

CHROMOSOMAL MICRODELETIONS: DISSECTING *DEL22Q11* SYNDROME

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Identifying the genes that underlie the pathogenesis of chromosome deletion and duplication syndromes is a challenge because the affected chromosomal segment can contain many genes. The identification of genes that are relevant to these disorders often requires the analysis of individuals that carry rare, small deletions, translocations or single-gene mutations. Research into the chromosome 22 deletion (*del22q11*) syndrome, which encompasses DiGeorge and velocardiofacial syndrome, has taken a different path in recent years, using mouse models to circumvent the paucity of informative human material. These mouse models have provided new insights into the pathogenesis of *del22q11* syndrome and have established strategies for research into chromosomal-deletion and -duplication syndromes.

HAPLOINSUFFICIENCY

When loss of function of one gene copy leads to an abnormal phenotype.

SEGMENTAL ANEUSOMY

Disorder that results from the inappropriate dosage of crucial genes in a genomic segment.

Chromosome rearrangements are a notable cause of embryonic lethality and birth defects. In 1998, Brewer and colleagues¹ identified 283 chromosomal bands, the deletion of which was associated with malformations, indicating that HAPLOINSUFFICIENT loci are not rare in the human genome. Some chromosomal trisomy disorders, such as **Down syndrome**, can involve hundreds of genes, only a fraction of which will prove to be crucial for the pathogenesis of the disorder — presumably those for which proper function requires exact gene dosage.

The SEGMENTAL ANEUSOMY syndromes, which are caused by chromosomal deletions and duplications, or by imprinting defects that involve a chromosome segment², are similarly caused by inappropriate gene dosage. Microdeletion syndromes are a subset of the segmental aneusomy syndromes that are not visible by standard cytogenetic analysis, although some can be seen using high-resolution cytogenetic approaches. Although more tractable than the larger chromosomal rearrangements, microdeletions commonly encompass 10–30 genes, and identifying the vital genes has proved to be a surprisingly difficult task.

Single-gene mutations are responsible for several microdeletion syndromes, namely **Alagille syndrome**^{3,4}, **Angelman syndrome**^{5,6} and **Rubinstein–Taybi syndrome**⁶, whereas mutations in two genes, *PAX6* and

WT1, are responsible for the main phenotypic features of another microdeletion syndrome, Wilms tumour–aniridia–genitourinary anomalies–mental retardation (**WAGR**) syndrome. There is also evidence that more than one gene is responsible for both **Williams syndrome**^{7–9} and **Langer–Giedion syndrome**¹⁰. For other microdeletion syndromes, it remains to be determined whether they are single- or multi-gene disorders, and which genes are responsible for the various components of their clinical phenotypes.

Patients with unusual deletions can help to identify the key genes that are involved in a particular syndrome. However, these patients are often very rare, especially for those syndromes in which deletion size is ‘set’ by the presence of repetitive sequence elements, as discussed in more detail below. Instead, creating chromosomal rearrangements that include or exclude given sets of genes in an experimental model is another way to resolve the genetics of these genomic disorders. This has been the recent strategy of several groups, whose research focuses on unravelling the molecular genetics of the chromosome 22 deletion (*del22q11*) syndrome.

Its occurrence in an estimated 1 in 4,000 live births¹¹ makes *del22q11* syndrome the most common microdeletion syndrome. The syndrome comprises DiGeorge syndrome (**DGS**), velocardiofacial syndrome (**VCFS**) and **conotruncal anomaly face syndrome**. These

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Table 1 | Common clinical features of *del22q11* syndrome

Clinical features	Developmental defect (known or presumed)
<p>Congenital cardiovascular defects: Tetralogy of Fallot[*], interrupted aortic arch type B, persistent truncus arteriosus[†], right aortic arch[‡], aberrant right subclavian artery, ventricular septal defects, overriding aorta, transposition of the great arteries, aortic valve stenosis, coarctation of the aorta[¶].</p>	Abnormal development of the cardiac outflow tract and of pharyngeal arch artery IV.
<p>Hypocalcaemia — low serum calcium levels might cause seizures</p>	Lack of parathyroid hormone due to impaired/failed development of the parathyroid glands (derived from pharyngeal pouch III).
<p>Recurrent ear and respiratory infections</p>	Lack of, or impaired function of, T cells due to impaired development of the thymus gland (derived from pharyngeal pouch III). This feature might be secondary to craniofacial defects that predispose to ear infection.
<p>Craniofacial anomalies: Receding or abnormally small jaw, widely spaced eyes, broad nasal root, midface hypoplasia, cleft palate (overt or submucosal), external ear anomalies.</p>	Impaired development of structures derived from pharyngeal arches I and II.
<p>Behavioural defects: Deficits of learning and memory, motor development, speech and language development, attention-deficit disorder.</p>	Unknown
<p>Psychiatric disorders: Schizophrenia, schizoaffective disorder, bipolar disorder.</p>	Unknown

*A complex heart defect that comprises ventricular septal defect, overriding aorta, right ventricular hyperplasia and right ventricular outflow stenosis or atresia. †When a single vessel exits the heart instead of two. ‡Loops to the right as a mirror image of the normal left loop (see FIG. 3). ¶Congenital narrowing of the descending aorta, usually close to the ductus arteriosus.

three clinical entities are united by a common microdeletion (*del22q11*) in the proximal long arm of chromosome 22 (REFS 12–15). We refer to the phenotype caused by the microdeletion as *del22q11* syndrome, to distinguish it from those cases with a DGS-like phenotype that are caused by certain *in utero* insults, such as fetal exposure to excessive levels of alcohol, retinoic acid or maternal diabetes, or by chromosome 10p deletions. Affected individuals carry the deletion on only one of the chromosomes 22, so it is presumed to be a gene-haploinsufficiency syndrome. In most cases, the deletion occurs *de novo*, but in about 10% of cases it is inherited from a mildly affected parent¹⁶, thus behaving as an autosomal-dominant trait. The deletion is estimated to encompass ~30 genes, but it is not known which of these genes (or gene) are responsible for the clinical features of the disorder.

Recent research into *del22q11* syndrome has been aided by the availability of the human and mouse genomic sequences and by the novel application of established chromosome-engineering techniques. It has focused on two main areas: the mechanics of the deletion, and the use of mouse mutants to study the biology of the human syndrome. In this review, I discuss recent research that has revealed the chromosomal basis of the human deletion and that has, through the use of mouse models, identified a new disease gene that is essential for pharyngeal development. I also consider the potential applicability of generating precisely targeted deletions in the mouse to identify crucial genes involved in other diseases that are caused by chromosomal rearrangements.

***Del22q11* syndrome — a complex disorder?**

The symptoms of *del22q11* syndrome are many and diverse (TABLE 1). Each symptom might or might not be

present in an affected individual and, if present, symptoms can occur with varying degrees of severity. So, distinct clinical features of *del22q11* syndrome can show variable EXPRESSIVITY and incomplete PENETRANCE. Nevertheless, there are some key clinical traits that can be classified according to whether they affect pharyngeal or neurobehavioural development.

The 'pharyngeal' phenotype encompasses the most characteristic features of *del22q11* syndrome, namely congenital cardiovascular defects, craniofacial anomalies and aplasia or hypoplasia of the thymus and parathyroid glands. These features are thought to arise owing to the abnormal development of the pharyngeal apparatus (BOX 1) during early fetal development. The 'neurobehavioural' phenotype manifests in early childhood as learning difficulties, cognitive deficits and attention-deficit disorder. In adolescence and adulthood, some patients develop various psychiatric disorders, mainly schizophrenia, SCHIZOAFFECTIVE DISORDER and BIPOLAR DISORDER. The basis of the neurobehavioural phenotype is unknown. All *del22q11*-syndrome patients manifest at least some components of the pharyngeal and neurobehavioural phenotypes with varying degrees of severity. In addition, some patients have various other symptoms, such as growth delay, minor skeletal defects and renal defects^{17,18}.

The mechanics of the 22q11 deletion

In contrast to the clinical heterogeneity of this syndrome, the *del22q11* genetic lesion is remarkably homogeneous in affected individuals, with only a handful of exceptions. Approximately 90% of patients have a typically deleted region (TDR) of ~3 Mb (REFS 19–22), which encompasses an estimated 30 genes (FIG. 1 and TABLE 2), whereas about 8% of patients have a smaller, nested deletion of ~1.5 Mb (REFS 19–22), which encompasses 24

EXPRESSIVITY

The extent to which a particular organ or structure is affected by a particular genotype. *Del22q11* syndrome is characterized by variable expressivity because apparently identical deletions can result in mild or severe disease.

PENETRANCE

The proportion of affected individuals among the carriers of a particular genotype. If all individuals with a disease genotype show the disease phenotype, then the disease is said to be completely penetrant.

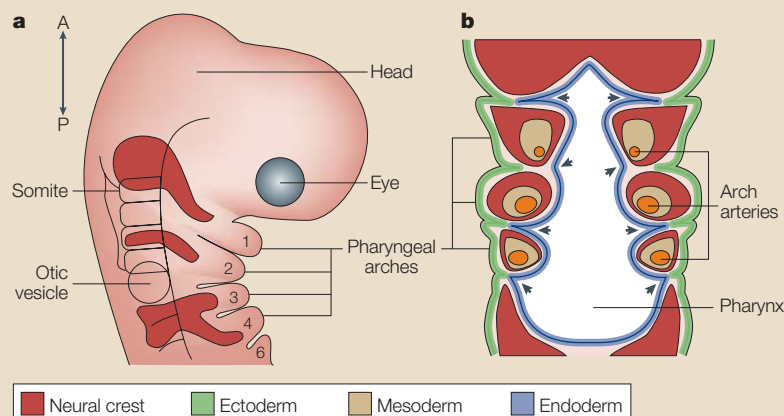
SCHIZOAFFECTIVE DISORDER

A psychotic illness that comprises both schizophrenia and affective (mood) disorder.

BIPOLAR DISORDER

A mood disorder that is characterized by periodic swings between exaggerated elation and depression.

Box 1 | **Pharyngeal development**



The PHARYNGEAL ARCH and PHARYNGEAL POUCH complex (or pharyngeal apparatus) is a vertebrate-specific system that develops as a series of bulges on the lateral aspect of the head (panel a). All three embryonic tissue layers (ectoderm, mesoderm and endoderm) contribute to the pharyngeal apparatus, each giving rise to distinct tissues, as shown in the frontal section in panel b. Each pharyngeal arch has an outer ectodermal layer (green), which gives rise to the epidermis and to the neuronal tissues of arch-associated ganglia. There is an inner endodermal layer (blue), which forms the epithelial lining of the pharynx. The pharyngeal endoderm ‘outpouches’ in the direction of the ectoderm, forming a series of pharyngeal pouches (arrowheads) that contain the endocrine tissues of the pharyngeal glands (thymus, thyroid and parathyroid). Between the ectoderm and endoderm lies a core of ectomesenchymal neural crest (red), which forms skeletal and connective tissue, and mesodermal cells (yellow), which form the pharyngeal musculature and the endothelial cells that surround the arch arteries. Each pharyngeal arch surrounds an arch artery that connects the heart, by means of the aortic sac, to the dorsal aortae. Although the pharyngeal arches all have the same basic plan, they also have separate identities according to their anteroposterior position. For example, in all vertebrates, the most anterior arch forms the jaw and parts of the middle ear; the second arch forms the hyoid apparatus and part of the middle ear; and the posterior arches form the throat cartilages. In *del22q11* syndrome patients, structures that derive from all three components of the pharyngeal apparatus are affected. (A, anterior; P, posterior). (Modified with permission from REF. 68)

PHARYNGEAL ARCHES
The tissue that lies between the paired pharyngeal pouches.

PHARYNGEAL POUCHES
Paired embryonic structures formed by the folding of the endodermal lining of the primitive foregut.

LOW-COPY REPEATS
1–200-kb blocks of genomic sequence that are duplicated in one or more locations on a chromosome, and thought to be of recent evolutionary origin because they have very high sequence identity and are absent in closely related species.

genes. The lack of variety in the extent of deletion has been attributed to the presence of intrachromosomal LOW-COPY REPEATS (LCRs), also referred to as segmental duplications, which flank the TDR and the 1.5-Mb deletion^{21,23–25}. It has been proposed that LCRs might confer instability to the region by mediating aberrant homologous recombination and unequal crossing-over events during meiosis due to high sequence similarity between the repeat segments, thereby generating chromosomal rearrangements of a uniform and predictable size^{22–25}.

A total of eight LCRs have been identified in the 22q11 region (LCR22s)^{26–28} (FIG. 1). The LCR22s comprise blocks of genomic sequence that contain genes, pseudogenes and gene fragments, each of which shares up to 99% similarity with the equivalent sequence in another repeat, although each LCR22 differs in its overall size and organization^{22,27}. The LCR22s have been implicated not only in deletions, but also in segmental aneusomies that result from chromosomal duplications — namely, *cat eye syndrome*^{27,29}, *Der(22) syndrome*^{30,31},

and duplication of the *del22q11* syndrome TDR²⁷ — and in two somatic rearrangements that are associated with cancer^{32,33}. LCRs are also associated with several segmental aneusomy syndromes that involve other chromosomes, including Williams syndrome^{34,35}, *Smith–Magenis syndrome*³⁶, 17p11.2 duplication syndrome³⁷, *Prader–Willi syndrome*^{38,39}, *Charcot–Marie–Tooth disease, type 1A* and hereditary neuropathy with liability to pressure palsies (*HNPP*)⁴⁰, and it is likely that similar mechanisms generate these specific chromosomal rearrangements. In the future, other genetic disorders will probably be found to be associated with LCRs, as analysis of the human genome sequence indicates that >3% of the human genome comprises intrachromosomal segmental duplications, most of which are more than 10 kb in length^{41,42}.

Patients with *del22q11* syndrome who have either the 3-Mb or 1.5-Mb deletion, and a patient with a balanced chromosome translocation breakpoint in the region (ADU in FIG. 1)⁴³, all have similar phenotypes. Therefore, it has not been possible to correlate deletion size with phenotype to narrow down the region for gene-identification purposes. Furthermore, the rare patients that have different deletions or rearrangements in the region^{43–48} have not been helpful in localizing disease genes because some of these rearrangements are non-overlapping (FIG. 1). The presence of the characteristic phenotype in patients with non-overlapping deletions considerably complicates gene identification by traditional positional-cloning strategies because, by definition, no unique crucial region harbouring the disease gene can be identified. It was this unpromising situation that spurred investigators to turn to the mouse, with the goal of modelling the disease in an animal that was amenable to genetic manipulation.

Studying *del22q11* syndrome in the mouse

Initial approaches to modelling *del22q11* syndrome in the mouse boded well, as the gene content of the human 22q11 region was found to be highly conserved in a region of mouse chromosome 16 (REFS 49–53) (FIG. 2). Using the then recently developed chromosome-engineering techniques (BOX 2), three research groups generated deletions in mice that encompassed subsets of the genes deleted in patients with *del22q11* syndrome^{54–56}. We generated the first such mouse deletion, named *Df1* (REF. 54), which encompassed mouse homologues of 18 out of the 24 genes that are deleted in patients with the 1.5-Mb deletion (FIG. 2). Mice carrying one copy of the deleted chromosome (*Df1/+*) had cardiovascular defects similar to those seen in human patients (FIG. 3, TABLE 1). Furthermore, the cardiovascular defects could be corrected in mice that were bred to carry the *Df1* deletion on one chromosome 16, and a reciprocal duplication (*Dp1*) on the other, which restored normal gene dosage. This genetic-rescue experiment showed that a gene(s) within the *Df1* region is involved in heart development and is haploinsufficient in these mutant mice. Two other mouse deletions were subsequently reported (FIG. 2) that partially overlapped with the *Df1* deletion: the first deletion encompassed 7 genes⁵⁵, and

Table 2 | Genes encompassed by *del22q11*

Gene (synonym)	Gene product	Product function	References
<i>DGCR6</i>	γ -laminin 1-like protein	Putative adhesion protein	98
<i>PRODH</i>	Proline dehydrogenase	Enzyme involved in proline catabolism	99
<i>DGCR2 (IDD/LAN)</i>	Integral membrane protein; C-type lectin	Putative adhesion receptor	100,101
<i>TSK (STK)</i>	Serine/threonine kinase	Signal transduction	49
<i>DGSI (ES2eI)</i>	Nuclear protein	Essential for early embryonic development	49,102
<i>GSCL</i>	Goosecoid-like protein	Putative transcription factor	103,104
<i>SLC25A1 (CTP)</i>	Citrate transporter protein	Catalyses citrate transport across the inner mitochondrial membrane	105
<i>CLTCL1* (CLTD)</i>	Clathrin heavy chain-like protein	Vesicle-mediated intracellular transport	49,106
<i>HIRA (DGCR1)</i>	Protein with WD40 domains	Putative transcriptional regulator	107
<i>NLVCF</i>	Nuclear protein	Unknown	108
<i>UFD1L</i>	Ubiquitin degradation 1-like protein	Putative role in ubiquitin-dependent protein degradation	109
<i>CDC45L</i>	Cell-cycle initiator protein	Putative role in initiation of DNA replication	110
<i>CLDN5 (TMVCF)</i>	Claudin 5	Component of tight junctions	111
<i>PNUTL1 (CDCREL1)</i>	Septin-like protein	Putative role in cytokinesis	112
<i>GP1BB</i>	Platelet glycoprotein β -subunit	Component of transmembrane protein	113
<i>TBX1</i>	T-box 1	Putative transcription factor	61
<i>GNB1L (WDR14)</i>	G protein β -subunit	Component of signal-transducing G proteins	114
<i>TR (TRXR2)</i>	Thioredoxin reductase	Regulation of the redox protein thioredoxin	115
<i>COMT</i>	Catechol-O-methyltransferase	Catecholamine metabolism	116,117
<i>ARVCF</i>	Catenin-like protein	Putative role in adherens junctions	111
<i>T10</i>	Serine/threonine-rich protein	Unknown	118
<i>RANBP1</i>	RAN-binding protein 1	Intracellular transport	52
<i>ZNF74</i>	Protein with 12 zinc-finger domains	Putative transcription regulator	119
<i>CRKL</i>	Adaptor protein with SH2/SH3/SH3 domains	Protein binding	26
<i>LZTR1 (TCFL2)</i>	Putative DNA-binding protein	Putative transcription factor	120

*Mouse homologue not identified, SH2, Src-homology domain 2; SH3, Src-homology domain 3.

the second encompassed 12 genes⁵⁶. Mice that were heterozygous for either deletion had normal heart development, indicating that the gene responsible for the cardiovascular defects in *Df1/+* mice lay in the interval between the genes *Arvcf* (armadillo repeat gene deleted in velocardiofacial syndrome) and *Ufd1l* (ubiquitin fusion degradation 1 (Ufd1)-like), which contains eight genes (FIG. 2). Within that interval, mice with null mutations in two genes, catechol-O-methyltransferase (*Comt*)⁵⁷ and *Pnutl1* (also known as *Cdcrel1*)⁵⁸, had been reported, but neither had cardiovascular defects.

To ascertain which of the remaining six genes in the critical region was responsible for the cardiovascular phenotype, two of the groups used a combination of genetically engineered nested deletions and duplications (BOX 2), transgenesis and single-gene targeting^{58,59}. In key experiments, mice carrying transgenes that encompassed large genomic fragments from the critical region were bred with mice heterozygous for the *Df1* (REF. 58) or the *Lgde*^{F9} deletions (FIG. 2). Both groups identified a genomic DNA fragment that contained four genes — *Gnb1l* (guanine-nucleotide-binding protein (G protein) β -polypeptide 1-like), *Tbx1* (T-box 1),

Gp1bb (glycoprotein Ib, β -polypeptide) and *Pnutl1* — which corrected the cardiovascular defects when introduced into the *Df1* (REF. 58) or the *Lgde*^{F9} deletion mice. This indicated that at least one of the genes present in this genomic fragment was responsible for the cardiovascular phenotype. In the case of *Df1/+* mice, the cardiovascular defects were completely rescued by the transgene, whereas for *Lgde*^{F9/+} mice, the rescue was partial. This discrepancy might relate to the fact that the transgene that partially rescued the *Lgde*^{F9/+} cardiovascular phenotype⁵⁹ was derived from a human genomic clone, whereas the transgene that fully rescued the *Df1/+* cardiovascular phenotype⁵⁸ was derived from a mouse genomic clone. This finding indicates that the human gene product possibly cannot substitute entirely for the mouse gene product, perhaps because of differences between the two gene products or because the human gene is expressed at a reduced level or is not regulated normally in the mouse. Whether the transgene that rescues the cardiovascular phenotype also rescues the non-cardiovascular defects reported in *Lgde*^{F9/+} embryos⁵⁹ and *Df1/+* embryos in certain genetic backgrounds⁶⁰ is not known.

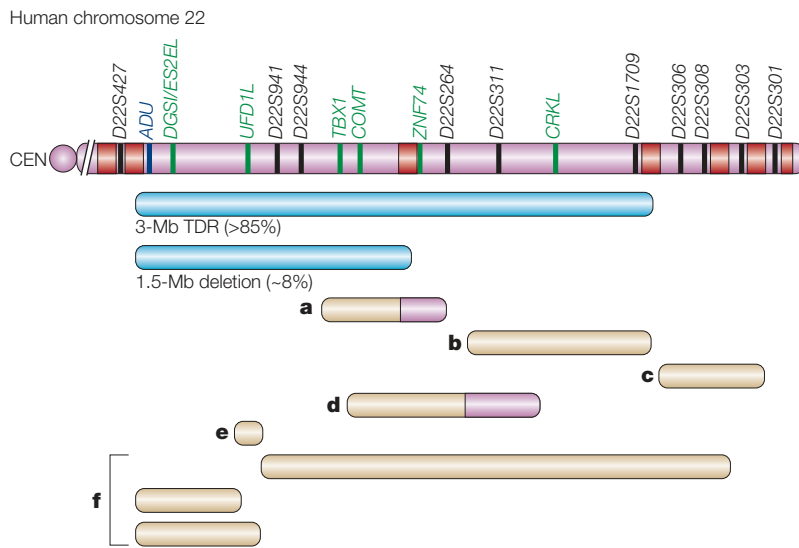


Figure 1 | Human *del22q11* region. The *del22q11* region on human chromosome 22, on which selected genes (green) and molecular markers (black) that have been used to characterize patient deletions are shown. Red blocks represent low-copy repeats (LCRs). ADU (blue) is a patient with DiGeorge syndrome and a balanced chromosomal translocation. The common 3 Mb typically deleted region (TDR), which is present in more than 85% of *del22q11* patients and the 1.5-Mb deletion are shown (turquoise). **a–f** | Individual patients with unusual deletions. In **a** and **d**, purple indicates where the distal deletion breakpoint is not precisely known. Deletions shown in **a–f** were reported in the following studies: **a** | REF. 45; **b** | REF. 44; **c** | REF. 47; **d** | REF. 97; **e** | REF. 48; and **f** | REF. 45. (CEN, centromere; *COMT*, catechol-O-methyltransferase; *CRKL*, v-crk avian sarcoma virus CT10 oncogene homologue-like; *DGS1*, DiGeorge-syndrome-critical-region gene 1; *TBX1*, T-box 1; *UFD1L*, ubiquitin fusion degradation 1-like; *ZNF74*, zinc-finger protein 74.)

To identify the gene responsible for the cardiovascular phenotype, both groups took a candidate-gene approach, selecting to knock out *Tbx1* in the mouse. This gene is a member of the T-box family of genes, and was considered to be the strongest candidate because during embryonic development it is highly expressed in the pharyngeal apparatus⁶¹ (BOX 1, FIG. 4), which, as mentioned above, gives rise to the structures that are most commonly affected in *del22q11* syndrome. At the same time, another group had appreciated the merits of *Tbx1* as a potential candidate gene, and also made a *Tbx1* knockout mouse⁶². The finding by all three groups of cardiovascular defects in *Tbx1*^{+/−} mice that were identical to those found in *Df1*^{+/+} and *Lgdell*^{+/+} mice (FIG. 3), provided compelling evidence that *Tbx1* alone is responsible for that phenotype. The phenotype of *Tbx1*-null mice, which is discussed in the following section, was remarkably reminiscent of severe forms of *del22q11* syndrome⁶², indicating that human *TBX1* might be involved in both cardiovascular and non-cardiovascular aspects of the *del22q11*-syndrome phenotype.

Pharyngeal development and *Tbx1*

Developmental disorders of the pharyngeal apparatus are a common source of birth defects. These include craniofacial abnormalities, palatal clefting, odontogenesis defects, thymic and parathyroid defects, and some of the most common cardiovascular defects

(~30% of all cases of congenital heart disease). We do not know why these abnormalities are so common, but several explanations are possible: first, even the most severe pharyngeal abnormalities might be compatible with vertebrate development; second, many genes might contribute to pharyngeal development; or third, the pharyngeal apparatus might be sensitive to a range of environmental insults. Whichever is the case, elucidating the development of the pharyngeal apparatus, and analysing the molecular and genetic components that underlie its development, should greatly affect our understanding of the biology and genetics of birth defects.

DGS has been referred to as III–IV pharyngeal pouch syndrome^{63,64}, a name that is appropriate and that has been justified by classical embryological studies. The pharyngeal pouches, and the blood vessels in between them, are the primordia of the structures that are most commonly affected in *DGS/del22q11* syndrome: the aortic arch, thymus and parathyroid glands (BOX 1). However, it is important to note that most of the pharyngeal apparatus, not only the third and fourth pouches, contributes to the pathogenesis of the pharyngeal component of the *del22q11*-syndrome phenotype described above.

The *Tbx1*-null mutant phenotype is a near perfect fit for the typical *del22q11*-syndrome phenotype⁶² and is an excellent entry point for studying the developmental genetics of the pharyngeal apparatus. *Tbx1*-null mice, which die at birth, have PERSISTENT TRUNCUS ATERIOSUS, a hypoplastic pharynx, lack thymus and parathyroid glands, and have ear, jaw and vertebral anomalies. The embryological basis of these abnormalities is the failed development of the pharyngeal arches and arch arteries 2–6, and of the pharyngeal pouches 2–4 (REFS 58,62). The severity and extent of the embryological lesion indicates that *Tbx1* might be required for the segmentation of the pharyngeal endoderm⁵⁸, an event that initiates the development of the entire pharyngeal apparatus. Indeed, no other gene so markedly and specifically affects pharyngeal morphogenesis. Interestingly, however, a zebrafish mutant, named vangogh (*vgo*), has a phenotype that closely resembles the *Tbx1*-null phenotype as it also lacks pharyngeal arches and pouches^{65,66}. The mutant phenotype has been attributed to a failure of the pharyngeal endoderm to segment and form pouches, and consequently, to a failure of the endodermal–mesenchymal tissue interactions that are required for development of the pharyngeal arches⁶⁷. So, although the gene that is disrupted in *vgo* has yet to be identified, the similarity of the phenotype in the two mutants is so striking that it indicates that a thorough investigation of the role of *Tbx1* in pharyngeal endoderm development is warranted, because it might shed light on the pathogenesis of *del22q11* syndrome.

We have learned at least two, perhaps unexpected, facts from the mouse *Tbx1*-null mutants: first, the severe disruption of the development of the pharyngeal apparatus *per se* does not affect embryo viability, and second, the loss of function of a single gene can

PERSISTENT TRUNCUS ATERIOSUS

A severe heart defect in which a single vessel exits the heart instead of the normal two (the aorta and pulmonary trunk). It reflects the abnormal persistence of an earlier embryonic state.

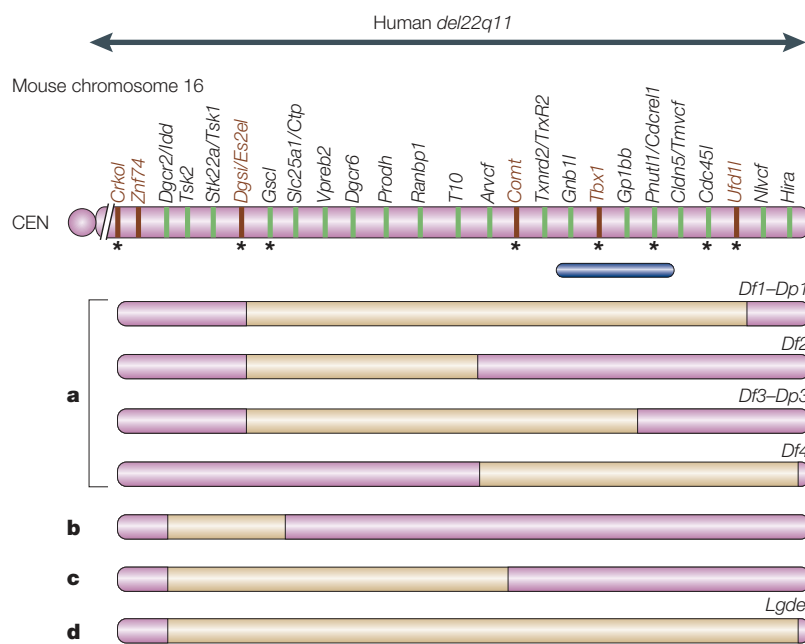


Figure 2 | The mouse chromosome 16 region that is in conserved synteny with 22q11.

The gene content of the region deleted in *del22q11*-syndrome patients is highly conserved in the mouse genome, although several ancestral rearrangements have led to changes in gene order between the two species. Mouse mutants have been reported for the genes that are marked with an asterisk. **a-d** | Mouse chromosome-16 deletion mutants that have been made by different groups. The deletions shown were published in **a** | REFS 54, 58; **b** | REF. 55, **c** | REF. 56, and **d** | REF. 59. Yellow indicates deleted or duplicated, and purple indicates non-deleted or non-duplicated, chromosome segments. The blue bar shows the extent of a transgene that rescues cardiovascular defects in *Df1/+* and *Lgdel/+* mutants. (*Arvcf*, armadillo repeat gene deleted in velocardiiofacial syndrome; *Cdc45l*, cell division cycle 45-like; CEN, centromere; *Cldn5*, claudin 5; *Comt*, catechol-O-methyltransferase; *Crkol*, v-crk avian sarcoma virus CT10 oncogene homologue-like; *Df*, deficiency; *Dgcr2/ldd*, DiGeorge syndrome gene c; *Dgcr6*, DiGeorge syndrome region gene 6; *Dgsi/Es2el*, expressed sequence 2 embryonic lethal; *Dp*, duplication; *Gnb11*, guanine-nucleotide-binding protein β -polypeptide 1-like; *Gp1bb*, glycoprotein 1b β -polypeptide; *Gscl*, goosecoid-like; *Hira*, histone cell-cycle-regulation defective homologue A; *Nlvcf*, nuclear localization signal protein absent in velocardiiofacial patients; *Prodh*, proline dehydrogenase; *Ranbp1*, Ran-binding protein 1; *Slc25a1/Ctp*, solute carrier family 25 member 1; *Stk22a/Tsk1*, serine/threonine kinase 22a; *Tbx1*, T-box 1; *Tsk2*, serine/threonine kinase 22b; *Txnrd2/TxrR2*, thioredoxin reductase 2; *Ufd11*, ubiquitin fusion degradation 1-like; *Vpreb2*, pre-B-lymphocyte gene 2; *Znf74*, zinc-finger protein 74.)

affect the development of the entire pharyngeal apparatus. *Tbx1* is clearly necessary for growth and patterning of the pharyngeal endoderm, but whether *Tbx1* is also sufficient to induce these processes, or whether signals from other genes are required, remains to be resolved. Possible sources of these signals might be the endoderm itself⁶⁸, or the notochord⁶⁹. It has been proposed for many years that neural crest abnormalities underlie DGS pathogenesis. However, it seems unlikely that migrating neural crest cells carry information for these inductive processes, as experiments have shown that pharyngeal patterning is not affected in chick embryos in which the neural crest has been ablated^{70,71}.

So, is this the end of the hypothesis that neural crest defects cause the pathology of DGS? This hypothesis pre-dates the identification of the 22q11 deletion⁷², and was based on the contribution of the neural crest to the pharyngeal apparatus. It was supported by neural crest

ablation experiments in chick embryos, which produced a DGS-like phenotype that was characterized by persistent truncus arteriosus, abnormal patterning of the great arteries and aplasia or hypoplasia of the thymus and parathyroid glands^{73,74}. As it seems that neural crest cells do not have a primary role in the *Tbx1*-null phenotype, and as *Tbx1* is not expressed in the neural-crest-derived mesenchyme of the pharyngeal arches^{61,62,75}, perhaps it is time to lay this hypothesis to rest. However, neural crest cells might have a secondary role in the disorder as targets of *Tbx1*-driven signalling, which might emanate from the pharyngeal endoderm or the core mesenchyme of the pharyngeal arches, both of which express *Tbx1*.

FIGURE 4b illustrates the relationship between *Tbx1* expression and the effect of *Tbx1* heterozygosity, which causes a reduction in the size, or the absence, of the fourth-arch artery. The *Tbx1* gene is expressed in the pharyngeal endoderm, but is not expressed in the mesenchyme that surrounds the fourth-arch artery or in the artery itself (E.A.L., unpublished observations). In addition, *Tbx1* expression in the core mesenchyme of the pharyngeal arches does not extend into the fourth arch (it is restricted to pharyngeal arches 1, 2 and 3). Therefore, one could speculate that *Tbx1*, which is a putative transcription factor, induces the expression of diffusible signalling molecules in the pharyngeal endoderm that are directed towards the underlying mesenchymal cells, which, in turn, support the growth of the vessel. Hence, cells derived from the neural crest might be at the receiving end of *Tbx1* signalling, which is most likely to come from the pharyngeal endoderm. The identification of the molecular targets of *Tbx1* should address these issues.

Insights and questions from mouse models

The mouse models of *del22q11* have begun to answer some questions about the pathogenesis of this syndrome and, inevitably, have also raised many more questions. The mouse *Tbx1* haploinsufficiency phenotype indicates that deletion of one copy of *TBX1* might be responsible for the cardiovascular phenotype of the *del22q11* syndrome. Furthermore, the *Tbx1*-null phenotype indicates that the gene might also be responsible for the thymic, parathyroid and craniofacial abnormalities associated with this disorder. These differences between the human and mouse phenotypes could be explained by differences in gene-dosage sensitivity between the two species. However, some clinical features of *del22q11* syndrome cannot obviously be attributed to reduced *TBX1* dosage, either because there is no evidence of them in *Tbx1*-null mutants, (such as renal abnormalities, for example), or because *Tbx1* is not expressed in the affected organ. For example, *Tbx1* is not expressed in brain, so its haploinsufficiency does not obviously account for the behavioural and neuropsychiatric problems of affected individuals. These, and other less common, phenotypes might be caused by the secondary effects of *TBX1* haploinsufficiency, or they might be caused by the reduced dosage of other deleted genes.

POSITIVE SELECTION

When a specific chemical is added to a culture medium, the cells that express a positive selectable marker gene, such as the neomycin- or puromycin-resistance genes, survive and are selected for.

Despite the strong evidence from the mouse that *Tbx1* mutations cause a *del22q11*-syndrome-like phenotype, there is no direct proof that human *TBX1* mutations cause *del22q11* syndrome, because such mutations have not been found in affected individuals who do not carry the deletion^{58,76}. One explanation for this is that mutation searches have been limited to the analysis of coding regions. This strategy would not detect certain kinds of mutations, including: those in introns, such as

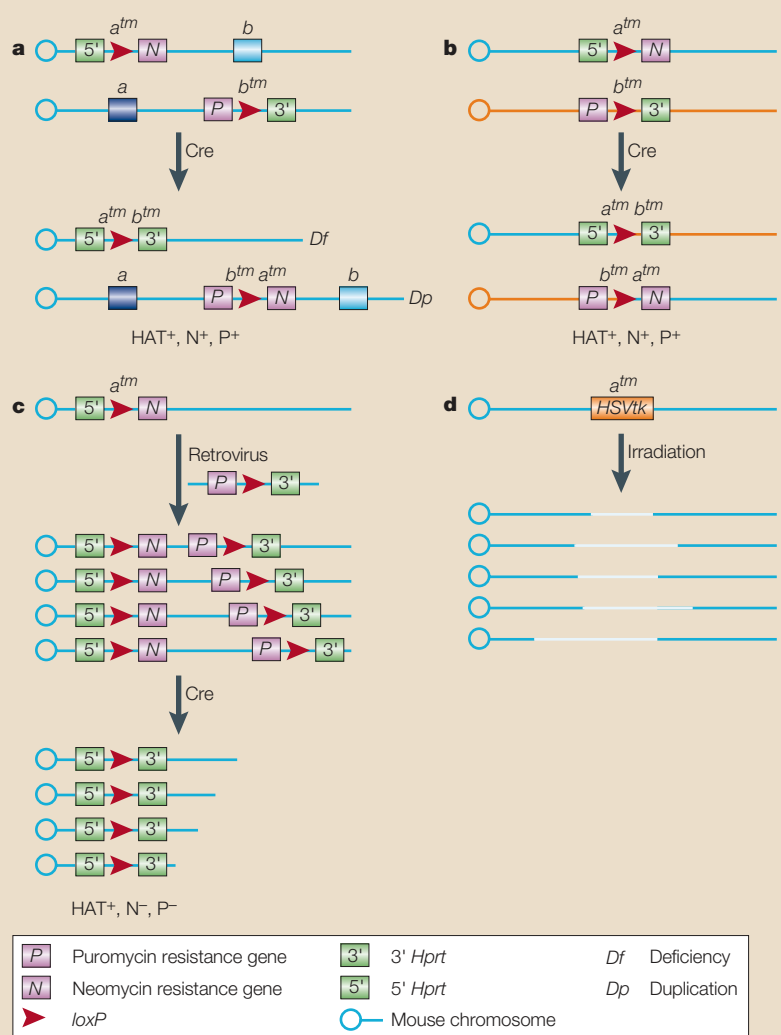
those that occur in **Friedreich ataxia**⁷⁷ and **spinocerebellar ataxia type 10**⁷⁸; the deletion of several exons, which would not be detected by standard exon amplification using the polymerase chain reaction (PCR); or mutations in yet-to-be identified regulatory elements located outside the *TBX1* coding region. It is also possible that point mutations are extremely rare in the population, in which case, non-deleted patients who are selected on the basis of a suggestive phenotype might be phenocopies of

Box 2 | Engineering chromosomal rearrangements

Chromosome-engineering strategies have been developed that enable precisely defined deletions, duplications and translocations to be created in embryonic stem (ES) cells^{89–95}. These cells are then used to establish the rearrangements in mice to analyse their phenotypic consequences *in vivo*.

The figure shows strategies for generating targeted (panels a and b) or ‘semi-targeted’ (panels c and d) rearrangements in ES cells. Panel a shows a chromosome-engineering strategy that was used by Lindsay and co-workers⁵⁴ to generate a precisely targeted deletion (*Df*) and a complementary duplication (*Dp*) in the same ES cell. Chromosome-engineering cassettes that contain *loxP* sites and different POSITIVE SELECTION markers — the neomycin resistance gene (*neo*) and the puromycin resistance gene (*puro*) — are targeted by homologous recombination to loci of interest (*a* and *b*), which serve as anchor points of the deletion or duplication. If these loci are genes, targeting can be used to inactivate them (*atm*, *btm*; *tm*, targeted mutation). Cre-mediated homologous recombination between *loxP* sites generates the *Df* and *Dp* alleles. Cells in which recombination has occurred can be positively selected for because each selection cassette contains half of an *HPRT* MINIGENE (5' *Hprt* or 3' *Hprt*). These two halves are joined together after recombination, conferring HAT (hypoxanthine, aminopterin and thymidine) resistance to recombined cells with the desired rearrangement. Because even carefully engineered chromosomal deletions can cause unwanted effects on neighbouring, undeleted genes, the phenotypic effects of which could be erroneously attributed to the deleted genes, engineered chromosomal duplications are extremely useful tools for establishing whether a phenotype is due to haploinsufficiency. This is because a chromosomal duplication can restore normal gene copy number. The use of chromosomal duplications for phenotypic-rescue experiments also overcomes some of the problems associated with using transgenes for such studies, such as high copy number, inappropriate transgene expression and genetic-background effects — which pose an important problem because haploinsufficient phenotypes can be profoundly affected by genetic background^{60,82,83} (most transgenic facilities use only one particular mouse strain, which is often different to that used for gene-targeting experiments).

The strategy shown in panel a can also be used to create balanced chromosome translocations by targeting *loxP*-containing cassettes into loci on different chromosomes (panel b). Nested-deletion and duplication alleles, such as those shown in FIG. 2, can be created by targeting the first chromosome-engineering cassette to a specified locus, and then inserting the second cassette randomly into the same ES cells using a recombinant retroviral vector (panel c). Cre-mediated homologous recombination between the *loxP* sites creates the deletions, and marker selection identifies the cell lines that carry them. An alternative strategy for generating semi-targeted deletions targets a NEGATIVE SELECTION cassette, which encodes herpes simplex thymidine kinase (*HSVtk*), into a specific locus (panel d). The targeted ES cell line is then irradiated, and clones that have lost the *HSVtk* marker are selected for in culture. This method allows many clones with random deletion breakpoints that flank a selected locus to be generated, but because the deletion breakpoints are not tagged, further work is required to map the extent of the deletion. For a more detailed description of chromosome-engineering strategies, see REF. 96. (*Hprt*, hypoxanthine phosphoribosyl transferase.)



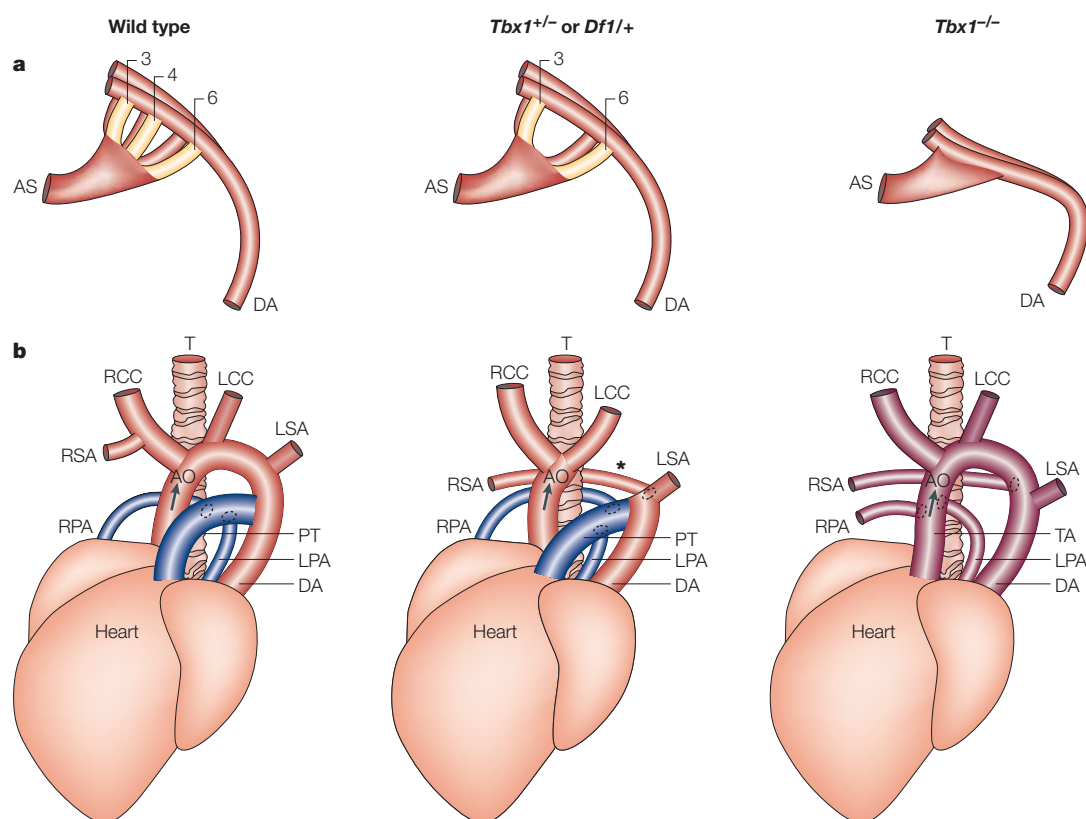


Figure 3 | Cardiovascular defects in mutant embryos. a | At embryonic day (E) 10.5, *Tbx1*^{+/-} embryos and *Df1*^{+/+} embryos have abnormally small or absent fourth aortic-arch arteries. In *Tbx1*^{-/-} embryos, there is no development of the pharyngeal apparatus distal to arch two. As a result, arch arteries three to six do not form, and the aortic sac (AS) connects directly with the dorsal aorta (DA). **b** | In near-term embryos (E18.5), *Tbx1*^{+/-} embryos and *Df1*^{+/+} embryos have abnormalities in the structures that derive from the fourth aortic-arch arteries, which are the root of the right common carotid (RCC) and a segment of the aortic arch. The resultant abnormalities include aberrant right subclavian artery (RSA), which originates from the aortic arch instead of from the RCC, and an interrupted aortic arch (asterisk). *Tbx1*^{-/-} embryos have persistent truncus arteriosus (PTA), in which a single vessel exits the heart instead of the normal two, the aorta (AO) and pulmonary trunk (PT). Blue and red vessels indicate pulmonary and aortic arterial flow, respectively. Purple indicates that in the presence of the PTA abnormality, blood from both sides of the heart is mixed. Arrows indicate the direction of blood flow from the heart. (LCC, left common carotid; LPA, left pulmonary artery; LSA, left subclavian artery; RPA, right pulmonary artery; T, trachea; TA, truncus arteriosus.)

HPRT MINIGENE
(hypoxanthine phosphoribosyl transferase (*Hprt*)). This is divided into two complementary, but non-functional, fragments: 5'*Hprt* contains exons 1–2 and 3'*Hprt* contains the remaining exons, 3–9. Each *Hprt* fragment is linked to a *loxP* site, and Cre-mediated recombination between them unites the 5' and 3' cassettes and restores *Hprt* activity, which is required for purine biosynthesis and allows desired recombination events to be selected for in HAT (hypoxanthine, aminopterin and thymidine) medium.

NEGATIVE SELECTION
When a specific chemical is added to a culture medium to kill the cells that still express a negative selectable marker gene, such as the herpes simplex virus thymidine kinase gene (*HSVtk*). Cells that no longer express the marker gene survive.

HSVTK
Herpes simplex virus thymidine kinase (HSVtk) is essential for thymidine-nucleotide biosynthesis by means of a salvage pathway, and is often used as a negative selectable marker in gene targeting.

AORTICOPULMONARY SEPTUM
In early heart development, the heart outflow tract comprises a single tube, the truncus arteriosus, which is later divided into two separate vessels, the aorta and the pulmonary trunk, by the formation of the aorticopulmonary septum.

the syndrome. Until mutations are identified in non-deleted patients, *TBX1* will remain a candidate gene, albeit a tantalizing one, for *del22q11* syndrome.

A few rare patients have deletions that do not include *TBX1*. This has led to the intriguing hypothesis that there might be several loci within 22q11.2 that can independently cause a similar phenotype. In support of this proposal, Guris and colleagues⁷⁹ have reported that mice that lack *Crkol*, which encodes a member of the *Crk* family of adaptor proteins, have cardiovascular and thymic defects that are similar to those seen in *del22q11*-syndrome patients. The human homologue (*CRKL*) is deleted in patients who carry the common 3-Mb deletion, but not in those with the 1.5-Mb deletion⁴⁴, whereas the mouse homologue maps to the equivalent region of mouse chromosome 16 (FIG. 2). A comparison of *Tbx1* and *Crkol* mutants indicates that although both mutants have similar heart defects at birth, *Crkol* acts at a later stage of development than *Tbx1*. For example, the AORTICOPULMONARY SEPTUM forms normally in *Crkol*^{-/-}

mutants, whereas it is absent in *Tbx*^{-/-} mutants. Furthermore, the pharyngeal arch arteries, which are normal in *Crkol*^{-/-} mutants until embryonic day (E) 11.5, are already abnormal in *Tbx1*^{+/-} mutants at E10. These differences in phenotype indicate that *Tbx1* and *Crkol* might function in different genetic pathways. The high mortality rate of *Crkol*^{-/-} embryos, despite their relatively mild pharyngeal-related phenotype, indicates that they might have severe problems elsewhere that have yet to be described. *Crkol*^{+/-} mice are normal, but it would be interesting to establish whether compound *Tbx1*^{+/-}/*Crkol*^{+/-} heterozygotes have a more severe phenotype than *Tbx1*^{+/-} mice. Unfortunately, none of the multi-gene deletion mouse mutants reported to date include both genes (FIG. 2). However, a combined haploinsufficient effect is unlikely to occur in patients with *del22q11* syndrome, because those who carry the 3-Mb deletion, who are *TBX1*^{+/-}/*CRKL*^{+/-}, have essentially the same phenotype as those who have the 1.5-Mb deletion, who are *TBX1*^{+/-}/*CRKL*^{+/+}.

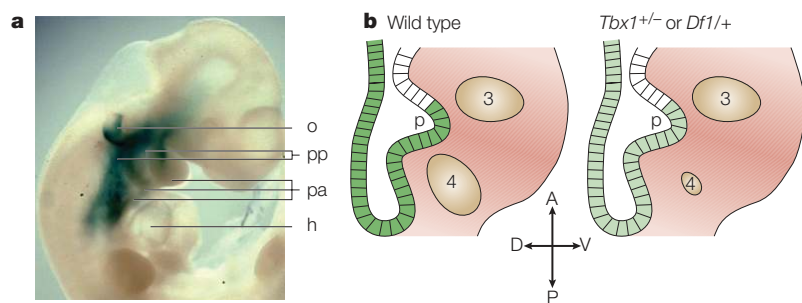


Figure 4 | *Tbx1* expression in early mouse embryogenesis. *Tbx1* expression shown by a *lacZ* reporter gene that has been inserted into the *Tbx1* locus. **a** | On embryonic day (E) 10.5, *Tbx1* is expressed in the pharyngeal arches (pa), the pharyngeal pouches (pp) and in the otocyst (o), which will form the inner ear. (h, heart.) **b** | At E10.5, *Tbx1* is strongly expressed in the pharyngeal endoderm (green, light shade in *Tbx1*^{+/-} indicates 50% reduction of gene dosage), which lines the developing pharynx (p). The fourth pharyngeal-arch artery (4) is small or absent in *Tbx1*^{+/-} and *Df1*/⁺ embryos. 3, third pharyngeal-arch artery. (A, anterior; P, posterior; D, dorsal; V, ventral.) (Image in panel **a** courtesy of Francesca Vitelli and Antonio Baldini.)

A more difficult issue to address is whether rearrangements within the *del22q11*-syndrome region (small deletions and translocations) can have long-range negative effects on the expression of neighbouring genes^{44,80}. Such effects do occur, for example, in some cases of **campomelic dysplasia**⁸¹, a syndrome that is characterized by skeletal abnormalities and sex reversal. If long-range effects were also relevant to *del22q11* syndrome, it would explain the phenotypic similarities between patients who have non-overlapping rearrangements in the region, as one could speculate that all such rearrangements affect *TBX1* expression, whether or not they physically disrupt the gene. Again, mouse studies have provided at least a partial answer to this question. Transgenic rescue experiments have shown that a 140-kb mouse genomic fragment that contains the mouse *Tbx1* gene can completely rescue the *Tbx1* haploinsufficient phenotype⁵⁸. The same experiment was carried out with a human genomic fragment of about the same size with similar results, although the rescue was incomplete⁵⁹. These results show that a relatively small genomic fragment contains all the necessary genetic elements to ensure the normal functioning of *Tbx1* during cardiovascular development. However, these studies do not establish whether the transgenes can rescue the non-cardiovascular phenotype, such as the thymus and parathyroid defects^{59,60}, or whether putative long-range effects could be due to changes in chromatin conformation rather than to the deletion of distant regulatory elements. Both these issues could be addressed in the mouse by transgenic rescue of the *Tbx1*^{-/-} phenotype, and by producing further deletion alleles that surround, but do not include, the *Tbx1* locus.

Future directions

The *Tbx1* and deletion mutants described here provide the necessary tools to address, for the first time, the pathogenesis of *del22q11* syndrome. A key step

will be to identify the genes that are targets of *Tbx1*, to define the molecular pathways in which *Tbx1* acts in the control of cardiovascular and pharyngeal development. Establishing the role of *Tbx1* in the development and function of the pharyngeal endoderm will be important for understanding the *Tbx1* heterozygous phenotype. It will also be important to establish whether mutations in *Tbx1* alone can account for the entire phenotypic spectrum of *del22q11* syndrome. If this is not the case, the nested-deletion strategy could be used to identify further genes. However, confirming the role of *TBX1* in the *del22q11*-syndrome phenotype can only come from the identification of *TBX1* mutations, or other DNA rearrangements that affect gene function, in patients without deletions.

An important development to emerge from the recent mouse studies into *del22q11* syndrome is a general approach for modelling human chromosomal disorders. Indeed, it has been shown, for the first time, that engineered deletions and duplications can be used to pinpoint the gene responsible for a haploinsufficient phenotype in a mouse model of a large deletion. This approach will not, of course, be useful for resolving haploinsufficiency disorders if there is not conserved synteny in the region of interest, or if the critical genes are not dosage sensitive in mice. Many of the loss-of-function mutations are normal in the heterozygous state, as has been seen in many published mouse-knockout studies. However, one should take into account that haploinsufficient phenotypes can be subtle, incompletely penetrant, and/or strain dependent^{60,62,82,83}, and might therefore require extensive phenotypic analysis and breeding on different genetic backgrounds to show the full extent of the mutant phenotype.

An alternative strategy to creating mouse models of specific haploinsufficiency disorders would be to scan the entire mouse genome for haploinsufficient loci by generating overlapping sets of deletions, a technically feasible and medically relevant undertaking. To facilitate this analysis, and to decrease the risk of loss of animal viability, deletions should not be too large (~1–2 Mb). So, coverage of the entire mouse genome would require ~1,500–3,000 lines of precisely tiled deletions. This is not an enormous number of mutant lines compared with large-scale mutagenesis programmes^{84–86}. In fact, a large-scale deletion project could actually complement mutagenesis programmes because it would greatly facilitate the mapping of point mutations induced by chemical mutagens⁸⁷. A viable alternative to the generation of thousands of mouse lines would be to generate a bank of ES cell lines that carry deletions from which individual investigators could pick their deletion of interest and establish it in mice⁸⁸.

The technical strategies discussed in this review, together with future technical advances for modelling chromosome syndromes in mice, promise to shed light on the molecular-genetic basis of diverse segmental aneusomy syndromes, by allowing research to advance in the absence of rare informative patients.

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