Developmental perspectives on copy number abnormalities of the 22q11.2 region


The 22q11.2 chromosomal landscape predisposes to genomic rearrangements that are associated with a variety of clinical phenotypes. The most well known of these include the 22q11.2 deletion and Cat-eye syndromes (CES), but more recently other copy number abnormalities have been recognised, especially with increased use of microarrays in the investigation of patients with congenital malformations or cognitive impairment. In addition, mutations in the \( \text{TBX1} \) gene have been found in patients with phenotypes reminiscent of 22q11.2 syndromes. Recent advances in our understanding of 22q11.2 genes and their interactions provide insight into the mechanisms underlying the phenotypic variability of the 22q11.2 syndromes, and suggest a possible common developmental pathway perturbed by copy number abnormalities of this locus.

The chromosome location 22q11.2 is best known for the proximal 22q11.2 microdeletion syndrome (OMIM#192430), a term that etiologically unifies nomenclature such as velocardiofacial syndrome (VCFS), DiGeorge sequence (DGS) and conotruncal anomaly face syndrome; however, the recent use of diagnostic microarrays has allowed the delineation of additional syndromes at this locus. Although the 22q11.2 syndromes have overlapping phenotypes, often with craniofacial, cardiac and cognitive involvement, it is becoming increasingly clear that they are cytogenetically and clinically distinct.

It is timely to review the 22q11.2 syndromes in light of the advances in our understanding of the biological function of genes within this region. We focus on the structural malformations of the syndrome, as the neurocognitive phenotypes are beyond the scope of this paper.

Chromosomal landscape of the 22q11.2 region

The 22q11.2 region is prone to chromosomal rearrangement because of the existence of segmental duplications, or low copy repeat (LCR) sequences that mediate non-allelic homologous recombination resulting in unequal crossover rearrangements (1). The eight characterised LCRs are labelled 2–8 (2) or A–H (3) from the centromeric to telomeric ends (4); the two largest being LCR22-2 and LCR22-4 (Fig. 1). Less common abnormalities with breakpoints not within any of the known LCRs are probably mediated by other repeats, such as SINE/Alu elements (5–8). High-resolution single nucleotide polymorphism (SNP) and comparative genomic hybridisation microarray technologies have identified atypical breakpoints that do not correlate with known repeats (9, 10) suggesting an even greater level of genomic complexity underlying the 22q11.2 syndromes.

Syndromes of the 22q11.2 region

The salient clinical features of each 22q11.2 syndrome are summarised in Table 1. An expanded nomenclature for these syndromes has been proposed, based on the precise segment affected by a deletion or duplication (11).
Fig. 1. Red boxes indicate deletions and green boxes indicate duplications or tetrasomic changes (CES cases). Orange boxes indicate largest and most frequently involved LCR22s, blue boxes indicate smaller and less frequently involved LCR22s. Note that due to space limitations, not all genes in the interval are listed. References are indicated for copy number abnormalities other than the typical 3 and 1.5 Mb proximal deletions/duplications and the CES cases. Abbreviated references: F 09 (52); Y (112); K 1997 (102); ISC 2008 (103); D’Ang (140); Rauch (65). *Concurrent microduplication extends from LCR22-2 to LCR22-4 (69).

Cat-eye syndrome

Individuals with cat-eye syndrome (CES; OMIM# 115470) have a dicentric bisatellited supernumerary marker chromosome 22 resulting in the duplication of the short arm and a variable interval of the proximal long arm. The tetrasomic CES critical region is proximal to LCR22-2 (12). The breakpoints of each CES marker chromosome tend to occur within LCR22-2 and/or LCR22-4, with variability resulting in symmetrical or asymmetrical duplications (3, 13, 14). The marker chromosome may be present in mosaic form, even in familial cases (15).

The cat-eye syndrome phenotype is highly variable, but frequently includes pre-auricular tags or pits, iris coloboma, anal anomalies and cardiac defects, particularly total anomalous pulmonary venous return (TAPVR) (16, 17). Urogenital defects, skeletal anomalies, and gastrointestinal malformations are less commonly described. Intelligence can be normal and cognitive impairment, if present, is not related to the presence of other malformations or the degree of mosaicism (16).

There is no correlation between the phenotype and the extent of duplicated material, or the degree of mosaicism. Although some familial mosaic cases were milder than their non-mosaic relatives (15), there are other reportedly normal patients with the marker chromosome in non-mosaic form (18). This implies that there are other phenotypic determinants beyond gene dosage alone.

Microdeletions proximal to LCR22-4

Most individuals with the proximal microdeletion syndrome have a 3-Mb deletion spanning from LCR22-2 to LCR22-4, whereas a minority has a 1.5-Mb deletion from LCR22-2 to LCR22-3a (8) or atypical deletions (19–21).

Individuals with a proximal 22q11.2 microdeletion have a distinctive craniofacial phenotype, and other features including cardiovascular defects (especially conotruncal anomalies), thymic hypoplasia leading to variable degrees of T-cell deficiency, hypoparathyroidism and neonatal hypocalcaemia, feeding difficulties, and renal defects (22–24). Cognitive deficits are found in the majority, often independent of associated congenital anomalies (25). Neuropsychiatric disorders are diagnosed at increased frequency compared with the general population (26, 27).

The proximal 22q11.2 microdeletion phenotype displays great inter and intrafamilial variability (22). There is little discernible difference
Table 1. Phenotypes of 22q11.2 genomic disorders

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Cat-eye syndrome</th>
<th>Proximal deletion LCR22-2 to LCR22-4 (VCFS)</th>
<th>Proximal duplication LCR22-2 to LCR22-4</th>
<th>Distal deletion LCR22-4 to LCR22-8</th>
<th>Distal duplication LCR22-4 to LCR22-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherited or de novo (%)</td>
<td>Often de novo</td>
<td>80% de novo</td>
<td>Frequently inherited</td>
<td>80% de novo</td>
<td>Only 1/14 confirmed de novo</td>
</tr>
<tr>
<td>Craniofacial Downslanting palpebral fissures; hypertelorism; epicanthic folds; iris and retinal colobomas; flat nasal bridge; cleft palate; micrognathia; pre-auricular pits and tags; low-set ears</td>
<td>Prominent tubular nose; hooded eyelids; mild hypertelorism; ear anomalies; asymmetric crying facies; flat midface; palatal anomalies including cleft and VPI; Robin sequence; craniosynostosis infrequent</td>
<td>More variability; superior placement of eyebrows; broad nasal bridge; hypertelorism; up or downslanting palpebral fissures; cleft palate and VPI; ear anomalies; occasionally pre-auricular pits and tags; rarely craniosynostosis</td>
<td>Subtle dysmorphology; arched eyebrows; hypoplastic midface; hypertrophic atri al nasi, smooth philtrum; thin upper lip; microcephaly; choanal atresia or stenosis; pre-auricular tags; palatal or uvular defects; Goldenhar syndrome phenotype</td>
<td>Widely variable, gestalt not discernible; microcephaly and macrocephaly; frontal bossing; broad forehead; pre-auricular pits; VPI; hyponasal speech</td>
<td></td>
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<tr>
<td>Cardiac TAPVR; septal defects; TOF</td>
<td>Wide range, including conotruncal heart defects, for example, IAA, TOF; vascular anomalies, for example, tracheal ring, aberrant carotids or subclavian arteries</td>
<td>Reported in less than 50% patients; TOF, IAA, HLHS, TAPVR and heterotaxy (n = 1) (50)</td>
<td>Truncus arteriosus; bicuspid aortic valve; IAA; VSD</td>
<td>Much less commonly reported; usually minor; PDA; patent foramen ovale, anomalous right subclavian artery; VSD and tricuspid regurgitation</td>
<td></td>
</tr>
<tr>
<td>Immune</td>
<td>Not reported</td>
<td>Thymic hypoplasia; T-cell deficiency</td>
<td>Rarely thymic aplasia</td>
<td>T-cell deficiency (n = 1) (21)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Central nervous system Normal to mild cognitive impairment</td>
<td>Mild to moderate cognitive impairment; increased risk of psychiatric issues; behavioural problems; seizures occasionally; hearing loss occasionally; structural defects uncommon</td>
<td>Wide range of cognitive ability from normal to moderate impairment; behavioural problems; hearing loss common; seizures occasionally</td>
<td>Normal to mild impairment;</td>
<td>Wide variability from normal to profound disability; occasionally seizures</td>
<td></td>
</tr>
<tr>
<td>Endocrine</td>
<td>Not reported</td>
<td>Hypoparathyroidism leading to hypocalcaemia; hypothyroidism; growth hormone deficiency</td>
<td>Neonatal hypocalcaemia (n = 1) (11)</td>
<td>Borderline hypocalcaemia (n = 1) (63)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Anal</td>
<td>Atresia, stenosis, malposition</td>
<td>Anal anomalies infrequent</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Imperforate anus (n = 1) (11)</td>
</tr>
<tr>
<td>Urogenital Ectopic or horseshoe kidney; hydronephrosis; renal agenesis; VUR; cryptorchidism</td>
<td>Renal agenesis; multicystic dysplasia; hydronephrosis; VUR; cryptorchidism and hypospadias uncommon</td>
<td>Uncommon; urethral stenosis; hydronephrosis; hypospadias; cryptorchidism rare; bladder extrophy</td>
<td>Hypospadias (n = 1) (63)</td>
<td>Duplicated collecting system; VUR; cryptorchidism</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Feeding difficulties; hypotonia; frequent infections</td>
<td>Hypotonia; frequent infections and otitis media; poor growth</td>
<td>Various minor skeletal defects, for example, digital hypoplasia; prematurity; pre- and post-natal growth restriction; malignant rhabdoid tumour</td>
<td>Musculoskeletal problems, for example, scoliosis, contractures; variable hypotonia</td>
<td></td>
</tr>
</tbody>
</table>

HLHS, hypoplastic left heart syndrome; IAA, interrupted aortic arch; TAPVR, total anomalous pulmonary venous return; TOF, tetralogy of Fallot; VPI, velopharyngeal incompetence; VSD, ventriculo-septal defect; VUR, vesico-ureteric reflux.

References are included for single cases in the literature.
between individuals with the 3-Mb deletion and those with the nested 1.5-Mb deletion or smaller atypical deletions (8), making phenotype–genotype correlation difficult. Numerous non-overlapping atypical deletions have been described (2, 6, 19, 28–33), making the proposal of a minimal critical region less tenable than initially proposed (Fig. 1).

A particularly interesting family, reported recently, highlights the gene dosage sensitivity in humans. A phenotypically normal patient with a proximal microdeletion and a proximal microduplication on separate alleles fathered an affected daughter who had inherited his deleted chromosome, suggesting genomic compensation in the father (34).

**Human TBX1 point mutations**

TBX1 is the only gene in which point mutations have been found in non-deleted patients who manifest the clinical phenotype of the proximal 22q11.2 microdeletion syndrome (35–39); however, these patients are rare, as TBX1 mutations have not been found by other researchers studying non-deleted VCFS patients (40–42). Analyses of other genes within and outside the 22q11.2 region have failed to detect mutations in non-deleted patients (43, 44). Most patients with TBX1 point mutations were ascertained through features characteristic of the proximal 22q11.2 microdeletion syndrome (including the typical facial dysmorphism) but lacked a deletion (35, 37–39). These mutations are discussed further below.

**Microduplications proximal to LCR22-4**

The proximal 22q11.2 microduplication extends from LCR22-2 usually to LCR22-4, and, complementary to the microdeletion, some patients have a nested 1.5-Mb microduplication (45, 46) or a microduplication that extends beyond LCR22-4 (47, 48).

The phenotype is highly variable with the only features reported in more than 50% of patients being cognitive impairment and facial dysmorphism (49), although it is challenging to delineate a common facial gestalt from the reported patients. Even amongst family members carrying the identical microduplication, contrasting phenotypes are evident, such as a proband with a narrow face, downsloping palpebral fissures, developmental delay, hypotonia, failure to thrive, sleep apnoea, seizure-like episodes and velopharyngeal insufficiency, whose mother and maternal grandmother only had pre-auricular ear pits (3). Phenotypically normal carrier parents and siblings have been reported, even when the proband had congenital malformations, cognitive deficits or facial dysmorphism (50–52).

Clinical overlap with the 22q11.2 microdeletion syndrome was observed initially, but this may be due to ascertainment bias (46–48, 53, 54). Two studies screened patients referred with features of the proximal 22q11.2 microdeletion syndrome by interphase Fluorescent In Situ Hybridisation (FISH) and found none with microduplications, suggesting that other populations may need to be screened (55, 56).

Analysis of 275 females referred for Fragile X molecular analysis yielded two proximal microduplication patients (48), including a patient with a 22q11.2 triplication, an ‘expansion’ of her mother’s duplication. The other microduplication patient in this cohort had choriorietinal coloboma and pre-auricular skin tags, evoking the CES phenotype, even though the CES region was not duplicated (48). Patients with interstitial duplications of the CES region and the LCR22-2 to LCR22-4 region have manifested a predominantly CES phenotype (57, 58). Cardiac abnormalities reported in two series of 22q11.2 microduplication patients include tetralogy of Fallot, hypoplastic left heart syndrome, and interrupted aortic arch, but were diagnosed in less than half the patients (47, 48). Recent reports have identified proximal 22q11.2 microduplications in patients with non-syndromic bladder exstrophy (59, 60) as well as autism cohorts (61). Phenotypically normal parents, siblings and anonymous controls were also found to have the microduplications.

**Microdeletions distal to LCR22-4**

Rearrangements distal to LCR22-4 are mediated by molecular mechanisms similar to those in the proximal region (62). Initial reports of patients with microdeletions involving the region distal to LCR22-4 were ascertained through cohorts phenotypically similar to the proximal microdeletion syndrome, that were negative for the standard 3-Mb deletion (21, 63). More recently Ben-Shachar et al. ascertained microdeletions in 6 patients from more than 8000 patients with suspected chromosomal abnormalities by array comparative genomic hybridisation (aCGH), thus minimising ascertainment bias (64).

Facial dysmorphism associated with distal 22q11.2 microdeletions is distinct to that associated with microdeletions involving the proximal LCRs (64–67), with the most consistent findings being a long smooth philtrum and thin upper lip.
Additional phenotypic features include low birth weight and prematurity, and minor skeletal anomalies (64, 66, 67). Developmental delay is typically global, but expressive language is more severely affected. Cardiac anomalies have been observed in 50% of distal 22q11.2 microdeletion patients, usually septal defects (63, 65, 68–70) and occasionally conotruncal abnormalities (21, 63, 64); however, compared to patients with proximal 22q11.2 deletions, patients with distal 22q11.2 deletions are more likely to present with non-conotruncal cardiac defects (65). Defects not commonly found in the proximal microdeletion syndrome but were observed in distal microdeletion patients include bicuspid aortic valve (64), cardiac dextrorotation (69) and choanal atresia or stenosis (21, 65).

Most distal 22q11.2 microdeletions arise de novo (63–67), similar to the proximal 22q11.2 microdeletion syndrome (22, 24). When inherited, there does not appear to be a parent-of-origin effect, and phenotypic variability is observed (21, 65).

Three patients with distal 22q11 deletions have been reported with the Goldenhar syndrome (GS) phenotype (70, 71) (and Tan et al., manuscript submitted); raising the possibility that 22q11.2 may be one locus for GS and its associated phenotypes such as oculo-auriculo-vertebral spectrum (OA VS). Furthermore, OA VS has been reported in patients with the proximal 22q11.2 microdeletion (72, 73) and a cytogenetically visible duplication of 22q11.2q13.1 (74), lending more support to the hypothesis that the genes at this locus may play important roles in the pathogenesis of GS/OA VS.

Microduplications distal to LCR22-4

The distal 22q11.2 microduplications are the most phenotypically variable, often having been inherited from ostensibly unaffected parents (11, 75) and associated with apparently contradictory clinical features between different patients, such as macro- and microcephaly or upslanting and downslanting palpebral fissures and no clear facial gestalt (76). Neurological development ranged from normal to profound disability and no major cardiac phenotype was reported. Recently two additional distal 22q11.2 microduplication patients were ascertained via congenital heart defects only – tetralogy of Fallot (77) and single atrium, VSD, tricuspid valve dysplasia and RV hypoplasia (78).

Of 14 reported probands with parental samples available, only one had a de novo abnormality (11, 75, 76). There is no parent-of-origin effect (six maternal, seven paternal). Most had inherited the copy number abnormality from a reportedly normal parent, while some parents had mild learning difficulties or developmental delay, thus complicating the causal relationship between the copy number abnormality and phenotype.

Developmental pathogenesis of 22q11.2 syndromes

In general, the phenotypes of genomic disorders are a consequence of the dosage imbalance of one or more genes within the affected region. The interval between LCR22-2 and LCR22-4 contains over 40 genes, and the interval between LCR22-4 and LCR22-8 has over 50 (UCSC genome browser build hg18; http://genome.ucsc.edu). Except for CLTCL, the genes in the LCR22-2 to LCR22-3a interval are conserved in the mouse and located at a syntenic region on mouse chromosome 16 (79). Initially, perturbations of neural crest development were thought to underlie the proximal 22q11.2 microdeletion syndrome because many of the affected structures are neural crest derivatives (80). In addition, ablation of chick neural crest results in malformations similar to those seen in the human 22q11.2 deletion syndrome (81). However, although many 22q11.2 syndrome features may be classified as neurocristopathies, the neural crest cells (NCCs) do not generate the craniofacial complex in isolation, but depend heavily on signalling inputs from the surrounding tissue. Thus, many of the 22q11.2 syndrome features could be due to either a defect in the NCCs themselves or a defect in the supporting environment. To understand the pathogenesis and variability of the 22q11.2 phenotypes, it is important to review the developmental functions and interactions of genes within the region (Table 2).

Cat-eye syndrome mouse models

Transgenic mouse models of CES with ubiquitous and cardiac-specific increased dosage of Cecr1 had cardiac enlargement, ASD (cardiac-specific), and kidney and eye anomalies (ubiquitous expression, in addition to cardiac enlargement), implicating CECRI as an important phenotypic determinant in CES (82, 83). Not all patients with CES have increased dosage of the LCR22-2 to LCR22-4 region, yet the phenotype cannot be predicted between individuals with differing rearrangements, perhaps suggesting that the genes proximal to LCR22-2 are more important in the pathogenesis.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Sites of expression</th>
<th>Animal model</th>
<th>Mutation</th>
<th>Biological role or phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cecr1</strong> (within the centromeric CES region)</td>
<td>Cardiac outflow tract and atria, VII/VIII cranial nerve ganglia, notochord in human embryo</td>
<td>Transgenic mouse</td>
<td>β-actin promoter for ubiquitous CECR1 expression, α-myosin heavy chain promoter for cardiac CECR1 expression</td>
<td>Kidney and eye anomalies, cardiac enlargement. Cardiac enlargement, ASD</td>
<td>Riazi et al. (83) Riazi et al. (82)</td>
</tr>
<tr>
<td><strong>Cecr2</strong></td>
<td>Neural tube, rostral brain, pharyngeal arches</td>
<td>Mouse</td>
<td>Genetrap Cecr2\textsuperscript{Gt\textasciitilde}Gt\textasciitilde; Cecr2\textasciitilde\textasciitilde;</td>
<td>Exencephaly (strain-dependent). Lack of eyelids in some</td>
<td>Banting et al. (137)</td>
</tr>
<tr>
<td><strong>Dgcr6</strong></td>
<td>Pharyngeal arches, frontonasal mesenchyme, NCC derivatives</td>
<td>Chick</td>
<td>Retroviral antisense and partial sense (dominant negative) constructs</td>
<td>Subarterial VSD, double outlet right ventricle, absence of fourth pharyngeal arches</td>
<td>Herck et al. (124)</td>
</tr>
<tr>
<td><strong>Ufd1l</strong></td>
<td>Mouse pharyngeal arches 1–4, palatal precursors, frontonasal region, otocyst, medial telencephalon, limb bud, cardiac outflow tract</td>
<td>Mouse</td>
<td>Ufd1l\textasciitilde\textasciitilde; Ufd1l\textasciitilde\textasciitilde;</td>
<td>No cardiac phenotype Embryonic lethal</td>
<td>Lindsay et al. (84)</td>
</tr>
<tr>
<td></td>
<td>Chick frononasal region, pharyngeal arches, limb bud, neural tube, cardiac conotruncus</td>
<td>Chick</td>
<td>Ufd1l antisense retroviral infection of premigratory cardiac NCCs</td>
<td>Conotruncal septation defects</td>
<td>Yamagishi et al. (115)</td>
</tr>
<tr>
<td><strong>Hira</strong></td>
<td>Neural crest-derived craniofacial tissues, pharyngeal arches and pouches, somites, forelimb bud</td>
<td>Chick</td>
<td>Functional attenuation by exposure of premigratory cardiac NCCs to antisense oligonucleotides and orthotopic backtransplantation to untreated hosts</td>
<td>Persistent truncus arteriosus</td>
<td>Roberts et al. (118) Farrell et al. (122)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Mouse</td>
<td>Hira\textasciitilde\textasciitilde;</td>
<td>Embryonic lethal after E9.5 in inbred homozygotes, gastrulation defects at E6.5–E7</td>
<td>Roberts et al. (121)</td>
</tr>
<tr>
<td><strong>Tbx1</strong></td>
<td>Pharyngeal surface ectoderm, endoderm and mesodermal core of the pharyngeal arches; otic vesicle</td>
<td>Mouse</td>
<td>Tbx1\textasciitilde\textasciitilde;</td>
<td>Hypoplastic or absence of fourth pharyngeal arch arteries by E10</td>
<td>Chapman et al. (138) Calmont et al. (92)</td>
</tr>
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<td></td>
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<td></td>
<td>Tbx1\textasciitilde\textasciitilde;</td>
<td>Lack of development of third to sixth pharyngeal apparatus, cleft palate, micrognathia, ear anomalies, thymic and parathyroid anomalies</td>
<td>Jerome and Papaioannou (89) Lindsay et al. (90) Merscher et al. (91) Piotrowski et al. (96)</td>
</tr>
<tr>
<td></td>
<td>Zebrafish</td>
<td>van gogh (tbx1 null)</td>
<td></td>
<td>Defects in pharyngeal arch derivatives, rescued by wildtype endoderm transplants</td>
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<tr>
<td>Gene</td>
<td>Sites of expression</td>
<td>Animal model</td>
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<tr>
<td>Crkl</td>
<td>Pharyngeal arch derivatives, frontonasal process, limb and tail buds, dorsal neural tube</td>
<td>Mouse</td>
<td>Crkl(^{-/-})</td>
<td>Aortic arch defects, thyroid, thymus, parathyroid defects; post-migratory NCC defect. Involved in Ras/Mapk-dependent pathways</td>
<td>Guris et al. (105)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fgf8(^{+/+});Crkl(^{++/-})</td>
<td>Hypoplastic fourth pharyngeal arch arteries at E10.5, cardiovascular defects, glandular defects, cleft palate, micrognathia, appendicular skeleton defects</td>
<td>Guris et al. (109)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fgf8(^{++/-});Crkl(^{-/-})</td>
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</tr>
<tr>
<td>Erk2/Mapk1</td>
<td>Frontonasal process, forebrain, midbrain-hindbrain boundary, pharyngeal arches, foregut, limb buds, liver primordia, tail, placenta</td>
<td>Mouse</td>
<td>Erk1(^{-/-});Erk2(^{fl/fl})Wnt1:Cre</td>
<td>Craniofacial defects: maxillary and mandibular hypoplasia, cleft palate, absence of tongue, eye placement anomalies Cardiovascular defects: VSDs, persistent truncus arteriosus, double outlet RV Thymus and thyroid developmental defects</td>
<td>Corson et al. (139) Newbern et al. (44)</td>
</tr>
<tr>
<td>Ypel1</td>
<td>Mouse pharyngeal arch, cardiac outflow tract, limb bud</td>
<td>Mouse</td>
<td>N/A</td>
<td>N/A</td>
<td>Farlie et al. (132)</td>
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<td></td>
<td></td>
<td>Zebradish</td>
<td>Antisense morpholino oligonucleotides</td>
<td>Underdeveloped jaw, pharyngeal arch cartilage defects</td>
<td>Aerts et al (133)</td>
</tr>
</tbody>
</table>

NCCs, neural crest cells; VSD, ventricular septal defect.
of CES. The possible roles of genes in the LCR22-2 to LCR22-4 region in the pathogenesis of CES have yet to be elucidated.

Early mouse models recapitulate the human phenotype

Early approaches to understanding the developmental pathogenesis of the proximal 22q11.2 microdeletion syndrome focused on replicating in mice the chromosomal anomaly seen in humans (84). The Df1 mouse strain harboured a deletion encompassing 24 genes and was viable in the heterozygous state. Cardiovascular abnormalities, attributable to aberrant development of the fourth pharyngeal arch arteries, were found in heterozygous (Df1/+), mice, and these defects were rescued with the restoration of normal dosage by mating with transgenic mice carrying a duplication of the Df1 deleted region (84). Interestingly, no craniofacial, thymic or parathyroid gland defects were detected, suggesting that in mice, development of these features of the 22q11.2 microdeletion syndrome are less sensitive than cardiac development to dosage changes in the Df1 interval genes. The existence of genetic modifiers outside the deleted region was demonstrated by differences in the penetrance of the cardiovascular defects depending on the genetic background (85). These strain-specific modifier genes also affect the growth of the thymus and parathyroid glands because the presence of the Df1 allele in a purebred background resulted in hypoplasia of both organs. Together with reports of phenotypic discordance in monozygotic twins with proximal 22q11.2 microdeletion syndrome focussed on replicating many features of the proximal 22q11.2 microdeletion syndrome (89). Tbx1 is expressed in the pharyngeal surface ectoderm (92), endoderm and mesodermal core of the pharyngeal arches, but not in the neural crest-derived mesenchyme (93), suggesting that the pharyngeal maldevelopment observed in Tbx1 mutants is not due to a primary defect within the neural crest (94).

Cre-lox-mediated conditional inactivation of Tbx1 in pharyngeal endoderm results in mice with parathyroid, thyroid, thymus and cardiovascular defects (95). These results are consistent with a study of the tbx1 null zebrafish mutant van gogh which displays defective pharyngeal structures (96). Endoderm transplanted from wild-type zebrafish embryos rescued the pharyngeal defects in van gogh mutants, suggesting that the defects are primarily derived from inappropriate signalling from the endoderm resulting in secondary consequences for neural crest-derived structures. Furthermore, NCC migration along the caudal portions of the pharyngeal apparatus was disrupted in the Tbx1−/− mouse (94), supporting the proposal that maldevelopment of the pharyngeal endoderm did not allow penetration of NCC into the pharyngeal apparatus (90). Recent work has shown that failure of appropriate cardiac NCC migration and consequent pharyngeal arch artery defects in Tbx1−/− mice result from a collapse of pharyngeal surface ectoderm signalling involving Gbx2 and the Slit/Robo system (92).

Developmental consequences of Tbx1 point mutations

Of the eight Tbx1 mutations reported (Table 3), four are missense, two are frameshift, one is a polyalanine expansion, and one is a single nucleotide transversion in a 5′ untranslated region (UTR). The two Tbx1 frameshift mutations reported in patients with the proximal microdeletion phenotype lead to loss of a nuclear localisation signal at the C-terminal end of the Tbx1 protein, and hence loss-of-function (37, 39, 97). On the other hand, the missense mutations caused a gain-of-function, possibly through stabilisation of the protein dimer DNA complex, as shown by a transcriptional reporter assay (38). These findings are consistent with observations that mice harbouring a BAC transgene over-expressing Tbx1 had similar malformations as those observed in haploinsufficient mice (98). The BAC transgenic mice were

TBX1, a major proximal 22q11.2 phenotypic determinant

The existence of cardiac anomalies in the Df1 mouse enabled phenotypic comparison of additional mouse models with smaller deletions to identify candidate cardiac development genes within the region (87, 88). This strategy led to the identification of TBX1, a T-box DNA binding domain transcription factor which maps to the proximal region between LCR22-2 and LCR22-3a; its dosage is altered in most patients with proximal microdeletions and microduplications. In murine models, inactivation of one copy of Tbx1 results in cardiovascular defects due to abnormal development of the fourth pharyngeal arch arteries (89–91); inactivation of both copies results in more severe cardiovascular defects, in addition to craniofacial, ear, and glandular defects, recapitulating many features of the proximal 22q11.2 microdeletion syndrome (89). Tbx1 is expressed in the pharyngeal surface ectoderm (92), endoderm and mesodermal core of the pharyngeal arches, but not in the neural crest-derived mesenchyme (93), suggesting that the pharyngeal maldevelopment observed in Tbx1 mutants is not due to a primary defect within the neural crest (94).
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Inherited or de novo</th>
<th>Ascertainment</th>
<th>Clinical features</th>
<th>Predicted effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 4: 443T → A (F148Y)</td>
<td>Excluded in mother; father not tested</td>
<td>Non-deleted 22q11 phenotype</td>
<td>Female with facial dysmorphism, VPI, TOF, pulm atresia, ASD, MAPCA</td>
<td>Altered T-box function; Gain-of-function</td>
<td>Yagi et al. (37)</td>
</tr>
<tr>
<td>Exon 8: 928G → A (G310S)</td>
<td>De novo</td>
<td>Non-deleted 22q11 phenotype</td>
<td>Male with facial dysmorphism, VPI, IAA, VSD, thymic aplasia, parathyroid dysfunction, deafness</td>
<td>Alteration of conserved residue; gain-of-function</td>
<td>Yagi et al. (37)</td>
</tr>
<tr>
<td>Exon 9C: 1223delC frameshift</td>
<td>Inherited (mat)</td>
<td>Non-deleted 22q11 phenotype</td>
<td>Female with atypical facial dysmorphism, TOF, right aortic arch, absent thymus. Mother with atypical facial features. Brother with typical facial features and parathyroid dysfunction</td>
<td>Loss of nuclear localisation signal and transactivation domain at C-terminal region</td>
<td>Yagi et al. (37)</td>
</tr>
<tr>
<td>Exon 9C: 1320-1342del23bp</td>
<td>Inherited (mat)</td>
<td>Non-deleted 22q11 phenotype</td>
<td>Female with typical facial dysmorphism of proximal deletion syndrome, hypernasal speech in proband. Two affected sons: (i) pulm stenosis; (ii) TOF and Asperger syndrome.</td>
<td>Loss of transcriptional activation</td>
<td>Paylor et al. (39)</td>
</tr>
<tr>
<td>Exon 5: 582C → G (H194Q)</td>
<td>Inherited (pat)</td>
<td>Short stature, speech delay; facial features of prox del22q11</td>
<td>Male with short stature, microcephaly, clubfoot, cryptorchidism, innocent heart murmur, speech delay, facial dysmorphism of proximal deletion syndrome. Father short, facial dysmorphism, normal cognition</td>
<td>Gain-of-function</td>
<td>Zweier et al. (38)</td>
</tr>
<tr>
<td>Exon 9C: 1232T → C (L411P)</td>
<td>De novo</td>
<td>Non-deleted 22q11 phenotype</td>
<td>Male with TOF, mild T-cell deficiency, speech delay, no facial dysmorphism</td>
<td>Conserved residue affected</td>
<td>Torres-Juan et al. (36)</td>
</tr>
<tr>
<td>5' UTR Exon 2: -39C → T</td>
<td>Inherited (mat)</td>
<td>Non-deleted 22q11 phenotype</td>
<td>Two brothers with TOF; one with immunodeficiency. Mother phenotypically normal</td>
<td>In vitro translation experiments suggest an increase in TBX1 translation possibly by disruption of post-transcriptional regulation</td>
<td>Torres-Juan et al. (36)</td>
</tr>
<tr>
<td>Inherited (mat)</td>
<td>FraX-negative cognitive/behavior</td>
<td>Male with severe impairment, hypotonia, obesity, pontocerebellar atrophy, epilepsy, facial dysmorphism, frequent infections. Mother phenotypically normal</td>
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Table 3. Continued

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Inherited or de novo</th>
<th>Ascertainment</th>
<th>Clinical features</th>
<th>Predicted effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherited (mat)</td>
<td>FraX-negative</td>
<td>Developmental delay, polymicrogyria, lower limb asymmetry, facial dysmorphism, frequent infections. Mother phenotypically normal</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Unknown</td>
<td>FraX-negative</td>
<td>Mild impairment, aggressive behaviour, advanced puberty, cryptorchidism, hypophyseal anomalies</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Inherited (mat)</td>
<td>Non-deleted 22q11 phenotype</td>
<td>Male with DORV, pulm atresia, VSD, ASD; absence of thymus, partial T-cell immunodeficiency; low PTH; minor ear anomalies. Mother phenotypically normal. Same patient had paternally inherited exon 9 1334-1348del15bp (445-449delGYHPH). Phenotypically normal brother also had both variants</td>
<td></td>
<td>Gong et al. (42)</td>
<td></td>
</tr>
<tr>
<td>1399-1428dup30</td>
<td>Excluded in mother; father not available</td>
<td>Patients with TOF</td>
<td>Female with scoliosis, facial asymmetry, upslanting palpebral fissures, absence of PV, isolated left pulmonary artery. Normal development</td>
<td>Cytoplasmic aggregation of TBX1 protein resulting in loss of transcriptional activity</td>
<td>Rauch et al. (35)</td>
</tr>
</tbody>
</table>
proposed as a model for the proximal microduplication syndrome in which there is an increased dosage of \textit{TBX1} (98). Patients with \textit{TBX1} gain-of-function mutations may be a better phenocopy of the proximal 22q11.2 microdeletion because they have the typical facial gestalt of the syndrome (37, 38), as distinct to the features of the microduplication syndrome. The polyalanine expansion within \textit{TBX1} was shown to result in a reduction in transcriptional activity because of cytoplasmic aggregation (35).

A 5′ UTR mutation was initially reported as a non-pathogenic variant (42) because it was found in a patient with complex cardiac defects and other features suggestive of the proximal 22q11.2 microdeletion syndrome and his phenotypically normal mother. The proband also had a paternally inherited in-frame deletion of exon 9. His phenotypically normal brother had also inherited both variants. Subsequently, the 5′ UTR variant was found in two brothers with tetralogy of Fallot, one of whom also had immunodeficiency (36). The variant had been inherited from a phenotypically normal mother. The 5′ UTR mutation was detected in 3 of 200 Fragile X-negative patients referred for a predominantly cognitive and behavioural phenotype, and again, 2 of 3 were maternally inherited (36). The phenotypes of these three patients were somewhat dissimilar to each other and to other patients with \textit{TBX1} mutations. Functional studies using an \textit{in vitro} reporter construct suggest that the mutation increases translation levels of \textit{TBX1} because of disruption of post-transcriptional regulation. Four of the eight \textit{TBX1} mutations reported so far (two missense and both frameshifts) were inherited. The mechanism of reduced penetrance in an unaffected carrier parent remains unexplained.

Sequence variants of the non-deleted \textit{TBX1} allele have not been found to modify the cardiovascular phenotype in patients with a proximal 22q11.2 microdeletion (99).

Effects on murine morphogenesis of \textit{Tbx1} dosage changes

In order to better understand the effects of reduced \textit{Tbx1} dosage on mouse morphogenesis, mRNA levels of \textit{Tbx1} were manipulated over a range from 100% to 0% by interbreeding a series of mice, each harbouring one of the three mutant \textit{Tbx1} alleles (100). This study revealed striking differences in the sensitivity of different phenotypes to variations in total \textit{Tbx1} dosage. Importantly, this allelic series also showed that although there are distinct thresholds of \textit{Tbx1} dosage above which development predominantly proceeds normally and below which development predominantly proceeds abnormally, there is an intermediate level where phenotypic variability is maximised. Given that phenotypic variability is a hallmark of the 22q11.2 syndromes, this observation may have fundamental importance for understanding the developmental mechanisms underlying the human phenotypes.

To study the effects of increased \textit{Tbx1} dosage, a conditional \textit{Tbx1} over-expressing transgenic mouse was generated and crossed with mice expressing Cre in the \textit{Tbx1} domain, thus ultimately producing a mutant animal with a 30% elevation of \textit{Tbx1} expression within the endogenous domain (101). The presence of cardiovascular and thymic defects in the mutant animal confirms that these organs at least require a specific \textit{Tbx1} dosage and that similar developmental defects arise regardless of whether the dose is above or below that level.

Relationships between \textit{Tbx1}, \textit{Crkl} and other genes

\textit{CRKL} maps to a region between LCR22-3a and LCR22-3b, which is not deleted in patients with the ‘nested’ 1.5 Mb proximal deletion, and is not within the genes on mouse chromosome 16 deleted in the \textit{Df1} animal model. Patients with the nested 1.5 Mb deletion are often similarly affected to those with the 3-Mb deletion, suggesting that haploinsufficiency of \textit{CRKL} is not an essential pathogenetic determinant of the malformations associated with the proximal microdeletion syndrome. Patients who are deleted for \textit{CRKL} but not \textit{TBX1} have been reported (Fig. 1). Phenotypes reported in \textit{CRKL}-deleted patients include cardiac septal defects, absent or mild facial dysmorphism somewhat reminiscent of the proximal microdeletion syndrome (10, 28, 65, 102), and neuropsychiatric disturbance (103) (personal communication 2009, George Kirov).

To investigate the role of \textit{Crkl} as a phenotypic determinant, Guris et al. used compound heterozygote mice for \textit{Crkl} and \textit{Tbx1} null alleles, which are observed to have aortic arch, thymic and parathyroid defects, and these defects were more penetrant than in mice heterozygous for \textit{Crkl} or \textit{Tbx1} alone (104). \textit{Crkl}−/− mice exhibit craniofacial, thymic, parathyroid, cardiovascular and cranial ganglia abnormalities (105). In addition, retinoic acid signalling was upregulated in a dose-dependent manner in embryos lacking \textit{Crkl} and \textit{Tbx1}. Genetically reducing retinoic acid synthesis lowered the penetrance of thymic defects, but it did not appear to have an impact on
the cardiac anomalies. These data suggest that Tbx1 and Crkl play important dose-dependent roles in pharyngeal development at least in part through regulating the balance between retinoic acid production and degradation. In support of this, proteomic analysis of cells over-expressing Tbx1 showed down-regulation of genes involved in retinoic acid metabolism (106). In addition, repression of Tbx1 expression by retinoic acid in quail (107) and zebrafish (108) suggests reciprocal regulation of Tbx1 and retinoic acid signalling.

The dosage-sensitive genetic interaction between Tbx1 and Crkl suggests that the human proximal 22q11.2 microdeletion phenotype may be a contiguous gene syndrome, at least in those with the typical 3-Mb deletion (104). However, understanding the similar phenotype in those with smaller or atypical deletions remains challenging. The proposal that genetic modifiers outside the 22q11.2 region such as Fgf8 alter the sensitivity of the pharyngeal apparatus to dosage changes in a single 22q11.2 gene is supported by the work demonstrating the interactions between Crkl, Tbx1 and Fgf8 (109, 110). Using an allelic series of mouse mutants for Fgf8 and Crkl, fourth pharyngeal arch artery development was observed to be defective in mutants with combined deficiency of Fgf8 and Crkl, whereas this was not observed in Crkl−/− mutants alone (109). Similarly, the penetrance of cleft palate and mandibular hypoplasia was increased in Fgf8+/−;Crkl−/− mice compared with that of Fgf8+/−;Crkl+/− or Crkl−/− mutants. The pharyngeal defects were shown to be due to decreased survival of NCCs in mice with reduced dosage of Crkl and Fgf8. Moreover, Moon et al. showed that downstream activation of the Ras/Mapk (mitogen-activated protein kinase) pathway by Fgf8 was reduced in mouse embryonic fibroblasts from Crkl−/− mice compared with that of wildtype, providing compelling evidence for a role of Crkl in the transduction of intracellular signals in response to Fgf8 (109).

Although the differences in embryogenesis between mice and humans warrant caution in the interpretation of these findings, the insights provided by animal models have been useful in elucidating interactions in a complex molecular network. The epistatic relationships between Tbx1 and other genes were reviewed recently by Scambler (111).

Effects on morphogenesis of attenuating other proximal 22q11.2 candidate genes

A patient with a 20-kb microdeletion of exons 1–3 of UFD1L (and part of neighbouring gene CDC45L) in the LCR22-2 to LCR22-3a region presented with most of the major clinical features seen in patients with much larger deletions (112), suggesting that UFD1L haploinsufficiency may contribute to the phenotype of the proximal microdeletion syndrome. So far, UFD1L point mutations have not been identified in non-deleted patients (113, 114). Ufdll is expressed in mouse and chick pharyngeal arches, limb bud, and cardiac outflow tract, making it an attractive candidate for contributing to the structural malformations of the proximal microdeletion syndrome (112, 115). Ufdll was identified during a screen for murine genes dependent on dHAND (112), which is downregulated in endothelin-1−/− mice (116). Mice lacking functional endothelin-1 exhibited a cardiovascular phenotype similar to the human proximal microdeletion syndrome (117). Although Ufdll+/− mice displayed no cardiovascular phenotype at E18.5 and homozygous null mutants were embryonic lethal (84), functional attenuation of Ufdll in chick cardiac NCCs with antisense retroviral constructs resulted in conotruncal septation defects, but no defect in NCC migration or survival (115).

The murine and chick expression pattern of Hira (118, 119), another gene in the proximal 22q11.2 region, and its interaction with Pax3 [homozygous Pax3 null mice die in utero with cardiac outflow tract, parathyroid and thymic anomalies (120)] suggest that it may play a role in the pathogenesis of the human proximal microdeletion syndrome phenotype. Homozygous Hira null mouse embryos have severe gastrulation defects and are embryonic lethal (121). Attenuation of Hira in premigratory chick cardiac NCCs by exposure ex ovo to antisense oligonucleotides and orthotopic back transplantation to untreated hosts resulted in an increased incidence of persistent truncus arteriosus, but no defects in arch artery modelling, ventricular function or outflow tract development (122).

The expression pattern of murine and chick Dger6, the most centromeric gene in the 22q11.2 region, is similar to that of Ufdll (123, 124). Using similar experimental methods, chick Dger6 was attenuated with a retroviral construct, resulting in cardiovascular anomalies including subaortic VSD and double outlet right ventricle and abnormal fourth pharyngeal arch artery development (124). Furthermore, real-time polymerase chain reaction showed reductions in expression of Tbx1 and Ufdll, and an increase in Hira in infected chick cardiac and arch tissue, offering insight into the molecular interactions between several 22q11.2 genes and pharyngeal arch tissues.
Genetic relationships beyond the 22q11.2 locus

Advances in the fields of genetics and developmental biology have propelled research efforts into understanding the modifiers of the phenotypic consequences of 22q11.2 copy number abnormalities, especially in the setting of incomplete penetrance and variable expressivity.

Vascular endothelial growth factor (Vegf), expressed in the fourth pharyngeal arch endoderm, cardiac outflow tract, aortic sac, frontal and maxillary prominences, midline palate and thymus, has been proposed as a modifier of the proximal microdeletion phenotype (43). Alternative splicing of Vegf produces three isoforms: Vegf120, Vegf164 and Vegf188. Gene-targeted mice lacking the Vegf164 isoform, which differs from the other two in its receptor binding abilities, manifest malformations resembling the human phenotype. Furthermore, abnormalities of pharyngeal arch arteries and cartilage are induced in a dose-dependent manner by the injection of vegf morpholinos in tbx1 knockdown zebrafish. A VEGF promoter haplotype increased the risk of cardiovascular defects in 22q11.2 proximal microdeletion patients (43); however, this may not be applicable in all patient populations (125).

Some phenotypic overlap between the proximal 22q11.2 microdeletion syndrome and Coloboma, Heart defects, Atestia choanae, Retardation of growth and development, Genital defects, Ear anomalies syndrome (CHARGE; caused by mutations in CHD7 in 60% of cases) has been recognised previously (126–128). The epistatic relationship between Tbx1 and Chd7 was demonstrated in light of a patient with some features of a 22q11.2 microdeletion (without a FISH-detectable deletion or Tbx1 mutation) who was found to be hemizygous for CHD7 (129). Randall et al. found that the expression of Tbx1 and Chd7 in pharyngeal ectoderm were required for normal arch artery formation.

Possible genetic modifiers of the proximal microdeletion syndrome such as Fgf10, Gbx2, Pitx2, Shh, TgfB and retinoic acid have been reviewed recently (130). Molecular pathways involving Tbx1 are emerging, based on its relationships with retinoic acid (via Crkl), Fgfs in outflow tract development, and Shh in endoderm and mesoderm (130).

Genes in the distal 22q11.2 region (LCR22-4 to LCR22-8)

Functional and developmental data on genes in the distal 22q11 region are sparse compared with that of the proximal region. It is interesting to note that ERK2, also known as MAPK1, a downstream component of the Ras/MAPK pathway, maps to the distal 22q11 region. Newborn et al. investigated the role of ERK2 in the pathogenesis of the distal 22q11 microdeletion phenotype (44). They showed that the expression of ERK2 was reduced in cell lines derived from patients with distal deletions, consistent with haploinsufficiency. Homozygous conditional inactivation of Erk2 in mouse neural crest resulted in craniofacial defects such as shortened maxilla, mandibular hypoplasia and cleft palate, and variable penetrance of cardiovascular defects, specifically cardiac outflow septation defects (persistent truncus arteriosus). Concomitant deletion of Erk1 alleles exacerbated the phenotype. Thymus and thyroid gland anomalies were not seen in Erk2 conditionally inactivated mice, but manifested when Erk1 alleles were also deleted. Phenocopies of the Erk1/Erk2 inactivated embryos were generated by conditional inactivation of upstream molecules in the Ras/Mapk pathway Mek1 and Mek2, activating germline mutations of which are associated with the neurocardio-facio-cutaneous syndromes (reviewed by Tidyman and Rauen (131)). Similarly, inactivation of Srf, downstream of Erk2, resulted in a similar phenotype; although the resemblance was less complete, suggesting that Srf may only be responsible for certain aspects of the phenotype (44).

Another promising candidate gene for craniofacial anomalies in the distal 22q11.2 region is YPEL1, which is expressed in the pharyngeal arches and promotes an epithelial-like phenotype when overexpressed (132). Knockdown of ypel1 led to major craniofacial cartilage defects in zebrafish (133). Further research is warranted to elucidate its role in craniofacial development and the phenotypes of distal 22q11.2 copy number abnormalities and GS/OAVS.

PRAME, a gene encoding a tumour-associated antigen, is overexpressed in several solid and haematological cancers and is a dominant repressor of retinoic acid signalling, which is involved in cell proliferation arrest, differentiation and apoptosis (134, 135). Although the effects of germline haploinsufficiency of PRAME in embryonic development are unknown, the association with retinoic acid signalling is intriguing given the Tbx1 and Crkl context outlined above.

The similarity between the mouse mutants derived by conditional inactivation of elements in the Ras/Mapk/Erk2 pathway and patients with the distal 22q11.2 microdeletion syndrome suggests that this pathway is dosage-sensitive, although there may be differences in the sensitivity between
humans and mice. The relationship between Tbx1 and Fgf8 signalling in pharyngeal epithelia, and Erk2 activation in neural crest, also potentially mediated by Fgf8–Crkl signalling is intriguing, and suggests a common developmental process critical for pharyngeal and vascular morphogenesis disrupted by copy number abnormalities involving either the proximal or distal regions.

Concluding remarks

Much effort has gone into unravelling the developmental pathogenesis of the complex human phenotypes arising from copy number abnormalities at 22q11.2. Mounting evidence in animal models and some human studies suggest not only sensitivity to gene dosage changes during embryogenesis but also the existence of modifying factors that affect phenotypic outcome. Although the distal 22q11.2 syndromes are clinically distinct from those of the proximal region, copy number abnormalities of either region may influence dosage-sensitive developmental pathways involving multiple 22q11.2 components. The microdeletions and microduplications at this locus should be considered distinct entities rather than ‘blended’ (136), united by their relationship with the disrupted genes, but not phenotypically reciprocal. Further research using animal models and conditional manipulation of promising candidates such as Ypel1 will advance our understanding of the pathogenesis of the distal phenotypes. The possibility that deletions or duplications within the 22q11.2 region have positional effects on transcription of neighbouring genes has not been explored in detail. Along these lines, mouse models of distal 22q11.2 copy number abnormalities would be useful to investigate their impact on expression of Tbx1 and other proximal genes, whereas analysis of chromatin configuration in pharyngeal arch may serve to address the possibility of transcriptional co-regulation of several proximal and distal 22q11.2 genes. Incomplete penetrance and variable expressivity make counselling and prediction of the phenotypic consequences of 22q11.2 copy number abnormalities (especially microduplications) particularly challenging and emphasise the need for ongoing research.

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Conflict of interest

All authors declare that no conflicts of interest exist in the submission of this manuscript.

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