SEGMENTAL DUPLICATIONS: AN 'EXPANDING' ROLE IN GENOMIC INSTABILITY AND DISEASE

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The knowledge that specific genetic diseases are caused by recurrent chromosomal aberrations has indicated that genomic instability might be directly related to the structure of the regions involved. The sequencing of the human genome has directed significant attention towards understanding the molecular basis of such recombination 'hot spots'. Segmental duplications have emerged as a significant factor in the aetiology of disorders that are caused by abnormal gene dosage. These observations bring us closer to understanding the mechanisms and consequences of genomic rearrangement.

IMPRINTING
A genetic mechanism by which genes are selectively expressed from the maternal or paternal homologue of a chromosome.

UNIPARENTAL DISOMY
A condition whereby an individual or embryo carries two chromosomes inherited from the same parent.

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Several genetic disorders have been classified as segmental aneusomy syndromes, which are disorders that result from inappropriate dosage for crucial genes in a genomic segment. This definition applies when the dosage imbalance occurs by structural (deletion or duplication) or functional mechanisms (by IMPRINTING defects or UNIPARENTAL DISOMY). Recently, it is increasingly clear that a significant proportion of segmental aneusomy arises from the genomic restructuring caused by aberrant recombination that occurs at region- or chromosome-specific low-copy repeats, also known as segmental duplications[1–4]. The cytogenetic alterations mediated by segmental duplications include deletions, interstitial duplications, translocations, inversions and the formation of small marker chromosomes. These rearrangements can give rise to an altered copy number of a gene(s) or, alternatively, might disrupt the integrity of a single gene. Because a change at the genomic level is involved, these disorders have been referred to as genomic disorders[2].

Segmental duplications represent a new class of repetitive DNA element that has recently been identified in the human genome. Segmental duplications have resulted from the duplication of large segments of genomic DNA that range in size from 1 to 400 kb (REFS 4,5) and can be divided into two classes — interchromosomal and intrachromosomal. The interchromosomal class is duplicated on non-homologous chromosomes, and many members of this class localize to the pericentromeric and subtelomeric regions of human chromosomes[6–10]. The intrachromosomal duplications, also referred to as region- or chromosome-specific low-copy repeats, are typically found on a single chromosome or in a single chromosomal band[1–3]. Recent evidence has implicated many of these intrachromosomal segmental duplications in the aetiology of the chromosomal rearrangements associated with genomic disorders[1–3,11,12]. There seems to be a significant bias for genomic rearrangements that are mediated by intrachromosomal segmental duplications to be near centromeres and telomeres (FIG. 1). This has led to the suggestion that the pericentromeric and subtelomeric regions of chromosomes are more permissive to the generation and expansion of segmental duplications[3]. Furthermore, the processes responsible for the generation of segmental duplication and for disease-causing genomic rearrangements have been proposed to be molecularly interrelated[3]. However, a direct connection between these two processes has not yet been established. Although they seem to localize near centromeres and telomeres, intrachromosomal segmental duplications are more distant from centromeres and telomeres than are the pericentromeric and subtelomeric interchromosomal segmental duplications[3,6–10]. Also, not all intrachromosomal segmental duplications, or the genomic

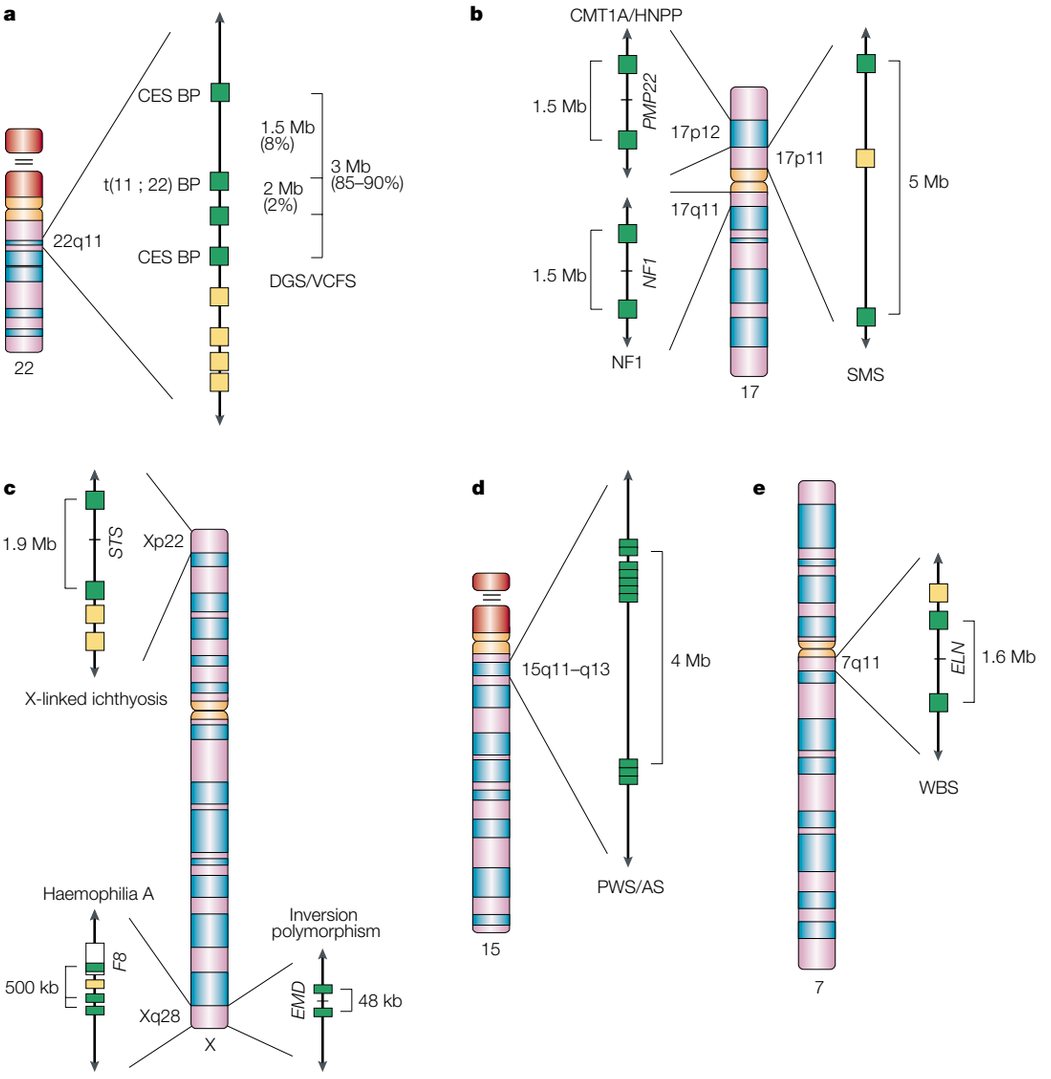

Figure 1 | **Chromosomal rearrangements mediated by segmental duplications.** Chromosomes involved in rearrangements mediated by segmental duplications are depicted by ideograms. The chromosomal bands or regions involved in rearrangement are indicated and expanded. Segmental duplications are shown as filled boxes: green boxes indicate those involved in recurrent chromosomal rearrangements, whereas yellow boxes indicate other copies that are rarely, if ever, involved in chromosomal rearrangements. **a** | On chromosome 22, recurrent deletions associated with DiGeorge and velocardiofacial syndromes (DGS/VCFS) are shown with the frequency of a particular deletion[25]. The proximal and distal breakpoints (BPs) for the marker chromosomes in cat eye syndrome (CES) and the t(11; 22) BP region are also indicated. **b** | On chromosome 17, the region on 17p12 that is duplicated/deleted in Charcot–Marie–Tooth disease type 1A and hereditary neuropathy with pressure palsies (CMT1A/HNPP) is shown with *PMP22* (peripheral myelin protein 22), the gene involved in their aetiology. Also on 17p11 is the region involved in the Smith–Magenis syndrome (SMS) deletions and the reciprocal duplication. On 17q11 is the region involved in neurofibromatosis type 1 (NF1) deletions. **c** | Three rearrangements of chromosome X include the region on Xp22 that contains the steroid sulfatase (*STS*) gene, which is deleted in X-linked ichthyosis; the region on Xq28 that contains the factor VIII (*F8*) gene, which is inverted in haemophilia A; and the region on Xq28 that contains a polymorphic inversion in the vicinity of the emerin (*EMD*) gene. *EMD* is involved in Emery–Dreifuss muscular dystrophy. **d** | The region on chromosome 15q11–q13 that is deleted in Prader–Willi and Angelman syndromes (PWS/AS) is shown with its numerous duplicated *HERC2* (hect domain and RLD 2) gene segments. **e** | On 7q11, the deletion associated with Williams–Beuren syndrome (WBS) is shown with elastin (*ELN*), the principal gene responsible for the supravalvular aortic STENOSIS of WBS.

rearrangements they mediate, are near centromeres and telomeres[1–3]. In this review, we describe the genomic organization of several clinically relevant intrachromosomal segmental duplications, the chromosomal abnormalities generated by them and the mechanisms by which they arise.

Misalignment, followed by recombination between non-allelic segmental duplications on homologous chromosomes, has been proposed to give rise to many genomic disorders, some of which are listed in TABLE 1. These disorders include Charcot–Marie–Tooth disease type 1A (CMT1A)/hereditary neuropathy with liability

STENOSIS
The blocking of a blood vessel that can be cleared by mechanical disruption.

Table 1 | **Genomic disorders mediated by segmental duplications**

Genomic disorder	Chromosomal rearrangement	Chromosomal location	Rearrangement size (Mb)	References
Charcot–Marie–Tooth disease type 1A (CMT1A)	Interstitial duplication	17p12	1.5	13,14
Hereditary neuropathy with pressure palsies (HNPP)	Deletion	17p12	1.5	13,14
Smith–Magenis syndrome (SMS)	Deletion	17p11.2	5	20
Duplication 17p11.2	Interstitial duplication	17p11.2	5	21
Neurofibromatosis type1 (NF1)	Deletion	17q11.2	1.5	15
Prader–Willi syndrome (PWS)	Deletion	15q11–15q13	4	16,17
Angelman syndrome (AS)	Deletion	15q11–15q13	4	16,17
Inverted duplication 15 (inv dup (15))	Supernumerary marker chromosome	15q11–15q14	4	73–76
Williams–Beuren syndrome (WBS)	Deletion	7q11.23	1.6	18,19
DiGeorge and velocardiofacial syndromes (DGS/VCFS)	Deletion	22q11.2	3	23–25
Cat eye syndrome (CES)	Supernumerary marker chromosome	22q11.2	3	22
X-linked ichthyosis	Deletion	Xp22	1.9	56–58
Haemophilia A	Inversion	Xq28	0.5	39,40

to pressure palsies (HNPP) on chromosome 17p11.2 (REFS 13,14), neurofibromatosis type 1 (NF1) on 17q11.2 (REF. 15), Prader–Willi/Angelman syndromes (PWS/AS) on 15q11–q13 (REFS 16,17), Williams–Beuren syndrome (WBS) on 7q11.23 (REFS 18,19), Smith–Magenis syndrome (SMS)/duplication 17p11.2 on 17p11.2 (REFS 20,21) and several rearrangements associated with 22q11, including DiGeorge and velocardiofacial syndromes (DGS/VCFS) and cat eye syndrome (CES)[22–25]. The genomic instability that is related to the structure of 22q11.2 was suspected to underlie the numerous nonrandom rearrangements that take place in this region long before the genomic sequence of chromosome 22 became available[26]. So far, a total of eight segmental duplications have been identified on chromosome 22q11 (REFS 5,26) (FIG. 1a). Four copies of the segmental duplications in proximal 22q11 have been identified within and flanking the DGS/VCFS region and at the end points of the CES duplications[22,23,25,27]. Furthermore, the breakpoint of the only recurrent, non-Robertsonian, CONSTITUTIONAL TRANSLOCATION [t(11; 22)] takes place in one of these duplications[28–31], also implicating the segmental duplications in non-homologous interchromosomal recombination events.

CONSTITUTIONAL TRANSLOCATION
A rearrangement between two chromosomes that occurs in the parental germ line or very early in embryonic development, such that every cell in the body contains the translocated chromosomes.

ICHTHYOSIS
A genetic disorder that causes the patient to have scaly skin.

Structure of segmental duplications

The segmental duplications that mediate the chromosomal rearrangements seen in genomic disorders show various organizational configurations. They can either be simple in structure or contain a complex arrangement of duplicated modules. The best-characterized example of a simple segmental duplication is the CMT1A-REP (REP, repeat) (FIG. 1b), which mediates the interstitial duplication or deletion associated with CMT1A or HNPP, respectively[13,32,33]. The two copies of CMT1A-REP that flank the duplicated/deleted region are 24 kb in size and share 98.7% nucleotide sequence identity[32]. Recent reports indicate that there might be other low-copy repeats near the CMT1A-REPs, but these do not seem to be directly responsible for the rearrangements that lead to CMT1A/HNPP[34]. Other simple segmental duplications include the S232 elements that mediate the deletions associated with X-linked ICHTHYOSIS[35–37], the two 11.3-kb inverted repeats on the X chromosome that mediate the inversion in the emerin gene (responsible for Emery–Dreifuss muscular dystrophy) gene and show >99% sequence identity[38], and the *int22h* (intron 22 homologous region) sequence, which mediates the inversion in the factor VIII gene that leads to haemophilia A (FIG. 1)[39,40].

In contrast to these simple structures, segmental duplications that are large and have a complex organization have been shown to mediate chromosomal rearrangements in several genomic disorders. The complex segmental duplications on 22q11 that mediate constitutional rearrangements, including deletions, interstitial duplications, supernumerary marker chromosomes, inversions and translocations, have been characterized extensively[5,24,25]. There are differences in the size, content and organization of duplicated modules in each copy of the 22q11 segmental duplication[25,26]. These segmental duplications contain several truncated gene segments or pseudogenes, which include *BCRL*, *HMPLPL* (*POM121L*), *GGTL*, *GGTrelL*, *V7 rel*, *E2F6L*, *KIAA0649L* and others[23,25]. A few of the 22q11 duplications also contain potentially recombinogenic sequences, which include palindromic (A+T)-rich repeats (PATRR) and variable-number tandem-repeat sequences[5,25]. The PATRR present in one of the duplication copies on 22q11 has been proposed to cause genomic instability as it has been shown to be directly involved in the constitutional t(11; 22), as well

as in other translocations that involve chromosome 22 (REFS 30,31,41,42).

Other large and complex segmental duplications include those on 17p11.2, which mediate the deletions associated with SMS and its reciprocal interstitial duplication product[20]. They contain at least four genes or pseudogenes[20,21]. The segmental duplications on 15q11–q13 that mediate the PWS/AS-associated deletions are also large and complex[16,17]. These are composed to a large extent of duplications of the *HERC2* (hect (homologous to the E6-AP carboxyl terminus) domain and RLD 2) gene[17,43]. It has been proposed that the *HERC2*-containing duplications have evolved a complex configuration by a complicated pathway that includes deletions and inversions[44]. Additional complexity in the organization of segmental duplications on 15q has been reported recently. These newly recognized duplications are found not only on 15q11–q13, but also on 15q24 and 15q26 (REF. 45). The three segmental duplications that mediate the deletions of 7q11.23 that are associated with WBS, also contain several genes or pseudogenes arranged in complex configurations[46]. Similarly, the segmental duplications that mediate the NF1 deletions, NF1-REPs, are 15–100 kb in size and contain at least four ESTs (expressed sequence tags) and an expressed *SH3GL* (SH3-domain GRB2-like) pseudogene[15].

Although only a few of the rearrangements that are mediated by segmental duplications have been analysed at the nucleotide level, there is evidence for recombination hot spots in the segmental duplications. The PATRR in one of the 22q11 segmental duplications discussed above has been shown to be a recurrent breakpoint for the t(11; 22) translocation[30,31,42]. The t(11; 22) breakpoints all localize in the PATRR, directly implicating them in the rearrangement mechanism[30]. In addition to the 22q11 PATRRs, recombination hot spots in segmental duplications on other chromosomes have also been described. The rearrangement breakpoints associated with CMT1A/HNPP seem to localize to a hot spot in the CMT1A-REP on 17p11.2 (REFS 47,48). The breakpoints of 21 out of 23 unrelated HNPP patients were mapped within a 557-bp region, further refining the hot spot within the CMT1A-REP[33]. The presence of a mariner transposon-like element very close to the CMT1A/HNPP breakpoints had led to the suggestion that the mariner-like element was somehow involved in the recombination[33,48]. However, there is no direct evidence to support the involvement of a mariner or any other specific sequence in the rearrangements associated with CMT1A/HNPP. In 46% ($n = 54$) of unrelated patients with the *NF1* deletion, the breakpoints in the NF1-REPs were localized within a 2-kb hot spot that contains a χ-like sequence[49]. In *Escherichia coli*, χ-elements stimulate recombination in nearby sequences. However, there is no direct evidence for the involvement of any particular sequence, including the χ-like sequences, in the *NF1* deletion.

The segmental duplications that are known seem to be limited to the genomes of primates. Phylogenetic analyses have indicated that most of these duplications have occurred relatively recently during primate evolution, ~1–25 million years (Myr) ago. This is based on the observations that there are differences in the copy number and location of the duplications on the chromosomes of representative members of the higher primates[5–9,16,50]. The duplications implicated in the rearrangement that leads to CMT1A — the CMT1A-REP — seem to be human and chimpanzee specific[32,34,47,51]. Similarly, the segmental duplications that mediate the rearrangements leading to PWS/AS are estimated to have originated ~15–20 Myr ago[16,43]. Comparative mapping of the mouse genome in the region of CONSERVED SYNTENY with human 22q11.2 has shown no evidence for the presence of the segmental duplications[52–55]. By contrast, duplicated sequences that are orthologous to the 22q11 segmental duplications have recently been identified in non-human primates, including great apes, and Old World and New World monkeys[5,25]. Furthermore, there seems to have been a sequential increase in copy number of duplications during primate evolution[5]. This indicates that the 22q11 segmental duplications have originated relatively recently during the evolution of the primate genome and, therefore, that segmental duplications might have been important in the evolution of the primate genome by rapidly generating genomic diversity between closely related primate species. It is tempting to speculate that the processes responsible for the generation and 'expansion' of segmental duplications are continuing and are therefore likely to have contributed to heteromorphism in the normal human population.

Segmental-duplication-mediated rearrangements

Deletions. In most patients with X-linked ichthyosis, the disorder is caused by a deletion of the steroid sulfatase (*STS*) gene on Xp[56–58]. Patients with STS deletions and X-linked ichthyosis have breakpoints that cluster in highly homologous sequence elements that flank the STS locus and are separated by 1.9 Mb on the short arm of the X chromosome[35] (FIG. 1c). The common repeat units are small, and the recombination events seem to involve repeats that are in the same orientation on the chromosome[35,36]. So, segmental duplications can lead to deletions through 'slipped pairing' (aberrant pairing between mismatched copies of the segmental duplication) and unequal crossing over in meiosis.

Excessive homologous recombination with unequal crossing over has been shown at the deletion end points of several deletion syndromes. WBS is due to a deletion of ~1.6 Mb in 7q11.23, which includes the elastin (*ELN*) gene[59] (FIG. 1e). It has been shown that there is a high frequency of recombined HAPLOTYPES in the segments that flank the *de novo* WBS deletions[60–62]. There is evidence that the WBS deletions are related to the presence of segmental duplications at the deletion breakpoints[63,64]. The region of the duplication has been characterized and shown to contain a transcribed gene[18]. Furthermore, the recombination event seen in several unrelated individuals seems to be similar, resulting in a junctional fragment that deletes the functional copy of this gene.

CONSERVED SYNTENY
The occurrence of genomic collinearity between homologous genes in different organisms.

HAPLOTYPE
An experimentally determined profile of genetic markers present on a single chromosome of any given individual.

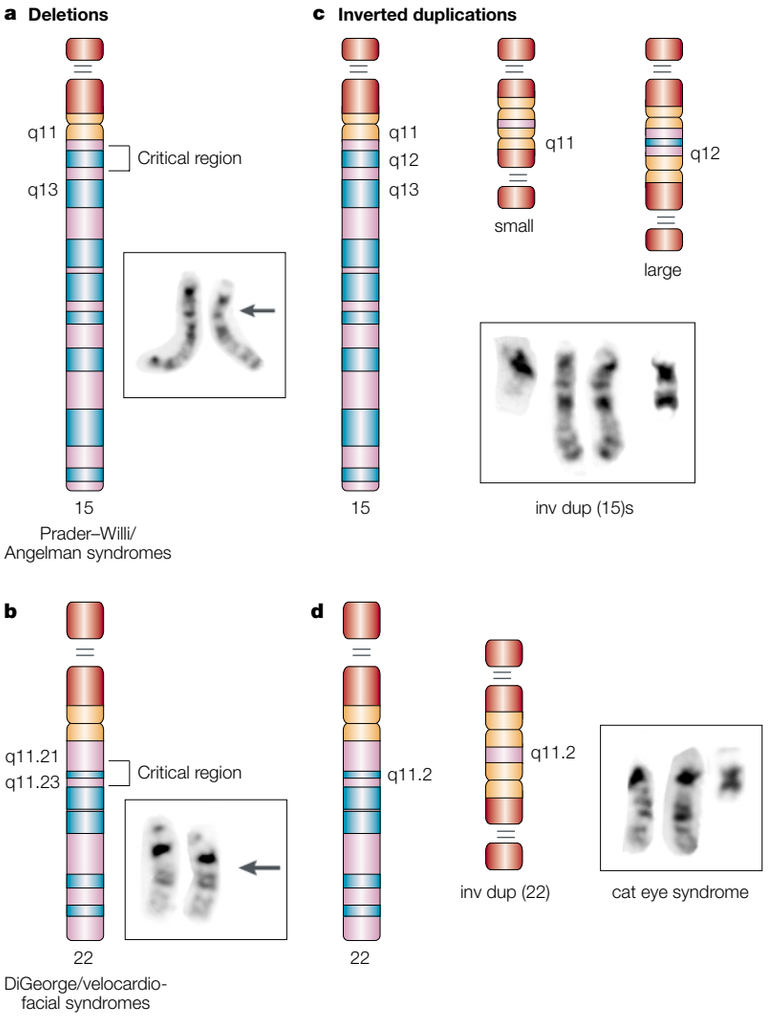

Figure 2 | **Chromosomes 15 and 22: deletions and inverted duplication chromosomes.** Ideograms and partial karyotypes are shown. For the deletions, the arrows indicate **a** | the deletion of chromosome 15q11–13 associated with Prader–Willi and Angelman syndromes, and **b** | the deletion of 22q11.21–11.23 (bottom) associated with DiGeorge and velocardiofacial syndromes. **c** | For the inverted duplications, the ideograms and partial karyotypes of two chromosome 15s show the two different sizes of inv dup (15) chromosomes. The smaller inv dup (15), on the left, is not associated with phenotypic abnormalities, whereas the larger inv dup (15), on the right, is associated with an abnormal phenotype (the central two are normal chromosome 15). **d** | The inv dup (22) (right) is associated with the cat eye syndrome. (Modified with permission from REF. 91.)

PWS and AS are most commonly associated with an ~4-Mb deletion of 15q11–q13 [del(15)(q11–q13)][16,17] (FIGS 1 and 2). There is evidence that both inter- and intrachromosomal rearrangements take place[65,66]: unequal crossovers during recombination, as well as intrachromosomal recombinational exchanges, occur. The chromosome 15 deletion breakpoints cluster in consistent hot spots, and large segmental duplications span the proximal and distal breakpoint regions[17]. NF1 has been reported to be due to a 1.5-Mb deletion that includes the *NF1* gene in a minority of patients (2–13%) (FIG. 1). These deletions seem to be mediated by segmental duplications that are referred to as NF1-REPs[15].

PULSED-FIELD GEL ELECTROPHORESIS
An electrophoretic technique used to separate large fragments of DNA (>20 kb and up to 10 Mb) on an agarose gel by periodically changing the orientation of the electric field applied to the gel.

The most common rearrangements associated with chromosome 22 are the deletions in 22q11.2 that are associated with DGS/VCFS. A typically deleted region (TDR) of ~3 Mb is observed in ~85–90% of patients with the 22q11.2 deletion (FIGS 1 and 2). So, there seems to be both a typical proximal and a typical distal deletion end point (DEP)[5,25]. In addition, there are two recurrent, variant distal DEPs. Furthermore, four out of the eight regions that contain the 22q11 segmental duplications coincide with the recurrent DEPs[5,24,25]. The direct involvement of these 22q11 segmental duplications in recombined deletion end-point junction fragments has been recently shown by PULSED-FIELD GEL ELECTROPHORESIS[25]. Hypotheses that would explain these findings have been constructed on the basis of the organization and orientation of the blocks of repeats[5,24].

Duplications/deletions. For several distinct clinical disorders, for example CMT1A (duplication) and HNPP (deletion), the existence of both reciprocal products of unequal crossovers in 17p has been observed[14]. These autosomal-dominant peripheral neuropathies result from unequal exchange between misaligned CMT1A-REP elements that are separated by 1.5 Mb on 17p12 (REFS 13,14,67) (FIG. 1b). This 1.5-Mb region contains the *PMP22* (peripheral myelin protein 22) gene, which seems to be dosage sensitive and is involved in the aetiology of the phenotype associated with CMT1A/HNPP[68]. A similar model has been shown for the SMS deletion[20] and the reciprocal interstitial duplication of the same region of 17p11.2, leading to a phenotype that is distinct from SMS[21]. This 17p11.2, deletion/duplication is ~5 Mb in most patients but the gene(s) that contribute to SMS and the reciprocal interstitial duplication are still unknown[20,21] (FIG. 1b).

Inversions. It has been shown that the local DNA-sequence environment has an important function in intragenic deletions or rearrangements[69]. A notable example of this is the rearrangement 'hot spot' that has been described for the factor VIII gene associated with ~45% of patients with haemophilia A. A functional 'deletion' that is caused by disruption of the factor VIII gene results from the presence of small segmental duplications that share a high level of sequence identity. These sequences, referred to as *int22h*, lie within intron 22 of the factor VIII gene; two additional copies are located in the reverse orientation ~500 kb telomeric to the factor VIII gene[39,40]. These sequence elements allow the distal long arm of the X chromosome to bend back on itself during meiosis, such that breakage and reunion causes an inversion that disrupts the factor VIII gene[70] (FIG. 1c). It is interesting that these inversions occur primarily in the male germ line, indicating that X-chromosomal pairing during meiosis in females might inhibit the distal long arm of the X chromosome from doubling back on itself and self pairing[71].

Another, perhaps more striking, example comes from the analysis of the genomic region that surrounds the emerin gene on Xq28, which is flanked by two large inverted repeats (FIG. 1c). Characterization of an emerin

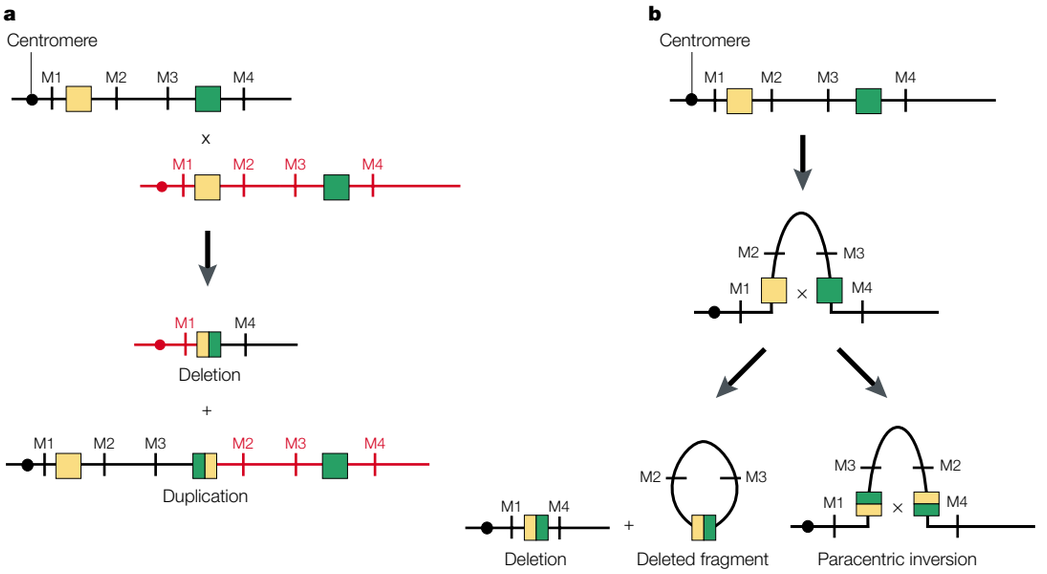

Figure 3 | **Models for formation of deletions and duplications.** Chromosomes are shown as lines. Black and red are used to distinguish the two homologues. Segmental duplications are shown as yellow or green boxes. **a** | Interchromosomal recombination between the two homologues of a particular chromosome leads to a reciprocal deletion and duplication. **b** | Intrachromosomal recombination between segmental duplications on the same chromosome leads to either a deletion or a paracentric inversion. (Modified with permission from REF. 5.)

deletion in a patient with Emery–Dreifuss muscular dystrophy showed — in addition to the gene deletion — a partial duplication of nearby sequences, recombination between the inverted repeats and an inversion of this region in 33% of normal females[38]. So, in this instance, the inversion represents a benign, population-based variant that is mediated by the presence of duplicated sequences.

Marker chromosomes. The second most common rearrangement associated with chromosome 15, after the deletions associated with PWS/AS, is the inverted duplication (inv dup (15)), which seems to be the most common marker chromosome in newborns[72]. There are several types of inv dup (15) marker chromosome, based on their size and rearrangement breakpoints[73–76] (FIG. 2). Most of the inv dup (15) chromosome breakpoints also seem to cluster within the segmental duplications in 15q11–q13[16,73,75,77]. In CES, a BISATELLITED chromosome that results from an inverted duplication of proximal 22q11 is present as a supernumerary chromosome[22] (FIG. 2). The breakpoints of marker chromosomes in CES patients seem to localize to copies of the segmental duplications in 22q11. The more proximal, most common cat eye chromosome (CEC) breakpoint interval corresponds with the proximal DGS/VCFS deletion endpoint interval. The more distal duplication breakpoint of the CEC overlaps with the common distal DGS/VCFS deletion. The resultant marker chromosomes have been divided into three categories on the basis of their size and location of the breakpoints[22]. Furthermore, recent detailed sequence analysis has begun to examine the genes that are duplicated in CES[78].

Other rearrangements. At the chromosomal level, another class of frequently observed rearrangements consists of translocations. Translocations can be divided into Robertsonian and reciprocal translocations. Robertsonian translocations involve exchanges between the short arms of the ACROCENTRIC chromosomes and seem to be mediated by satellite DNA repeats close to the centromeres[79]. Reciprocal, non-Robertsonian constitutional translocations are the result of crossover between two non-homologous chromosomes and most are known to be unique events. So, little is known about the mechanisms or sequences involved in the formation of most reciprocal translocations. The t(11; 22)(q23; q11) is the only known recurrent, non-Robertsonian constitutional translocation in humans. Balanced translocation carriers have no clinical symptoms, and clustered breakpoints have been reported in numerous unrelated families[28,29,31].

The t(11; 22) breakpoint on 22q11 localizes to one of the segmental duplications on 22q11 (REFS 25,28,30,31). Although chromosome 22 has been almost entirely sequenced, the segmental duplication at which the t(11; 22) breakpoint resides still contains a gap[5,25,26]. It has been shown that palindromic (A+T)-rich sequences surround the breakpoints on chromosomes 11 and 22 (REFS 30,31,42). Computer analysis of the DNA sequence that flanks the breakpoints predicts the formation of hairpin or cruciform structures[30]. It is likely that these unstable DNA structures in 22q11 and 11q23 facilitate the recurrent t(11; 22) translocation. Also, the segmental duplication on 22q11 that contains the chromosome 22 breakpoint of the t(11; 22) seems to be a hot spot for other reciprocal translocations. These include a bal-

BISATELLITED
A chromosome that contains two copies of the satellited acrocentric short arm, often as a result of an inverted duplication. It is usually present as a supernumerary marker chromosome in a cell.

ACROCENTRIC
This refers to a chromosome the centromere of which lies very close to one end, such that one arm of the chromosome is much larger than the other.

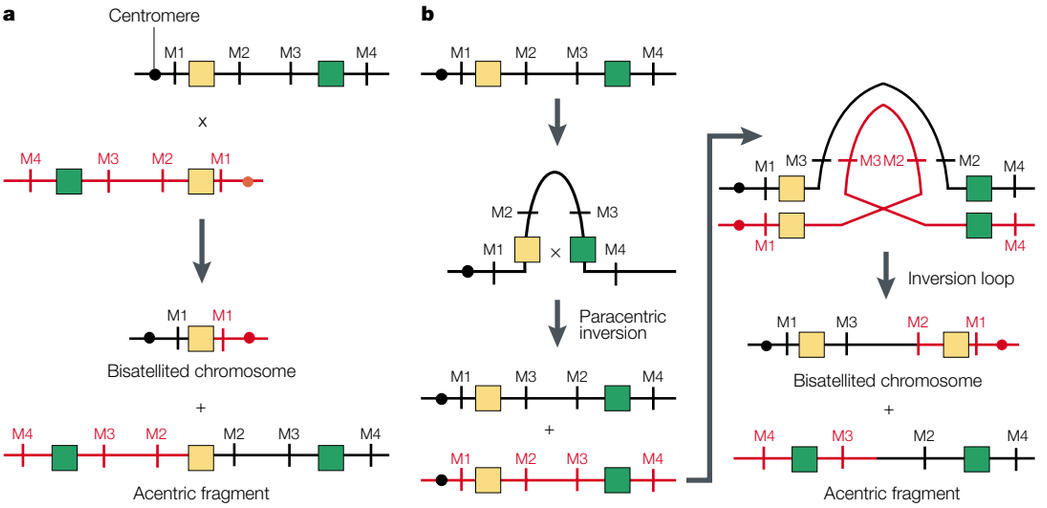

Figure 4 | **Models for formation of cat eye syndrome or inv dup (15) marker chromosomes.** Chromosomes are shown as lines. Black and red are used to distinguish the two homologues. Segmental duplications are shown as yellow or green boxes. **a** | Interchromosomal recombination between the two homologues of a particular chromosome, or **b** | paracentric inversion in one homologue of a chromosome, followed by recombination in an inversion loop, leads to the formation of a bisatellited marker chromosome and an acentric fragment. Both models can be used to explain all three described types of cat eye chromosome and the formation of the inv dup (15) marker chromosome. (Modified with permission from REF. 5.)

anced t(20; 22)[80], three unbalanced [t(12; 22), t(4; 22), t(17; 22)][81] and a balanced t(1; 22)[82]. So, it seems that this copy of the 22q11 duplication might contain sequences or secondary structures that are permissive to translocation.

Mechanistic models for rearrangements

The sequence analysis of segmental duplications has shown 96–99% sequence identity between duplicated copies over their entire length[4]. On the basis of this high level of sequence identity, several models can be proposed to explain the chromosomal rearrangements that are mediated by segmental duplications.

Deletions, duplications/deletions and inversions. Deletions could be explained using one of two possible models (FIG. 3). In the first model, an interchromosomal misalignment might occur during meiosis I between the two homologues of a particular chromosome. This misalignment might be mediated by the segmental duplications, or by modules within them that lie in direct orientation with respect to each other. Crossing over would lead to reciprocal deletion and duplication events (FIG. 3a). This mechanism has been used to explain the deletions associated with WBS[60–62] and those associated with SMS, as well as the reciprocal interstitial duplication[20,21], some of the reciprocal interstitial duplications/deletions associated with CMT1A and HNPP[14,83–85], and some of the deletions associated with PWS/AS[17,65,66] and DGS/VCFS[5,23,25,62,86].

The formation of deletions can also be explained by intrachromosomal recombination between segmental duplications during mitosis or meiosis. In this model, the segmental duplications, or modules within them, that lie in inverse orientation with respect to one another might form a 'stem–loop' intermediate. Recombination between the duplicated modules forming the 'stem' would then lead to the deletion of intervening DNA present in the 'loop' (FIG. 3b). This mechanism has been used to explain the deletions associated with NF1 (REF. 15), a few deletions associated with HNPP[83] and some deletions associated with DGS/VCFS[5,23,25,62]. Another possible outcome of the intrachromosomal misalignment between inverted segmental duplications is a paracentric inversion (FIG. 3b). This mechanism might explain the intrachromosomal inversions observed in the factor VIII gene that lead to haemophilia A[70], the polymorphic but benign inversion found close to the emerin gene[38] and the inversions that disrupt the iduronate sulfatase (*IDS*) gene in Hunter syndrome[87].

Inter- and intrachromosomal recombination events have been reported for the standard 3-Mb deletion associated with DGS/VCFS[23,62,86], as well as for the genomic aberrations that occur in PWS/AS[65,66] and CMT1A/HNPP[83–85]. Furthermore, the existence of individuals that are mosaic for deletions of 22q11 indicates that mitotic instability does occur[88–90]. Interestingly, although the deletions associated with DGS/VCFS are seen frequently, the reciprocal interstitial duplication event is rarely observed[24]. This is presumed to be the result of a mild and/or nonspecific phenotype.

Marker chromosomes. The formation of a bisatellited, supernumerary marker chromosome that is mediated by segmental duplications can be explained using one of two possible models. In the first model, interchromosomal misalignment occurs between the two homologues of a particular chromosome by virtue of the segmental duplications or modules within them that lie in opposite orientation with respect to one another. Recombination between these inverted sequences could

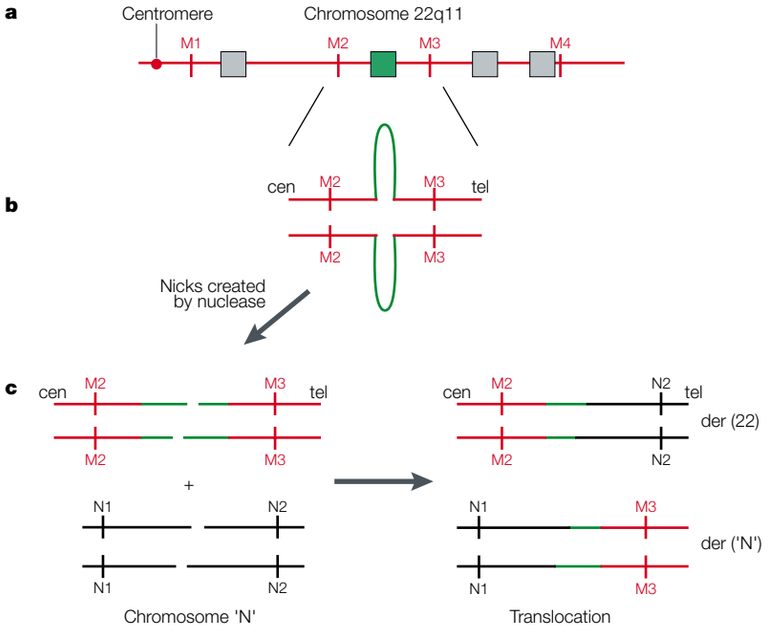

Figure 5 | **Model for translocations in 22q11. a** | The segmental duplication to which the translocation breakpoint localizes is shown as a green box. Other segmental duplications are shown as grey boxes. **b** | An expanded view of the region that contains the translocation breakpoint region is shown. The centromeric (cen) and telomeric (tel) ends are indicated. The palindromic sequences (green lines) are predicted to form a hairpin/cruciform structure on chromosome 22. The tips of the cruciforms could be prone to nicking by nucleases. **c** | A chromosome 22 with double-stranded breaks within the palindrome could recombine with another chromosome (chromosome 'N') that has similar double-stranded breaks. This would lead to a translocation between chromosome 22 and chromosome 'N' and result in the formation of two derivative (der) chromosomes: der (22) and der ('N'). Most often the 'N' is chromosome 11. (Modified with permission from REF. 5.)

lead to the formation of a bisatellited chromosome and an ACENTRIC fragment (FIG. 4a). This model can be used to explain all three types of CEC that have been identified[22]. Therefore, if the misalignment and recombination occur between the proximal copies of the 22q11 segmental duplication on both homologues (as shown in FIG. 4a), they would give rise to a type I CEC. Alternatively, the symmetrical type II CEC would result from a misalignment and recombination between the distal copies of the 22q11 segmental duplication (green boxes in FIG. 4a). Finally, misalignment and recombination between any one proximal (yellow box) and any one distal (green box) copy of the segmental duplication would result in the formation of an asymmetrical type II CEC.

In the second model, intrachromosomal recombination — facilitated by the segmental duplications during mitosis or meiosis — could first lead to a PARACENTRIC INVERSION (FIG. 4b) in one of the homologues of a particular chromosome. Subsequently, a single crossover event between paired homologues within the inversion loop could lead to the formation of the duplication/deficiency CES marker chromosome and an acentric fragment (FIG. 4b). This model might explain the formation of some of the asymmetrical CES chromosomes[22]. It is likely that the inv dup (15) and other bisatellited marker chromosomes result from one or a combination of the models proposed for the CES marker chromosome formation.

Other rearrangements. The chromosome 22 breakpoint of the recurrent, constitutional t(11; 22) has been localized to one of the 22q11 segmental duplications[30]. Many other balanced and unbalanced translocation breakpoints also cluster in the same region of 22q11. This further indicates that this region of 22q11 might contain unstable sequences that predispose it to be involved in translocations. The recent cloning and sequencing of the recurrent, constitutional t(11; 22) breakpoint and the identification of PATRRs at the site of the translocation strongly support this hypothesis[30,42]. Analysis of the available sequence indicates the presence of large palindromes that flank the t(11; 22) breakpoint regions on both chromosomes. It has been proposed that these palindromic sequences lead to the formation of hairpins or cruciforms at physiological temperature[30,31]. The translocation breakpoints localize to the tips of the hairpin/cruciform (B.S.E., unpublished data). Because the tip of the hairpin is sensitive to nucleases, the initiating step of the translocation might be a double-stranded break mediated by this hairpin-nicking activity. Recombination between the nicked chromosome 22 and any other chromosome with similar nicks could lead to a translocation between the two chromosomes (FIG. 5). Although the recurrent, constitutional t(11; 22) seems to be mediated by such a mechanism, there could be additional factors that facilitate this particular recombination event[30,42].

Conclusions and future directions

Based on the examples discussed above, it is clear that segmental duplications can create genomic instability by predisposing certain chromosomes or chromosomal bands to rearrangements through misalignment and unequal crossing over. The proposed inter- or intrachromosomal recombination between copies of the segmental duplications might result in deletions, interstitial duplications, inversions, translocations and marker chromosomes. An analysis of the draft human genome sequence indicated that up to 5% of the human genome might be comprised of segmental duplications greater than 10 kb in size[4]. As the human genome sequence approaches completion, many more segmental duplications will probably be identified. So, in the future, more instances of segmental duplications will, no doubt, be shown to be responsible for various genomic disorders. We expect these regions of genomic instability to be principal contributors to the burden of cytogenetic abnormalities seen in a clinical setting.

Several models, including those presented here, have been proposed for the mechanisms that are involved in chromosomal rearrangements that are mediated by segmental duplications. It is unclear whether there are precise sequences in the complex, segmental duplications that are directly associated with facilitating the rearrangements. Recombination-promoting sequences have been identified in the

ACENTRIC
A chromosome or chromosomal fragment that lacks a centromere.

PARACENTRIC INVERSION
An inversion of a chromosomal segment that does not contain the centromere.

22q11 segmental duplications[5,25,30], CMT1A-REP[47,48] and NF1-REP[49]. Based on the example of t(11; 22), it is likely that the unstable, recombination-susceptible sequences present at the breakpoints might lead to double-stranded breaks, which in turn promote the observed chromosomal rearrangements. Further investigation of the precise breakpoints in several individuals with each type of genomic disorder will shed additional light on the mechanism of such recombination and rearrangement events.

Genetic change, besides being a principal driving force for evolution, is also a source of human disorders. Segmental duplications represent an under-appreciated source of genetic change owing to their ability to act as substrates for aberrant genomic rearrangements. Although many segmental duplications in the human genome have been identified and sequenced, very little is known about their formation and amplification in the genome. Furthermore, the extent that these segmental duplications contribute to normal human variation is not known. To better understand segmental duplications and their effect on human disease, future efforts will need to be aimed at elucidating the mechanisms that are involved in their formation and spread in the human genome. It should also be interesting to determine whether segmental duplications have amplified during recent human evolution, which might have resulted in genomic variability in the human population. Such a finding should help to assess the involvement of segmental duplications in creating genetic variation that could lead to the genomic instability that is associated with human genetic disorders.

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Online links

DATABASES

The following terms in this article are linked online to:

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
ELN | emerin | *HERC2* | *IDS* | *NF1* | *PMP22* | *STS*

OMIM: http://www.ncbi.nlm.nih.gov/Omim/
Angelman syndrome | cat eye syndrome | Charcot–Marie–Tooth disease type 1A | DiGeorge syndrome | Emery–Dreifuss muscular dystrophy | haemophilia A | hereditary neuropathy with liability to pressure palsies | Hunter syndrome | neurofibromatosis type 1 | Prader–Willi syndrome | Smith–Magenis syndrome | velocardiofacial syndrome | Williams–Beuren syndrome