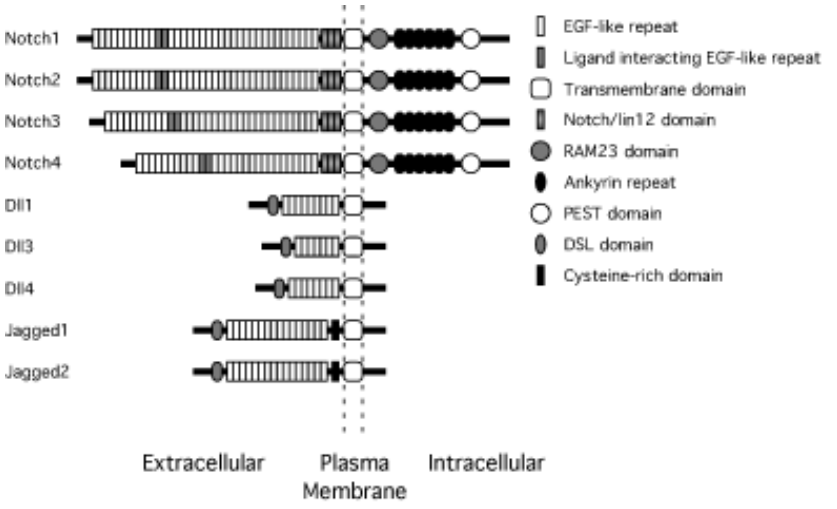

Fig. 1. Mammalian Notch receptors and ligands; *a schematic representation of their structure.*

brane domain, and translocation of the Notch intracellular domain (NICD) to the nucleus of the cell receiving the signal. Here, the NICD interacts with transcription factors of the CSL family, converting them from repressors to activators of transcription, and thus causing direct activation of target genes. These include transcription factors and signalling molecules that can either inhibit or promote differentiation along particular developmental pathways (reviewed in Mumm and Kopan, 2000). Genetic studies in *Drosophila* and *C. elegans* have revealed that the basic pathway is modulated at a variety of different levels by a large array of accessory proteins (reviewed in Panin and Irvine, 1998 and Mumm and Kopan, 2000), the majority of which are also represented in mammals.

During development, this signalling pathway controls the specification of cell fate via three mechanisms that have been well defined in *Drosophila* (reviewed in Bray, 1998). Firstly, lateral inhibition is a mechanism by which isolated cells within a field of cells with equivalent developmental potential can undergo specification and simultaneously inhibit the neighbouring cells from following the same developmental pathway. This process acts through a positive feedback loop which amplifies small differences in the levels of Notch activation between neighbouring cells, and often involves induction of the HES family of transcriptional repressors which inhibit the expression of tissue-specific transcription factors. It has been suggested that in this process Notch signalling is acting to repress a "default" differentiation pathway, and promote a "secondary" fate. Notch signalling is also involved in lateral specification that drives the lineage decisions of progenitor cells with the potential to differentiate into more than one cell type. In this process, cells are triggered to commit to a specific fate by the reception of a signal via the Notch receptor. An example of this process is in lymphocyte development (see below). Finally, Notch signalling is a key component in the process of forming boundaries between different types of cells. The best described examples are the formation of the dorsal/ventral boundary in both the *Drosophila* wing imaginal disc and in the vertebrate wing (Irvine and Vogt, 1997); and somite formation in vertebrates (see below).

Defects in Notch Signalling Cause Human Genetic Disease

Since Notch signalling is involved in a wide variety of processes, it is not surprising that mutations of either receptors or ligands has been found to be the underlying cause of human genetic disorders. Four such diseases have been identified and studied in detail, and the mechanisms of these reflect the multiple roles of the Notch pathway in both development and differentiation (Table 1). They include two developmental disorders, a cancer, and a degenerative disease. We will briefly discuss three of these disorders, and then focus in more detail on the syndrome spondylocostal dysplasia.

A subset of T-cell acute lymphoblastic leukaemias were originally associated with the *NOTCH1* gene by the discovery of three patients carrying a chromosomal translocation between chromosomes 7 and 9. Such translocation occurs by somatic mutation, and thus is not inherited. It results in the overexpression of truncated Notch1 polypeptides that lack most of the extracellular domain (ECD) and constitutively activate the Notch pathway (Ellisen *et al.*, 1991), thus acting through a gain-of-function mechanism. This disease highlighted the role of Notch signalling in lymphocyte development, and subsequent studies have shown that this pathway acts at three critical stages in this process (reviewed in Deftos and Bevan, 2000 and Anderson *et al.*, 2001). Initially lymphoid progenitor cells can differentiate towards either the B-cell or T-cell lineages. T-cell precursors can then either form $\alpha\beta$ or $\gamma\delta$ cells, and finally $\alpha\beta$ cells can form either helper T cells (CD4+) or cytolytic cells (CD8+). It is not clear how overexpression of the truncated Notch receptor causes the disease, but transgenic mice expressing the truncated Notch receptor have higher levels of CD8+ cells. Also in mice, similar translocations have frequently been observed in T cell tumours arising in $MMTV^{D}$/myc transgenic mice infected with murine leukaemia virus (MuLV), suggesting that overexpression of the constitutively active Notch1 receptor in mice causes the same effect as in humans, with the viral LTR acting as a strong promoter. In addition, *in vitro* transformation of T cells and fibroblasts has been achieved using overexpression of various truncated Notch1 proteins (Hoemann *et al.*, 2000; Anderson *et al.*, 2001).

Another example of a disease caused by mutation in a Notch receptor is provided by CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy, MIM125310). The key feature of this disorder is relapsing strokes affecting relatively young adults of both sexes, leading to a progres-

TABLE 1

HUMAN GENETIC DISEASES CAUSED BY MUTATION OF NOTCH PATHWAY COMPONENTS

Gene	Locus	Disease	MIM
NOTCH1	9q34.3	Acute T-cell lymphoblastic leukaemia	190198
NOTCH3	19p13.1-2	CADASIL	125310
JAG1	20p12.2	Alagille syndrome	118450
DLL3	19q13.1	SCDO1	277300

MIM details were derived from Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), www.ncbi.nlm.nih.gov/omim/.

sive dementia. The mutant gene is *NOTCH3*, which was isolated by a positional cloning approach after mapping of the disorder to chromosome 19 by linkage analysis in a large affected family (Joutel *et al.*, 1996). Analysis of a large number of *NOTCH3* mutations suggest that in 90% of cases this disease is caused by a missense mutation that changes the number of cysteine residues in a single EGF repeat, frequently in repeats 1-5. These protein domains contain six highly conserved cysteine residues that form disulphide bonds critical to the correct folding of the polypeptide. The mutations reported either delete or insert a single cysteine residue. It is not yet clear how mutation of *NOTCH3* causes CADASIL, however the underlying defect involves a deterioration of the vascular smooth muscle cells throughout the body. To date the mouse *Notch3* has not be targeted, and thus it is not known whether such a system would be a useful model for studying CADASIL.

The first disease shown to be caused by mutation in a Notch ligand was Alagille syndrome (AGS MIM118450). This is an autosomal dominantly inherited developmental disorder characterised by five key features, including neonatal jaundice due to intrahepatic cholestasis; posterior embryotoxon and retinal pigmentary changes in the eye; pulmonic valvular stenosis as well as peripheral arterial stenosis in the heart; abnormal ("butterfly") vertebrae; and characteristic facies with prominent forehead and chin, and deep-set eyes. Cytogenetic analysis of some patients revealed either deletion or translocation of chromosome 20p12, and the defective gene was identified as *JAGGED1* by positional cloning techniques (Li *et al.*, 1997; Oda *et al.*, 1997). 3-7% of all patients have a complete deletion of the *JAGGED1* gene. Of the remainder, some 233 intragenic mutations have been identified (reviewed in Spinner *et al.*, 2001). The majority of these lead to premature termination codons (72%), and the remainder are comprised of 13% missense mutations and 15% splice junction mutations. Because there is little if any phenotypic difference between AGS patients with total deletion of the *JAGGED1* gene and those with premature termination codons or missense mutations, it is generally assumed that AGS is due to haploinsufficiency. In mice, a *Jagged1* null mutant has been created by gene targeting (Xue *et al.*, 1999). Mice heterozygous for the null allele exhibit eye dysmorphology, but do not show any of the other phenotypic abnormalities associated with AGS in humans. Interestingly, homozygous nulls are embryonic lethal, with defects of the remodelling of yolk sac and embryonic vasculatures, especially apparent in the cranial vasculature. This underlines the importance of Notch signalling in the formation of the vasculature and may provide some hints to the vascular defect in CADASIL. There are two other mouse mutants of *Jagged1*. The *coloboma* line of mice has been shown to carry a large deletion of mouse chromosome 2 including the entire *Jagged1* locus (Xue *et al.*, 1999). The *slalom* allele was generated in an ENU mutagenesis screen, and has been shown to be a missense mutation that replaces a conserved proline residue with a serine in EGF repeat 2 (P269S; Tsai *et al.*, 2001). Like the targeted mouse line, neither of these mouse mutants replicates the AGS phenotype.

It is interesting to note that the reported mutations in all three diseases seem to be restricted to extra-cellular and transmembrane domains of the receptor and ligand. A possible explanation for this observation comes from studies in *Drosophila* where deletion of the intracellular domain (ICD) of either Delta or Serrate produces a dominant negative phenotype (Sun and Artavanis-Tsakonas, 1996). Thus a Notch receptor or ligand carrying such a mutation in the ICD might be expected to affect a wide range of Notch signalling processes, and thus severely impair many developmental processes.

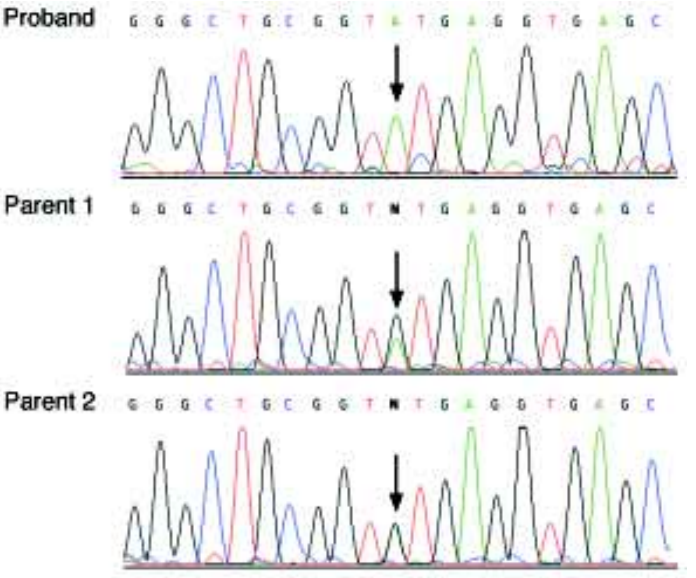

Fig. 2. A new mutation in DLL3 identified in an SCDO1 patient. *Electropherograms documenting the mutation are shown in the proband and both parents.*

Some Cases of Spondylocostal Dysplasia (SCD) are Caused by Mutation in the *DLL3* Gene

A second example of a human developmental disorder caused by mutation in a Notch ligand is provided by spondylocostal dysplasia (SCD, MIM277300). This is a group of vertebral malsegmentation syndromes with reduced stature resulting from axial skeletal defects. Previous studies have shown that some cases of autosomal recessive SCD are due to mutation of the *DLL3* gene (SCDO1, Bulman *et al.*, 2000). In order to study the function of *DLL3*, we have initiated a study to identify new mutations of *DLL3* in SCDO1, with the aim of using these in *in vitro* experiments to map the functional domains of the protein. To date we have analysed the *DLL3* gene of an Australian SCDO1 patient and their family, and have identified a new missense mutation (G926A; Fig. 2), resulting in the substitution of a tyrosine residue for cysteine 309 in EGF repeat 3. Structural predictions of the *DLL3* EGF repeat domain using the sequence threading algorithm (see Methods) suggest that this cysteine residue is involved

TABLE 2

POLYMORPHISMS IN THE *DLL3* CODING SEQUENCE

Exon	DLL3 nucleotide	Nucleotide change	Amino acid change	Protein region
3	318	GGG→GGC	G106	NT
5	425	CTG→CAG	L142Q	NT
5	515	TTC→TGC	F172C	NT
5	546	GCC→GCG	A182	DSL
6	653	CTG→CCG	L218P	EGF1
7	1029	CCC→CCT	P343	EGF4

The reference *DLL3* nucleotide sequence is derived from the genomic sequence of GenBank accession no. AC011500. Positions are based on the A of the ATG codon as nucleotide +1. NT, amino terminal region; DSL, Delta/Serrate/Lag-2 domain; EGF#, EGF repeat number #.

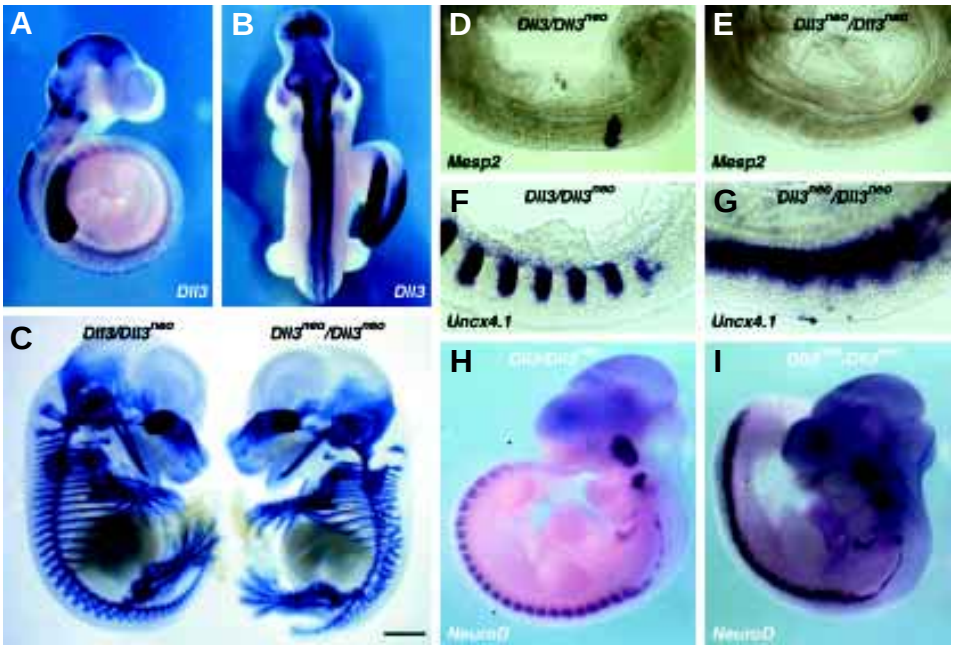

Fig. 3. *Dll3^{neo}/Dll3^{neo}* mutants have a defect in somitogenesis. *(A,B) Wholemount RNA* in situ *hybridisation showing* Dll3 *gene expression in 25-somite embryos viewed laterally* **(A)** *and dorsally* **(B)**. Dll3 *transcripts are detected in the mid- and hindbrain, the sensory cranial ganglia, the sympathetic chain, and along the length of the spinal cord. Expression is also detected in the dorsal root ganglia, the anterior margin of the nascent somites, the presomitic mesoderm and in the primitive streak/tailbud.* **(C)** *Lateral view of alcian blue stained embryos (14.5 dpc) with* Dll3/Dll3^{neo} *to the left and* Dll3^{neo}/Dll3^{neo} *to the right. (D,E) Lateral views of epithelial somites dissected from 9.5 dpc embryos following RNA wholemount* in situ *hybridisation with* Mesp2 *marking the site where the somite boundary forms.* **(D)** *Regularly sized and shaped somites are present in* Dll3/Dll3^{neo} *embryos. The distance between the band of* Mesp2 *expression and the last formed somite is equivalent to one somite width.* **(E)** *No epithelial somites are detectable in this* Dll3^{neo}/Dll3^{neo} *embryo immediately rostral to* Mesp2 *expression. Irregular somites are detected in the rostral PSM. (F,G) Anterioposterior identity is disrupted in* Dll3^{neo}/Dll3^{neo}. *Lateral views of epithelial somites dissected from 9.5 dpc embryos following RNA wholemount* in situ *hybridisation with* Uncx4.1. *Rostral to the left, caudal to the right.* **(F)** *Expression shows clear periodicity in* Dll3/Dll3^{neo} *embryos, and is restricted to the posterior of epithelial somites.* **(G)** Uncx4.1 *expression is reduced and periodicity is lost in* Dll3^{neo}/Dll3^{neo} *embryos. (H,I) Lateral views of 10.5 dpc embryos after RNA wholemount* in situ *hybridisation with* NeuroD Dll3/Dll3^{neo}**(H)** *and* Dll3^{neo}/Dll3^{neo} **(I)** *embryos.* NeuroD *expression is unaffected in the cranial ganglia of* Dll3^{neo}/Dll3^{neo} *embryos, but expression in dorsal root ganglia shows that these are unsegmented in* Dll3^{neo}/Dll3^{neo} *mutants. Scale bars: 400 μm (A,B); 1.35 mm (C); 400 μm (D,E); 200 μm (F,G); 700 μm (H,I).*

in a critical disulphide bond, and such a mutation would cause a major disruption of EGF repeat structure. As discussed above, this type of mutation in the EGF repeats of *NOTCH3* are responsible for CADASIL, and in addition similar mutations are known for *JAGGED1* in AGS. The only previously identified missense mutation in *DLL3* (G1154A) results in the substitution of glycine 385 (in EGF repeat 5) with an aspartic acid (Bulman *et al.*, 2000). Based on the threading analysis, it is unlikely that this substitution would affect the folding of the polypeptide chain, as it lies in a non-structural loop extending from the core of the EGF repeat. However, this residue is highly conserved between EGF repeats of several Notch ligand as well as across species, and may be essential for protein-protein interactions.

A number of single nucleotide polymorphisms (SNP) were identified in the course of this investigation (Table 2). Although some of these are silent mutations or introduce conservative amino acid substitutions, two of these cause relatively non-conservative amino acid substitutions. However these are not likely to have significant structural and functional consequences since such polymorphisms are present in both affected and unaffected individuals. We are currently sequencing 100 chromosomes from a control group of unaffected and unrelated individuals to provide a genetic baseline which will enable us to increase the confidence levels of distinguishing SCDO1-causative mutations from polymorphisms present in the general population.

Dll3 Null Mice Provide a Genetic Model for the Developmental Origins of SCDO1

In the mouse, *Dll3* is normally expressed in low levels in the epiblast (5.5-6 dpc), and later in higher levels in the primitive streak. By 8.0 dpc, *Dll3* is expressed robustly in the presomitic mesoderm (PSM), with lower transcript levels in the forming somite and detectable only in the anterior margin of nascent somites, and this persists throughout somitogenesis. *Dll3* is also expressed in a punctate pattern in the mid- and hindbrain and spinal cord of the ventricular layer, with prominent expression in the nasal pits, sensory ganglia, the sympathetic chain and dorsal root ganglia (Fig. 3 A,B; Dunwoodie *et al.*, 1997). To study the function of *Dll3*, we generated a loss of function mutation in the mouse *Dll3* gene using a gene targeting approach (Dunwoodie *et al.*, 2002). Homozygous null embryos (*Dll3^{neo}/Dll3^{neo}*) exhibit severe axial skeletal malformations consisting of highly disorganised vertebrae and costal defects, and appear to be a phenocopy of SCDO1 (Fig. 3C). Our analysis of these mutants shows that the developmental origins of the skeletal defects lie in delayed and irregular somite formation (Fig. 3 D,E). Analysis at the molecular level by RNA wholemount *in situ* hybridisation revealed a perturbation of anterioposterior somite polarity as detected by markers such as *Uncx4.1* (Fig. 3 F,G) and *mCer1* (Dunwoodie *et al.*, 2002). These aberrations are likely to be founded in the disruption of the segmentation clock which intrinsically oscillates within PSM (see below). A second mutant mouse line carrying a mutation in the *Dll3* gene is pudgy (*pu*), which was generated in an X-ray mutagenesis screen at the Oak Ridge National Laboratory, and has a four nucleotide deletion in exon 3, leading to a truncation prior to the DSL domain (Grüneberg, 1961; Kusumi *et al.*, 1998). The phenotype of homozygous *Dll3^{pu}* mice is very similar to those of our *Dll3^{neo}* null mice, and thus it is likely that they are functionally equivalent null alleles (Dunwoodie *et al.*, 2002).

Dll3 Null Mice have Central Nervous System Defects

Given the expression of *Dll3* in the nervous system (Dunwoodie *et al*., 1997; Kusumi *et al*., 2001), we also analysed the neural phenotype of *Dll3^{neo}/Dll3^{neo}* embryos in detail. Initially the neuroepithelium was analysed by examination of the marker genes *Hes5*, *Mash1* and *NeuroD*. In *Drosophila*, when Notch signalling is inactivated, *E(Spl)* expression is lost leading to an upregulation of genes of the *achaete-scute (ac-sc)* proneural class and consequently to an increase in the number of neuroblasts (Skeath and Carroll, 1992). Although no change in the Hairy-enhancer-of-split homologue *Hes5* expression was detected in *Dll3^{neo}/Dll3^{neo}* mutants, the expression of *Mash1* (an *ac-sc* homologue; Guillemot and Joyner, 1993) was examined in case *Mash1* expression was responsive to genes other than *Hes5*. However, no difference in expression was observed between *Dll3* wildtype and mutant embryos (data not shown). The expression of *NeuroD* was also examined since it is a marker of later neural development and marks differentiating neurons in trigeminal ganglia and dorsal root ganglia, each sites of *Dll3* expression (Lee *et al*., 1995; Dunwoodie *et al*., 1997). In *Dll3^{neo}/Dll3^{neo}* mutants, *NeuroD* expression was normal except in the dorsal root ganglia where strips of expression were evident either side of the neural tube replacing discrete blocks of *NeuroD* expression (Fig. 3 H,I). It is unlikely that this is due to an alteration in *NeuroD* expression *per se* but rather reflects the fact that dorsal root ganglia are unsegmented in *Dll3^{neo}/Dll3^{neo}* mutants as previously described (Dunwoodie *et al*., 2002).

Histological analysis from 11.5 dpc to adult stages revealed no differences in the neural tube of the trunk between *Dll3/Dll3^{neo}* and *Dll3^{neo}/Dll3^{neo}* embryos. However in the brain, histological analysis revealed defects in the neuroepithelium of *Dll3^{neo}/Dll3^{neo}* mutant embryos (4/7 between 11.5 and 16.5 dpc). Specifically, affected embryos appeared to have an enlarged roof to the fourth ventricle and a reduction or absence of the neuroepithelium (Fig. 4A). The affected neuroepithelium lay within the region of the rhombencephalon that gives rise to the medulla oblongata. These observations in *Dll3^{neo}* are consistent with the presence of ependymal malformations in the lateral ventricles of *Dll3^{pu}* homozygous mutants (Kusumi *et al*., 1998). In addition, the choroid plexus of the fourth ventricle was diminished in size or absent in 3/4 of these cases (Fig. 4 A-D). The choroid plexus of the hindbrain develops from pseudostratified epithelium in the roof of the fourth ventricle. This epithelium differentiates into a thin layer of columnar epithelium and this transition is marked by the onset of *transthyretin (TTR)* gene expression which occurs in the mouse at about 11.5 dpc (Thomas *et al*., 1988, and Fig. 4 E,F). It is possible that the defect in choroid plexus formation is primary, or it may be secondary to abnormalities in the rhombencephalic region. Since nothing was known about *Dll3* and the choroid plexus, *Dll3* expression was examined in association with *TTR* gene expression and choroid plexus formation between 11.5 and 14.5 dpc (Fig. 4 E,F and data not shown). At 11.5 dpc both *Dll3* and *TTR* were expressed in the roof of the fourth ventricle in overlapping or adjacent domains with the *TTR* expression domains (Fig. 4E). At 12.5 dpc, *Dll3* and *TTR* expression was still coincident, with the size of the expression domains appearing more equal (Fig. 4F). Between 12.5 and 14.5 dpc *Dll3* expression was not detected in the choroid plexus of the fourth ventricle which at this stage is now morphologically identifiable (data not shown). *Dll3* may be involved in the formation of the choroid plexus, but its function in the process is unclear at this stage. Additional sites of *Dll3* expression included the rhombic lip, olfactory bulb, the optic chiasma and ventrally in the cerebral hemispheres (Fig. 4G).

SCDO1 patients have not been reported with neurological defects or mental retardation, although there are some cases with

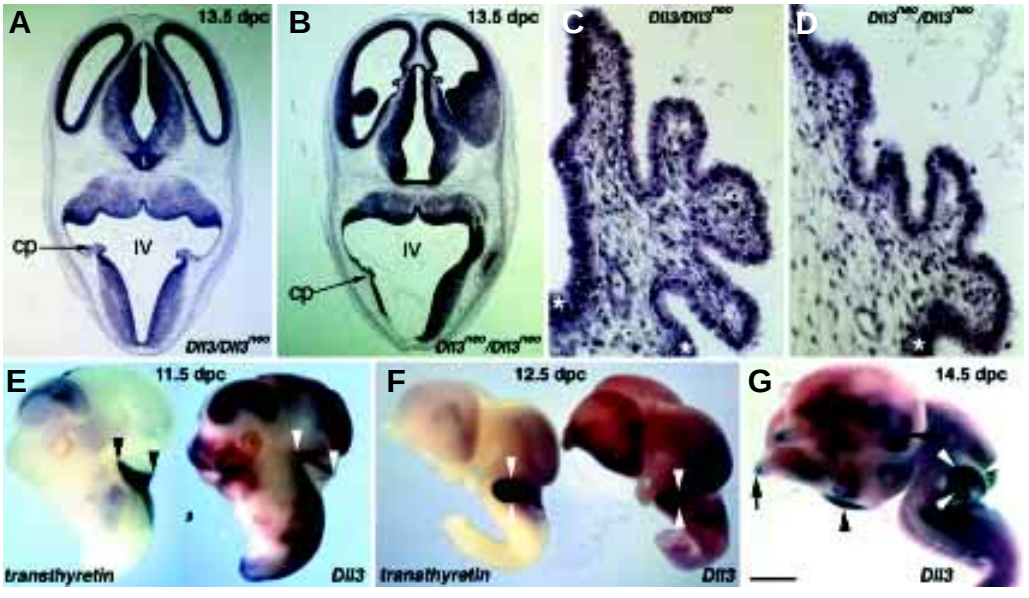

Fig. 4. Brain defects associated with the fourth ventricle are evident in some *Dll3neo/Dll3^{neo}* mutants. *(A-D) Haemotoxylin and eosin stained transverse sections (10 μm) show the developing brain at 13.5 dpc. In affected embryos, each of the sections was examined to ensure that the observed defects were not due to differences in the plane of section.* **(A)** Dll3/Dll3^{neo} *brains show even development of neuroepithelium surrounding the fourth ventricle (IV) and equivalent bilateral formation of the choroid plexus (cp).* **(B)** *The* Dll3^{neo}/Dll3^{neo} *brain shows uneven development of neuroepithelium surrounding the fourth ventricle and poor formation or absence of cp development.* **(C)** *Higher magnification of the cp taken from the embryo represented in (A).* **(D)** *Higher magnification of the cp taken from the embryo represented in (B). The white asterisks mark the proximal aspect of the cp. Note the broad proximal front and poor distal development of the cp in (D) compared to (C).* Transthyretin *and* Dll3 *expression in the developing brain using wholemount RNA* in situ *hybridisation (E-G). Lateral view of 11.5 dpc (E), 12.5 dpc (F) and 14.5 dpc (G) brains.* **(E)** Transthyretin *(left) and* Dll3 *(right) are expressed in the roof of the fourth ventricle, arrow heads define the dorsoventral extent of expression.* **(F)** Transthyretin *(left) and* Dll3 *(right) are expressed in the lateral recesses/ rhombic lip associated with the fourth ventricle; arrowheads mark the anteroposterior extent of expression.* **(G)** Dll3 *is expressed in the rhombic lip (white arrowheads), olfactory bulb (black arrow) and ventrally in the cerebral hemispheres (black arrowheads). Scale bars: 620 μm (A,B); 20 μm (C,D); 900 μm (E,F,G).*

craniofacial asymmetries (Turnpenny *et al.*, 1991; Kusumi *et al.*, 2001). The differences between the mouse model and SCDO1 may be explained in several ways. Firstly, in mice the neural defect is not completely penetrant and thus in SCDO1 neurological defects may be restricted to those patients with the most extreme phenotype. Since these patients occasionally die neonatally, then in those cases any neurological problems will most likely go unnoticed. Secondly, it is possible that slight neurological defects are present in older SCDO1 individuals, but are not superficially apparent. However, a more intensive investigation may uncover such defects, and thus our mouse model of SCDO1 may prove useful in uncovering further phenotypic indicators of SCDO1.

Somitogenesis and the Notch Signalling Pathway

The axial skeletal malformations of SCDO1 are caused by a defect in the developmental segmentation process known as somitogenesis (reviewed in Maroto and Pourquie, 2001). Somites are epithelial balls of tissue formed sequentially from the presomitic mesoderm (PSM) along the embryonic axis as it extends. Somites are formed at the rostral end of this unsegmented tissue in a regular repetitive fashion and subsequently differentiate into the axial skeleton, as well as cartilage, adaxial muscle and dermis. At the same time, at the caudal end of the PSM new cells are added continuously by cell division within the primitive streak and tail bud. Although the PSM has no obvious morphological segmentation, analysis of gene expression patterns within this tissue have suggested that a pre-pattern is established at an early stage. It is becoming clear that the underlying molecular mechanisms controlling the segmentation process are highly conserved between all vertebrate species, and that the Notch signalling pathway plays a central role in this process. The best explanation for the mechanism by which this occurs is the "clock and wavefront" model of Cooke and Zeeman (1976). This proposes that all the cells within the PSM oscillate between two internal states (the "clock"). At the same time, a slowly moving wavefront of competence progresses in a rostral to caudal direction through the PSM. As the wavefront passes a group of cycling cells, they stop oscillating and begin to differentiate. The interaction between the oscillation and the wavefront produces a temporal separation between adjacent groups of cells, allowing them to begin somite formation at different times in a regular metameric fashion. There is abundant evidence for the existence of the molecular clock from observations of "oscillatory" genes in the PSM of many vertebrate species from zebrafish to mouse. The first such observation was in the chicken with *chairy-1* (Palmeirim *et al.*, 1997). The number of such genes has rapidly grown and now includes *her1* (Sawada *et al.*, 2000), *Hes1* (Jouve *et al.*, 2000), *Hey2* (Leimeister *et al.*, 2000), *Lfng* (Forsberg *et al.*, 1998; McGrew *et al.*, 1998; Aulehla and Johnson, 1999), *Hes7* (Bessho *et al.*, 2001) and *Hox* genes (Zakany *et al.*, 2001). In all cases, these genes show regular waves of gene expression travelling from caudal to rostral in the PSM. Although these appear as waves, when viewed in isolation individual cells are turning gene expression on and off in a regular clock-like fashion. These genes are currently thought to be a read-out of the clock, rather than the clock itself. More recently, the wavefront has been suggested to be provided by *FGF8* expression within the PSM (Dubrulle *et al.*, 2001). Phenotypic analysis of mice which are mutant for Notch pathway genes, complemented with studies using ectopic expression of Notch pathway components in *Xenopus* and zebrafish, have suggested that the Notch signalling pathway is required to set up this metameric pattern at a number of levels, including border formation, anterior/posterior patterning of the nascent somites and perhaps even upstream of the clock mechanism itself (Dornseifer *et al.*, 1997; Jen *et al.*, 1997; Sparrow *et al.*, 1998; Jen *et al.*, 1999, and references in Table 3).

TABLE 3

NULL MUTANT MOUSE LINES WITH SOMITOGENESIS DEFECTS REPRESENT POTENTIAL CANDIDATE GENES FOR HUMAN SCD

Gene	Human	Reference chromosomal location	Short Tail
Notch pathway components			
NOTCH1	9q34.3	(Conlon *et al.*, 1995; Swiatek *et al.*, 1994)	Lethal by 11.5 dpc
DELTA-LIKE 1	6q27	(Hrabe de Angelis *et al.*, 1997)	Lethal by 12.0 dpc
RBPSUH	4p15.1	(Oka *et al.*, 1995)	Lethal by 10.5 dpc
LUNATIC FRINGE	7p22.2	(Evrard *et al.*, 1998; Zhang and Gridley, 1998)	√
PRESENILIN 1	14q24.2	(Shen *et al.*, 1997; Wong *et al.*, 1997)	√
Notch pathway targets			
HES7	17q13.1	(Bessho *et al.*, 2001)	√
MESP2	15q26.1	(Saga *et al.*, 1997)	√
Non-Notch pathway			
MESOGENIN	2p24.2	(Yoon and Wold, 2000)	√
PARAXIS	20p13	(Johnson *et al.*, 2001)	√
N-CADHERIN	18q12.1	(Radice *et al.*, 1997)	Lethal by 10.5 dpc
GCNF	9q33-q34.1	(Chung *et al.*, 2001)	Lethal by 10.5 dpc

Chromosomal locations were derived either from the publications cited or from searches of the Ensembl human genome server at the Wellcome Trust's Sanger Centre and the European Bioinformatics Institute (www.ensembl.org).

New SCD Candidate Genes

It is likely that mutation of other genes apart from *DLL3* are responsible for some cases of SCD since not all patients with autosomal recessive forms of SCD carry mutations in *DLL3*, and some cases of Jarcho-Levin syndrome/spondylcostal dysplasia have an autosomal dominant pattern of inheritance. The identification of such genes is important, and in the absence of extended pedigrees required for mapping studies and positional cloning, we are pursuing a candidate gene approach. Given the important role of the Notch signalling pathway in the process of somitogenesis, and the fact that the components of this system are very well characterised, these genes are a useful starting point to investigate the genetic origin of non-*DLL3* SCD cases. The phenotype of mouse lines carrying null mutations in various Notch pathway genes is an invaluable aid to selection of such candidates. Table 3 lists at least 7 different mouse lines containing null mutations in Notch pathway genes that show a similar defect in somitogenesis to the *Dll3/pudgy* mice. Note that mutation in *PSEN1* has been associated in humans with familial early onset Alzheimer disease, type 3 (AD, MIM104311). However this is due to a different function of this gene, distinct from its role in the Notch signalling pathway. Furthermore, in AD mutations in *PSEN1* are dominant, whereas in the *Psen1* targeted mouse line somitogenesis defects are only detected in homozygous null embryos. An additional source of candidate genes is provided by other mouse lines with similar somitogenesis defects. In these cases there is no obvious link with the Notch pathway, but these may represent genes further downstream of the primary Notch signalling events. Indeed, mutations

in such downstream genes may result in less severe segmentation defects and may be more likely to be present in spondylcostal dysplasia patients.

Other genes that cause somitogenesis defects in null mutant mice have been rejected, as they are already associated with other human genetic disease. These include *FGFR1* (Pfeiffer syndrome and myeloproliferative disorders; MIM 136350); *FOXC1* (Rieger anomaly, Axenfeld anomaly, and iris hypoplasia; MIM 601090); and *FOXC2* (lymphedema-distichiasis syndrome; MIM 602402). Another class of genes such as *Gdf11* (McPherron *et al*., 1999) show axial skeletal defects that are distinct from the fusions of SCDO1 and *Dll3* mice, and have also been excluded from consideration. It should be noted that not all genes associated with the Notch pathway in mammals show a somitogenesis phenotype in null mouse lines (e.g. *Hes1,* Ishibashi *et al*., 1995). In addition, mutant phenotypes for several downstream targets of Notch signalling in the PSM (for example *Hey1* and *HeyL*) have yet to be reported.

Another approach to identify further SCD candidate genes is to use a classical cytogenetic approach. To date, three SCD affected individuals have been reported with gross cytogenetic abnormalities (Crow *et al*., 1997; Dowton *et al*., 1997; Satar *et al*., 1997). These are respectively, a deletion of 18q22.2 –> qter; [fra(5)(q32)]; and monosomy 6q25->qter coupled with trisomy 15q11.1->pter. The last of these may be due to haploinsufficiency of *DLL1* (which is present at 6q27), although mice heterozygous for a null mutant of *Dll1* are phenotypically normal. The other cytogenetic abnormalities may provide clues to the location of other genes involved in SCD. Finally, a potential source of novel candidate genes may come from studies in zebrafish where large scale mutagenesis screens have isolated a group of five somitogenesis mutants (after eight, *aei*; fused somites, *fss*; deadly seven, *des*; beamter, *bea*; white tail; *wit;* van Eeden *et al*., 1996). Although *aei* is now known to be caused by mutation of the *deltaD* gene (Holley *et al*., 2000), a Notch ligand that has some similarity to mammalian *Dll3* in its expression pattern and effects on downstream targets of Notch signalling in null embryos (Durbin *et al*., 2000; Dunwoodie *et al*., 2002), the four remaining mutants are as yet unidentified and may represent novel genes involved in somitogenic processes.

If these candidate genes are not causative of spondylocostal dysplasia, they are likely to feature in other vertebral segmentation defects such as Klippel-Feil syndrome (MIM 148900) and others (Mortier *et al*., 1996).

The Mystery of the Missing Somites

As mentioned above, mutation in a number of genes in the mouse results in defective somitogenesis (Table 3). Where development proceeds to E14 and beyond, it is strikingly clear that not only is somite formation impaired, but there is also a loss of caudal tissue resulting in a very short tail. Typically in these cases the tail only has a few vertebrae, thus representing the lack of some 30 somites. This has, on the whole, escaped comment and no explanation has been proposed as to why caudal tissue is absent in these mutants. Primary trunk formation requires cells that have traversed the primitive streak and is responsible for generating the first 30 somites. Secondary trunk formation commences at E10, and in this case it is the tailbud that produces the caudal tissue necessary to produce the 35 somites required to make the entire tail. Cell labelling studies have provided evidence for a stem cell population in the tailbud (Tam and Tan, 1992; Nicolas *et al*., 1996; Wilson and Beddington, 1996), and this is the likely source of caudal tissue production required for the axial elongation of the embryo. Goldman *et al.* (2000) showed that the tailbud's ventral ectodermal ridge (VER) is required for the production of such caudal tissue. Ablation experiments showed that in its absence caudal tissue is not made, and consequently somites are not formed. This tissue is believed to act as a signalling centre, and exerts influence on the underlying population of stem cells. It is possible that in mutants where caudal tissue does not form, that the VER does not form or function properly. However, examination of the tailbuds of *Dll3* null embryos revealed that the VER was present (data not shown). Despite its presence, the VER may not be functional, or the underlying cells may be unable to respond to its influence. This can be further examined in mutants that lack caudal tissue by grafting mutant VER to wildtype tailbud mesenchyme and vice versa as performed by (Goldman *et al*., 2000). In *Xenopus*, Notch signalling is known to have a function in the tailbud as *XNotch1*, *XDelta1* and *XLfng* are expressed in specific tailbud tissues, and overexpression of a constitutively active Notch ICD results in tail outgrowth (Beck and Slack, 1999).

Conclusions

The diverse requirement for Notch signalling in many mammalian cell types is clear, and concomitant with these many roles, breakdown of this pathway is involved in several human disorders and is likely to be involved in many more. The conjunction of two powerful technologies, (targeted gene deletion and the human genome project) now make it possible for rapid progress to be made in understanding the basis of many human genetic diseases. We hope that this review underlines the fact that mouse models are useful for studying human developmental disorders, and such models may provide additional avenues for phenotypic analysis in affected individuals. In addition, the phenotypes of mutant mouse lines are invaluable in the selection of candidate genes for investigation in human genetic disorders for which the underlying cause is unknown.

Materials and Methods

Histology and Wholemount RNA In Situ *Hybridisation*

For histology, embryos were fixed in Bouin's fixative, dehydrated, embedded in paraffin wax, sectioned and stained with haematoxylin-eosin as described (Kaufman, 1992). Skeletal preparations were performed at 14.5 dpc according to (Jegalian and De Robertis, 1992). Wholemount RNA *in situ* hybridisation was performed as described (Harrison *et al*., 2000). *Dll3* (Dunwoodie *et al*., 1997) and *Mesp2* (Saga *et al*., 1997) probes were made as previously described. The *transthyretin* probe was synthesised from IMAGE clone (1078224) linearised with EcoRI and transcribed using T3 RNA polymerase.

Sequencing Primers and Method

Genomic DNA was amplified by PCR using Herculase proof-reading polymerase (Stratagene) and gel purified using QIAquick purification columns (Qiagen). Both strands were sequenced using a BigDye terminator Cycle Sequencing kit (PE Biosystems) according to the manufacturers instructions. Reactions were analysed on an ABI Prism 377 DNA Sequencer (PE Biosystems) at the UNSW DNA sequencing analysis facility. Primers used for amplification and sequencing were as follows:
exon 2: `TCCGCATTCATCCCTTTC` and `TGGGGCCGGGTAGCTGCCTG`

exon 3: CAGAGCCAGGTGGGAGGT and TTTCGCTGGCAGGGTTAG
exon 4: ACCCTAACCACTGTCTTTCATC and CTGTCAACACTCAGCACCAC
exon 5: ATGAGGGTGTTTTGGCCTC and CGAGTGACGCTCAGGAGG
exon 6: CCCAGATCTCCTCCCCAG and GAAGCAAGGTGGCTCAGG
exon 7: GGGACTTGAGACTGGACAAG and TGCCTAGAATAGGGCCTGG
exon 8: GACCGACCAGGGAAATGC, GCAGCGATGACAGAGCTG, TGCTACGCCCACTTCTCC and GCCGGAGGCAAAAGGTAGCG
exon 9: GCTGGTTTTGGGTTCCCC and AGAAGATGGCAGGTAGCTC

Threading

Structure predictions were done using the ProCeryon threading algorithm (ProCeryon Biosciences, release September 15, 1999 OR2.0) under the IRIX operating system on a SGI O2 workstation (Silicon Graphics Incorporated). The complete amino acid sequence of the human DLL3 protein (618 amino acids) was threaded against the library of selected unique folds (some 1300 structures) and analysed with the use of various scoring functions as described (Novotny *et al.*, 2001). The most conspicuous sequence/structure matches were found between residues 240-490 where several EGF repeat containing proteins aligned with very high scores. These were: human fibrillin; human blood factors VIIA and XA; Haementeria antistasin; wheat germ haemagglutinin; and mouse laminin. The alignment between the laminin structure (162 amino acids) and DLL3 residues 281-446 formed the basis of our analysis described in the text.

Acknowledgements

This work was supported by NHMRC Project Grant 142006 (SLD), the Clive and Vera Ramaciotti Foundation (SLD) and Core MRC Programme Support (RSPB). SLW is a Royal Society international fellow. K.K. is a Hitchings Elion Fellow of the Burroughs Wellcome Fund and supported by a Florence R.C. Murray Fellowship. We thank E. Grigorieva for excellent technical assistance. Consent was provided according to the guidelines of the NSW Genetics Service. The authors thank the family and clinicians who contributed to this study. We apologise to our colleagues whose contributions were not cited due to space constraints.

References

ANDERSON, A.C., ROBEY, E.A. and HUANG, Y.H. (2001). Notch signaling in lymphocyte development. *Curr. Opin. Genet. Dev.* 11: 554-560.

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.

AULEHLA, A. and JOHNSON, R.L. (1999). Dynamic expression of lunatic fringe suggests a link between notch signaling and an autonomous cellular oscillator driving somite segmentation. *Dev. Biol.* 207: 49-61.

BECK, C.W. and SLACK, J.M. (1999). A developmental pathway controlling outgrowth of the *Xenopus* tail bud. *Development* 126: 1611-1620.

BESSHO, Y., MIYOSHI, G., SAKATA, R. and KAGEYAMA, R. (2001). Hes7: a bHLH-type repressor gene regulated by Notch and expressed in the presomitic mesoderm. *Genes. Cells.* 6: 175-185.

BETTENHAUSEN, B., HRABE DE ANGELIS, M., SIMON, D., GUENET, J.L. and GOSSLER, A. (1995). Transient and restricted expression during mouse embryogenesis of Dll1, a murine gene closely related to *Drosophila* Delta. *Development* 121: 2407-2418.

BRAY, S. (1998). Notch signalling in *Drosophila*: three ways to use a pathway. *Semin. Cell. Dev. Biol.* 9: 591-597.

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.

CHUNG, A.C., KATZ, D., PEREIRA, F.A., JACKSON, K.J., DEMAYO, F.J., COONEY, A.J. and O'MALLEY, B.W. (2001). Loss of orphan receptor germ cell nuclear factor function results in ectopic development of the tail bud and a novel posterior truncation. *Mol. Cell. Biol.* 21: 663-677.

CONLON, R.A., REAUME, A.G. and ROSSANT, J. (1995). Notch1 is required for the coordinate segmentation of somites. *Development* 121: 1533-1545.

COOKE, J. and ZEEMAN, E.C. (1976). A clock and wavefront model for control of the number of repeated structures during animal morphogenesis. *J. Theor. Biol.* 58: 455-476.

CROW, Y.J., TOLMIE, J.L., RIPPARD, K., NAIRN, L., WILKINSON, A.G. and TURNER, T. (1997). Spondylocostal dysostosis associated with a 46, XX,+15,dic(6;15)(q25;q11.2) translocation. *Clin. Dysmorphol.* 6: 347-350.

DEFTOS, M.L. and BEVAN, M.J. (2000). Notch signaling in T cell development. *Curr. Opin. Immunol.* 12: 166-172.

DORNSEIFER, P., TAKKE, C. and CAMPOS-ORTEGA, J.A. (1997). Overexpression of a zebrafish homologue of the *Drosophila* neurogenic gene Delta perturbs differentiation of primary neurons and somite development. *Mech. Dev.* 63: 159-171.

DOWTON, S.B., HING, A. V., SHEEN-KANIECKI, V. and WATSON, M.S. (1997). Chromosome 18q22.2—>qter deletion and a congenital anomaly syndrome with multiple vertebral segmentation defects. *J. Med. Genet.* 34: 414-417.

DUBRULLE, J., MCGREW, M.J. and POURQUIE, O. (2001). FGF signaling controls somite boundary position and regulates segmentation clock control of spatiotemporal Hox gene activation. *Cell* 106: 219-232.

DUNWOODIE, S.L., CLEMENTS, M., SPARROW, D.B., SA, X., CONLON, R.A. and BEDDINGTON, R.S.P. Axial skeletal defects caused by mutation in the spondylocostal dysostosis/pudgy gene *Dll3* are associated with disruption of the segmentation clock within the presomitic mesoderm. *Development* in press.

DUNWOODIE, S.L., HENRIQUE, D., HARRISON, S.M. and BEDDINGTON, R.S. (1997). Mouse Dll3: a novel divergent Delta gene which may complement the function of other Delta homologues during early pattern formation in the mouse embryo. *Development* 124: 3065-3076.

DURBIN, L., SORDINO, P., BARRIOS, A., GERING, M., THISSE, C., THISSE, B., BRENNAN, C., GREEN, A., WILSON, S. and HOLDER, N. (2000). Anteroposterior patterning is required within segments for somite boundary formation in developing zebrafish. *Development* 127: 1703-1713.

ELLISEN, L.W., BIRD, J., WEST, D.C., SORENG, A.L., REYNOLDS, T.C., SMITH, S.D. and SKLAR, J. (1991). TAN-1, the human homolog of the *Drosophila* notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* 66: 649-661.

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.

FLEMING, R.J. (1998). Structural conservation of Notch receptors and ligands. *Semin. Cell. Dev. Biol.* 9: 599-607.

FORSBERG, H., CROZET, F. and BROWN, N.A. (1998). Waves of mouse Lunatic fringe expression, in four-hour cycles at two- hour intervals, precede somite boundary formation. *Curr. Biol.* 8: 1027-1030.

GOLDMAN, D.C., MARTIN, G.R. and TAM, P.P. (2000). Fate and function of the ventral ectodermal ridge during mouse tail development. *Development* 127: 2113-2123.

GRÜNEBERG, H. (1961). Genetical studies on the skeleton of the mouse: XXIX. *Genet. Res.* 2: 384-393.

GUILLEMOT, F. and JOYNER, A.L. (1993). Dynamic expression of the murine Achaete-Scute homologue Mash-1 in the developing nervous system. *Mech. Dev.* 42: 171-185.

HARRISON, S.M., HOUZELSTEIN, D., DUNWOODIE, S.L. and BEDDINGTON, R.S. (2000). Sp5, a new member of the Sp1 family, is dynamically expressed during development and genetically interacts with Brachyury. *Dev. Biol.* 227: 358-372.

HOEMANN, C.D., BEAULIEU, N., GIRARD, L., REBAI, N. and JOLICOEUR, P. (2000). Two distinct Notch1 mutant alleles are involved in the induction of T- cell leukemia in c-myc transgenic mice. *Mol. Cell. Biol.* 20: 3831-3842.

HOLLEY, S.A., GEISLER, R. and NUSSLEIN-VOLHARD, C. (2000). Control of her1 expression during zebrafish somitogenesis by a delta- dependent oscillator and an independent wave-front activity. *Genes Dev.* 14: 1678-1690.

HRABE DE ANGELIS, M., MCINTYRE, J., and GOSSLER, A. (1997). Maintenance of somite borders in mice requires the Delta homologue Dll1. *Nature* 386: 717-721.

IRVINE, K.D. and VOGT, T.F. (1997). Dorsal-ventral signaling in limb development. *Curr. Opin. Cell. Biol.* 9: 867-876.

ISHIBASHI, M., ANG, S. L., SHIOTA, K., NAKANISHI, S., KAGEYAMA, R. and GUILLEMOT, F. (1995). Targeted disruption of mammalian hairy and Enhancer of split homolog-1 (HES-1) leads to up-regulation of neural helix-loop-helix factors, premature neurogenesis, and severe neural tube defects. *Genes Dev.* 9: 3136-3148.

JEGALIAN, B.G. and DE ROBERTIS, E.M. (1992). Homeotic transformations in the mouse induced by overexpression of a human Hox3.3 transgene. *Cell* 71: 901-910.

JEN, W.C., GAWANTKA, V., POLLET, N., NIEHRS, C. and KINTNER, C. (1999). Periodic repression of Notch pathway genes governs the segmentation of *Xenopus* embryos. *Genes Dev.* 13: 1486-1499.

JEN, W. C., WETTSTEIN, D., TURNER, D., CHITNIS, A. and KINTNER, C. (1997). The Notch ligand, X-Delta-2, mediates segmentation of the paraxial mesoderm in *Xenopus* embryos. *Development* 124: 1169-1178.

JOHNSON, J., RHEE, J., PARSONS, S.M., BROWN, D., OLSON, E.N. and RAWLS, A. (2001). The anterior/posterior polarity of somites is disrupted in paraxis-deficient mice. *Dev. Biol.* 229: 176-187.

JOUTEL, A., CORPECHOT, C., DUCROS, A., VAHEDI, K., CHABRIAT, H., MOUTON, P., ALAMOWITCH, S., DOMENGA, V., CECILLION, M., MARECHAL, E. MACIAZEK, J., VAYSSIERE, C., CRUAUD, C., CABANIS, E. A., RUCHOUX, M.M., WEISSENBACH, J., BACH, J.F., BOUSSER, M.G. and TOURNIER-LASSERVE, E. (1996). Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature* 383: 707-710.

JOUVE, C., PALMEIRIM, I., HENRIQUE, D., BECKERS, J., GOSSLER, A., ISH-HOROWICZ, D. and POURQUIE, O. (2000). Notch signalling is required for cyclic expression of the hairy-like gene HES1 in the presomitic mesoderm. *Development* 127: 1421-1429.

KAUFMAN, M.H. (1992). *The Atlas of Mouse Development.* Academic Press, London.

KUSUMI, K., DUNWOODIE, S.L. and KRUMLAUF, R. (2001). Dynamic expression patterns of the pudgy/spondylocostal dysostosis gene Dll3 in the developing nervous system. *Mech. Dev.* 100: 141-144.

KUSUMI, K., SUN, E.S., 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.

LARDELLI, M., DAHLSTRAND, J. and LENDAHL, U. (1994). The novel Notch homologue mouse Notch 3 lacks specific epidermal growth factor-repeats and is expressed in proliferating neuroepithelium. *Mech. Dev.* 46: 123-136.

LEE, J.E., HOLLENBERG, S. M., SNIDER, L., TURNER, D.L., LIPNICK, N. and WEINTRAUB, H. (1995). Conversion of *Xenopus* ectoderm into neurons by NeuroD, a basic helix- loop-helix protein. *Science* 268: 836-844.

LEIMEISTER, C., DALE, K., FISCHER, A., KLAMT, B., HRABE DE ANGELIS, M., RADTKE, F., MCGREW, M.J., POURQUIE, O. and GESSLER, M. (2000). Oscillating expression of c-Hey2 in the presomitic mesoderm suggests that the segmentation clock may use combinatorial signaling through multiple interacting bHLH factors. *Dev. Biol.* 227: 91-103.

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. COSTA, T., PIERPONT, M.E., RAND, E.B., PICCOLI, D.A., HOOD, L. and SPINNER, N.B. (1997). Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat. Genet.* 16: 243-251.

LINDSELL, C.E., SHAWBER, C.J., BOULTER, J. and WEINMASTER, G. (1995). Jagged: a mammalian ligand that activates Notch1. *Cell* 80: 909-917.

MAROTO, M. and POURQUIE, O. (2001). A molecular clock involved in somite segmentation. *Curr. Top. Dev. Biol.* 51: 221-248.

MCGREW, M.J., DALE, J.K., FRABOULET, S. and POURQUIE, O. (1998). The lunatic fringe gene is a target of the molecular clock linked to somite segmentation in avian embryos. *Curr. Biol.* 8: 979-982.

MCPHERRON, A.C., LAWLER, A.M. and LEE, S.J. (1999). Regulation of anterior/posterior patterning of the axial skeleton by growth/differentiation factor 11. *Nat. Genet.* 22: 260-264.

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.

MUMM, J.S. and KOPAN, R. (2000). Notch signaling: from the outside in. *Dev. Biol.* 228: 151-165.

NICOLAS, J.F., MATHIS, L., BONNEROT, C. and SAURIN, W. (1996). Evidence in the mouse for self-renewing stem cells in the formation of a segmented longitudinal structure, the myotome. *Development* 122: 2933-2946.

NOVOTNY, J., RIGOUTSOS, I., COLEMAN, D. and SHENK, T. (2001). *In silico* structural and functional analysis of the human cytomegalovirus (HHV5) genome. *J. Mol. Biol.* 310: 1151-1166.

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. and CHANDRASEKHARAPPA, S.C. (1997). Mutations in the human Jagged1 gene are responsible for Alagille syndrome. *Nat. Genet.* 16: 235-242.

OKA, C., NAKANO, T., WAKEHAM, A., DE LA POMPA, J.L., MORI, C., SAKAI, T., OKAZAKI, S., KAWAICHI, M., SHIOTA, K., MAK, T.W. and HONJO, T. (1995). Disruption of the mouse RBP-J kappa gene results in early embryonic death. *Development* 121: 3291-3301.

PALMEIRIM, I., HENRIQUE, D., ISH-HOROWICZ, D. and POURQUIE, O. (1997). Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. *Cell* 91: 639-648.

PANIN, V.M. and IRVINE, K.D. (1998). Modulators of Notch signaling. *Semin. Cell. Dev. Biol.* 9: 609-617.

RADICE, G.L., RAYBURN, H., MATSUNAMI, H., KNUDSEN, K.A., TAKEICHI, M. and HYNES, R. O. (1997). Developmental defects in mouse embryos lacking N-cadherin. *Dev. Biol.* 181: 64-78.

SAGA, Y., HATA, N., KOSEKI, H. and TAKETO, M.M. (1997). Mesp2: a novel mouse gene expressed in the presegmented mesoderm and essential for segmentation initiation. *Genes Dev.* 11: 1827-1839.

SATAR, M., TEMOCIN, A.K., ATICI, A. and DEMIRHAN, O. (1997). A case of spondylocostal dysostosis with a fra (5) (q32). *Turk. J. Pediatr.* 39: 547-549.

SAWADA, A., FRITZ, A., JIANG, Y., YAMAMOTO, A., YAMASU, K., KUROIWA, A., SAGA, Y. and TAKEDA, H. (2000). Zebrafish Mesp family genes, mesp-a and mesp-b are segmentally expressed in the presomitic mesoderm, and Mesp-b confers the anterior identity to the developing somites. *Development* 127: 1691-1702.

SHAWBER, C., BOULTER, J., LINDSELL, C.E. and WEINMASTER, G. (1996). Jagged2: a serrate-like gene expressed during rat embryogenesis. *Dev. Biol.* 180: 370-376.

SHEN, J., BRONSON, R.T., CHEN, D.F., XIA, W., SELKOE, D.J. and TONEGAWA, S. (1997). Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell* 89: 629-639.

SHUTTER, J.R., SCULLY, S., FAN, W., RICHARDS, W.G., KITAJEWSKI, J., DEBLANDRE, G. A., KINTNER, C.R. and STARK, K.L. (2000). Dll4, a novel Notch ligand expressed in arterial endothelium. *Genes Dev.* 14: 1313-1318.

SKEATH, J.B. and CARROLL, S.B. (1992). Regulation of proneural gene expression and cell fate during neuroblast segregation in the *Drosophila* embryo. *Development* 114: 939-946.

SPARROW, D.B., JEN, W.C., KOTECHA, S., TOWERS, N., KINTNER, C. and MOHUN, T.J. (1998). Thylacine 1 is expressed segmentally within the paraxial mesoderm of the *Xenopus* embryo and interacts with the Notch pathway. *Development* 125: 2041-2051.

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.

SUN, X. and ARTAVANIS-TSAKONAS, S. (1996). The intracellular deletions of Delta and Serrate define dominant negative forms of the *Drosophila* Notch ligands. *Development* 122: 2465-2474.

SWIATEK, P.J., LINDSELL, C.E., DEL AMO, F.F., WEINMASTER, G. and GRIDLEY, T. (1994). Notch1 is essential for postimplantation development in mice. *Genes Dev.* 8: 707-719.

TAM, P.P. AND TAN, S.S. (1992). The somitogenetic potential of cells in the primitive streak and the tail bud of the organogenesis-stage mouse embryo. *Development* 115: 703-715.

THOMAS, T., POWER, B., HUDSON, P., SCHREIBER, G. and DZIADEK, M. (1988). The expression of transthyretin mRNA in the developing rat brain. *Dev. Biol.* 128: 415-427.

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. and STEEL, K.P. (2001). The mouse slalom mutant demonstrates a role for Jagged1 in neuroepithelial patterning in the organ of Corti. *Hum. Mol. Genet.* 10: 507-512.

TURNPENNY, P.D., THWAITES, R.J. and BOULOS, F.N. (1991). Evidence for variable gene expression in a large inbred kindred with autosomal recessive spondylocostal dysostosis. *J. Med. Genet.* 28: 27-33.

UYTTENDAELE, H., MARAZZI, G., WU, G., YAN, Q., SASSOON, D. and KITAJEWSKI, J. (1996). Notch4/int-3, a mammary proto-oncogene, is an endothelial cell-specific mammalian Notch gene. *Development* 122: 2251-2259.

VAN EEDEN, F.J., GRANATO, M., SCHACH, U., BRAND, M., FURUTANI-SEIKI, M., HAFFTER, P., HAMMERSCHMIDT, M., HEISENBERG, C.P., JIANG, Y.J., KANE, D.A. KELSH, R.N., MULLINS, M.C., ODENTHAL, J., WARGA, R.M., ALLENDE, M.L., WEINBERG, E.S. and NUSSLEIN-VOLHARD, C. (1996). Mutations affecting somite formation and patterning in the zebrafish, Danio rerio. *Development* 123: 153-164.

WEINMASTER, G., ROBERTS, V.J. and LEMKE, G. (1991). A homolog of *Drosophila* Notch expressed during mammalian development. *Development* 113: 199-205.

WEINMASTER, G., ROBERTS, V.J. and LEMKE, G. (1992). Notch2: a second mammalian Notch gene. *Development* 116: 931-941.

WILSON, V. and BEDDINGTON, R.S. (1996). Cell fate and morphogenetic movement in the late mouse primitive streak. *Mech. Dev.* 55: 79-89.

WONG, P.C., ZHENG, H., CHEN, H., BECHER, M. W., SIRINATHSINGHJI, D.J., TRUMBAUER, M.E., CHEN, H.Y., PRICE, D.L., VAN DER PLOEG, L.H. and SISODIA, S.S. (1997). Presenilin 1 is required for Notch1 and DII1 expression in the paraxial mesoderm. *Nature* 387: 288-292.

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.

YOON, J.K. and WOLD, B. (2000). The bHLH regulator pMesogenin1 is required for maturation and segmentation of paraxial mesoderm. *Genes Dev.* 14: 3204-3214.

ZAKANY, J., KMITA, M., ALARCON, P., DE LA POMPA, J.L. and DUBOULE, D. (2001). Localized and transient transcription of Hox genes suggests a link between patterning and the segmentation clock. *Cell* 106: 207-217.

ZHANG, N. and GRIDLEY, T. (1998). Defects in somite formation in lunatic fringe-deficient mice. *Nature* 394: 374-377.