DISEASE MECHANISMS

A genomic view of mosaicism and human disease

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Abstract | Genomic technologies, including next-generation sequencing (NGS) and single-nucleotide polymorphism (SNP) microarrays, have provided unprecedented opportunities to assess genomic variation among, and increasingly within, individuals. It has long been known that cancer is a mosaic genetic disorder, but mosaicism is now apparent in a diverse range of other clinical disorders, as indicated by their tissue distributions and inheritance patterns. Recent technical advances have uncovered the causative mosaic variant underlying many of these conditions and have provided insight into the pervasiveness of mosaicism in normal individuals. Here, we discuss the clinical and molecular classes of mosaicism, their detection and the biological insights gained from these studies.

Germline mosaicism
The diploid germ cell precursors in the gonad are heterogeneous: some have a mutation and some do not.

Somatic mosaicism
The non-germ cells of the body are heterogeneous: some have a mutation and some do not.

Lines of Blaschko
Streaky lines visible on the skin that radiate inferolaterally from the area over the dorsal spine. They are the consequence of the migration of neuroectodermal cells from the closure of the neural tube.

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Mosaicism is a biological phenomenon named after the intricate images created by craftsmen from small pieces of coloured tiles or glass; it describes an individual who has developed from a single fertilized egg and has two or more populations of cells with distinct genotypes[1]. This criterion of being formed from a single fertilized egg distinguishes mosaicism from the related phenomenon of chimerism, which describes an individual comprised of multiple cell lineages derived from distinct fertilized eggs (BOX 1).

Furthermore, the generation of genetically distinct cells from a single zygote necessitates postzygotic *de novo* mutational events[2] as the cause of mosaicism, and such mutations can result in sporadic disease. Note that *de novo* mutational events can also occur prezygotically; in these cases, it may be a parent who is mosaic (and usually unaffected), but the mutation might be inherited in the zygote and potentially in all cells of the developing offspring (that is, constitutional or non-mosaic) also to result in the formation of a *de novo* disease phenotype[2]. Beyond these general descriptions, there are more specific types of mosaicism that describe which parts of the body harbour the variant cells and the potential for transmission to offspring. These include germline mosaicism (also known as gonadal mosaicism), somatic mosaicism and gonosomal mosaicism (a combination of germline and somatic mosaicism) (FIG. 1). Although these classifications are useful in a practical sense, we acknowledge that they cannot be conclusively assigned owing to the limitations of tissue sampling. For example, the labelling of a patient with germline mosaicism is typically based on the detection of a mutation in multiple germ cells (typically sperm) and on the absence of the mutation in peripheral blood and/or skin fibroblasts; however, this analysis cannot formally exclude the presence of the mutation in other somatic cells.

Historically, mosaicism has been phenotypically recognized in humans and in animals. Coat colour variegation in animals, human dermatological disorders that follow the lines of Blaschko, heterochromia irides and other traits are readily recognizable as representing genetic differences. The pattern of the mosaic distribution of mutations is largely determined by normal embryological processes of cell replication, cell migration and apoptosis, and by the timing and pathophysiological effects of the mutation. For example, the cutaneous ectoderm and neural crest differentiate from ectoderm and then migrate radially from the dorsal neural tube[3]; this is the basis of the lines of Blaschko. Beyond these readily observable traits, it had been much more challenging to evaluate mosaicism until the advent of molecular techniques that can assay individual cells or subpopulations of cells. These techniques have demonstrated that mosaicism is not limited to grossly observable phenomena, such as dermatological disorders. These technological advances have also expanded the range of mutation types that can be detected. Chromosomal alterations (such as whole-chromosome aneuploidy, segmental aneuploidy (that is, aneuploidy of parts of chromosomes) and structural alterations) have been historically identified by cytogenetic analyses[4], but multiple advances in

Heterochromia irides
Describes an individual with irises that are of distinctly different colours.

Aneuploidy
A human cell with other than a multiple of 23 chromosomes.

resolution and throughput for DNA analysis tools[5] have facilitated the use of microarray-based and sequencing-based approaches to identify copy number changes, rearrangements and substitutions at single-nucleotide resolution.

Of course, one of the best known and clinically most important forms of mosaicism is that of cancer. The accumulation of hundreds to thousands of somatic alterations can convert a cell from normal into malignant. Because of the complexity of these myriad genomic changes and the clinical distinctiveness of cancer, coupled with the large number of excellent reviews on this topic[6,7], we have chosen not to cover this subject here and instead to focus on non-oncological disorders.

Box 1 | **Chimerism**

A chimaera is an individual composed of two or more genetically distinct cell lines originating from different zygotes. Chimerism can therefore be distinguished from mosaicism by the extent of genotypic differences. In mosaicism, nearly all loci are identical in the different cell populations as all cells are derived from the same zygotic genotype, but in chimerism there are divergent genotypes all across the genome. Chimerism may lead to medically important phenotypes, such as intersex phenotypes if the sex of the fertilized eggs is disparate[84]. Although chimerism can be established early in development (naturally or artificially by fusion of two fertilized eggs), it can also be established later in development, through placental vascular anastomoses in twin gestations. Chimerism can also be established by artificial means, such as cellular transplant. An individual who has undergone a successful allogeneic bone marrow transplant is a chimaera.

Differentiation of mosaicism from chimerism was difficult before the introduction of single-nucleotide polymorphism (SNP) arrays, as it required the genotyping of a battery of polymorphic markers after the suspicion of chimerism had been raised. In some patients, the potential chimerism could be identified on the basis of cytogenetic findings, such as a patient with both 46,XX and 46,XY cells, but in many patients, the two distinct cell lines may appear cytogenetically indistinguishable. Several individuals were reported in whom the two cells lines were cytogenetically distinguishable, with one abnormal cell line, such as reported for 45,X/46,XX and 46,XX/47,XY,+14 chimaeras[85,86]. Using SNP arrays, the diagnosis of chimerism is fairly straightforward, as the increased number of genotypes across all of the chromosomes in a chimeric individual can easily be recognized. By contrast, in a mosaic individual, typically only one part of the genome (such as a whole chromosome or a chromosome segment) is found to have the altered genotype frequencies that are consistent with mosaicism. In addition, it is possible to determine the mechanism of chimerism by analysis of the genotype patterns on the SNP array, with differentiation of possible mechanisms such as dispermic fertilization and parthenogenetic activation[29,86,87].

The use of SNP arrays has facilitated the discovery of unique cases of chimerism, including several in which one of the cell lines had uniparental disomy for all chromosomes[29,88]. We hypothesize that in this unique situation, the uniparental cell line was 'rescued' by the presence of the normal, biparental cell line. The phenotypes of individuals with complete uniparental disomy can include features of several different imprinting disorders, depending on the pattern of origin for the uniparental cell line. Reported patients with chimeric, whole-genome uniparental disomy presented with clinical features of Prader–Willi, Beckwith–Wiedemann and Russell–Silver syndromes[29,86,89].

Phenotypically normal individuals can also be chimeric, and this may come to light only if there is a reason to pursue genetic testing. An example of chimerism was brought to light when an adult female and her family underwent histocompatibility testing in preparation for a kidney transplant, and the results unexpectedly showed that she did not appear to be the biological mother of two of her three sons. After several lines of testing, she was found to be chimeric in some tissues, including fibroblasts, although there was no evidence of chimerism in her peripheral blood. In this case, both cell lines were 46,XX and had no obvious cytogenetic differences[90]. Clearly, chimerism could also have implications for forensic testing.

In this Review, we first describe a number of categories of mosaicism that have been clinically delineated. We then discuss some of the technical advances that have enabled mosaicism to be detected and discuss the different molecular types of mosaicism that are now becoming appreciated. Finally, we discuss the wider implications for medicine and biology and speculate on the future.

Clinical manifestations of mosaicism

Much of our early knowledge of mosaicism was based on clinical observations of readily recognizable intrapatient phenotypic variations. These included pigment variations and other abnormalities of skin development, such as hyperkeratosis, atrophy or hair distribution, which allowed clinicians to develop aetiological hypotheses that led to early models of mosaicism. The nuances of these clinical observations turned out to be prescient in that several hypotheses that were generated inspired molecular research that has led to remarkable insights into this field. These discoveries included various molecular mechanisms to account for the origins and pathological consequences of mosaicism and also how mosaicism can offer an explanation for unusual inheritance patterns and genetic test results that might otherwise have been assumed to be merely coincidence, chance reoccurrence or incorrectly assigned parentage.

Mosaic disorders that are clinically obvious fall into distinct categories. In all of these classes, the underlying postzygotic *de novo* mutation is likely to occur randomly to generate mosaicism and thus usually manifests clinically as sporadic disease in individuals with unaffected parents. However, the developmental timing and cell lineage affected, combined with the phenotypic consequences of the mutation, ultimately determine the tissue distribution of mosaicism (that is, somatic, germline or gonosomal) and also the patterns of disease reoccurrence within families. These clinical phenomena teach us important lessons about the biology of the genes that are mutated in these traits.

Mosaic manifestations of Mendelian disorders. A major class of mosaic disorders is those with mosaic forms of the same mutations that underlie disorders that are usually inherited in an autosomal dominant pattern. In the autosomal dominant disorders, the mutations are typically constitutional and are transmitted through the germ line to affected offspring; thus, these mutations are compatible with viability (albeit, a diseased state) when constitutional.

As a classic example, neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders in humans. Although the overwhelming majority of patients with NF1 harbour a germline constitutional mutation in *NF1*, some patients have been described who have so-called segmental NF1. These patients have clinical manifestations of NF1 limited to only a single portion of their body[8]. These postzygotic *de novo* mutations apparently arise in development during organogenesis and are unilateral, as evidenced by the topographically limited and lineage-restricted manifestations in these patients and the absence of disease in either parent. The

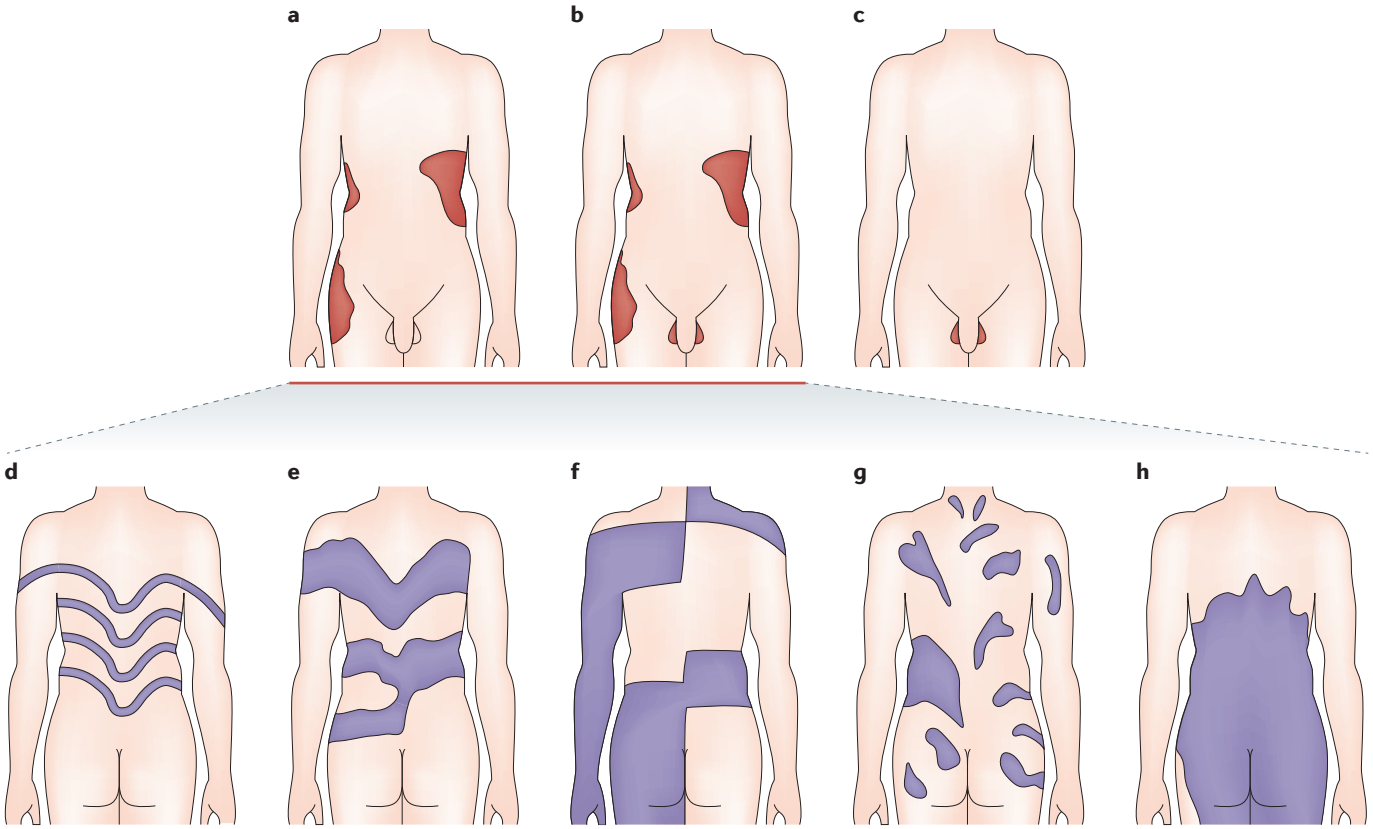


Figure 1 | **Types of mosaicism and patterns of cutaneous mosaicism.** The main types of mosaicism are somatic (**a**), gonosomal (**b**) and germline (**c**). Recognizable patterns of somatic mutations in the skin have been described. These include narrow lines of Blaschko (**d**), broad lines of Blaschko (**e**), checkerboard pattern (**f**), phylloid pattern (**g**) and patchy pattern without midline separation (**h**). The figure is modified, with permission, from REF. 9 © American Medical Association.

Structural alterations
Describes genomic changes that can be balanced, large-scale rearrangements without copy number changes (such as translocations and inversions) or large deletions and duplications that result in copy number changes.

Hyperkeratosis
A dermatological condition consisting of a thickening of the keratin-rich layer of the epidermis.

Atrophy
A condition of tissue volume loss due to disuse or lack of trophic stimulation.

Soma
All cells of the body other than the germ cells.

Expressivity
The degree to which a trait manifests in an individual who has some recognizable manifestation of the disorder.

distributions of these cutaneous manifestations have been categorized by Happle[9] into five distinct types, although these may be better considered as exemplars rather than as discontinuous clinical categories (FIG. 1). Some patients with such mosaicism have affected children[10]. In other cases, patients seem unable to pass the mutation on to offspring, suggesting that the mutation can be limited to a particular part of the soma and thus can be absent in their germ line. Interestingly, many dominantly inherited disorders, such as NF1, can have extraordinarily variable expressivity, and therefore chance variations in expressivity can be mistaken for apparently segmental mosaicism (BOX 2). Segmental NF1 can, like germline forms of the disease, be caused by a wide variety of *NF1* mutations, including point mutations[11] and copy number changes[12].

Other autosomal dominant disorders have been reported to manifest somatic mosaicism. A few examples in which the molecular aetiology has been demonstrated include hereditary haemorrhagic telangiectasia caused by endoglin (*ENG*) or activin A receptor type II-like 1 (*ACVRL1*) mutations[13,14] and Darier–White disease caused by mutations in *ATP2A2* (REF. 15). However, it is important to note that studies of the familial occurrences of these disorders had already identified the causative gene, thus facilitating the characterization of the molecular aetiology in the mosaic individuals.

Disorders that manifest only as mosaicism. In contrast to mosaic manifestations of Mendelian disorders, some mosaic disorders are caused by mutations that are seen only in mosaic form and that are incapable of germline transmission[16,17]. This might be caused by different mechanisms. For example, although a somatic mutation might occur in any cell, if this cell were a gonadal precursor cell and the mutation specifically caused apoptosis in the germ cells, the germ cell lineage would be extinguished by the mutation, such that the mutation and its phenotypic manifestations would be limited to non-germline cells. Thus this mutation would be truly somatic and unable to be transmitted though the germ line. An alternative mechanism for germline non-transmission is when a gonosomal mosaic mutation is lethal when constitutional. Although germ cells may harbour this mutation, it would be lethal in its constitutional state during subsequent embryonic development. In these patients, the mutation is never transmitted through the germ lineage and can manifest only as a somatic disorder.

The existence of this class of disorders was initially based on the observations that there were no familial occurrences, and for some disorders all patients appeared to manifest cutaneous symptoms in a segmental, rather than constitutional, pattern. This

Box 2 | **Variable expressivity**

Monozygotic twins provide a natural opportunity to distinguish mosaicism from variable expressivity: that is, whether distinct genotypes or varied expression of a common genotype underlie differences in the severity of disease manifestation. Recently, a pair of monozygotic twins who were discordant for the neurofibromatosis type 1 (NF1) phenotype and an *NF1* mutation has been described[91]. The affected twin met clinical diagnostic criteria for NF1 and had a c.4108C→T (p.Gln1370X) nonsense mutation in *NF1* in 30–40% of peripheral blood cells, 4% or less of buccal epithelia but not in excreted bladder epithelial cells. No mutations were detected in the blood, buccal epithelia or bladder epithelia of the unaffected twin or in the peripheral blood of the parents. By contrast, the monozygotic twins reported by Kaplan *et al.*[92] were discordant for NF1 but had a different molecular status. The affected twin was apparently constitutional for a c.5902C→T (p.Arg1968X) nonsense variant, whereas the unaffected twin was mosaic for that same alteration. These reports highlight several important features of mosaicism. First, monozygotic twins provide a special opportunity to observe somatic mutations because the mosaicism event can occur before or after twinning, can lead to diverse phenotypic consequences and can illuminate the mechanisms of mosaicism. Second, the mutational load and distribution of the mosaic genomic alteration can have dramatic effects on the clinical manifestation of the mutation, and this phenomenon demonstrates the important principles of penetrance and expressivity. The first set of twins described above showed that somatic mutations could arise in gestation after twinning and generate discordance primarily because the other twin has no mutated cells detected. The second set of twins showed that the somatic mutation can occur before twinning and can lead to discordance because the level of mosaicism did not reach that necessary for clinical expression in one of the twins.

In addition to mosaic genomic alterations, another possible explanation for the variable expressivity seen in monozygotic twins might be differences in epigenetics. Gene expression is affected not just by gene sequence but also by chemical modifications of DNA, such as cytosine methylation. Epigenetic differences have been shown to emerge over time in monozygotic twins and can be hypothesized to account for phenotypic discordance in genotypically identical monozygotic twins[93]. This hypothesis was tested in a cohort of twins with NF1, and evidence was found for an association of epigenetic differences with phenotypic discordance[94].

hypothesis was later refined to include the observation that these disorders are also observed in discordant monozygotic twin pairs, implying that the mutation occurred in the somatic cells of one twin rather than in the parental germ line or in the shared embryo pre-twinning.

The prototype of this class of disorders is McCune–Albright syndrome (MAS)[18,19], which manifests as bony hyperostoses, café-au-lait spots and endocrine dysfunction. Observations of MAS led Happle[20] to suggest his hypothesis of lethal genetic mutations that can manifest only as mosaics. The molecular aetiology of MAS was delineated in 1991 when it was shown to be caused by mosaic gain-of-function mutations in *GNAS1* using a classic forward genetics approach[21]. Other related disorders include Proteus syndrome[22] and linear nevus sebaceous syndrome (for a review, see REF. 23). The discoveries of the genetic cause of these other disorders awaited technological advances, as described below. There are also several chromosomal syndromes that are only seen in a mosaic form (Pallister–Killian syndrome and trisomies for multiple chromosomes, such as 8, 9 and 14), as discussed below.

Germline mosaicism. The final group of disorders that we want to delineate are those that apparently primarily manifest as germline mosaic disorders. This group of disorders comprises various conditions that are typically inherited in an autosomal dominant pattern but that occasionally manifest in a pattern that suggests autosomal recessive inheritance. The prototype of these disorders is osteogenesis imperfecta type II, which was observed to have frequent sibling recurrences with parents that are typically unaffected or that sometimes show a milder variant phenotype[24]. This is consistent with the mutation occurring during gametogenesis in one of the parents: the subset of parental gametes affected will rarely have a phenotypic consequence in that parent but can be passed on constitutionally to multiple offspring. This disorder has aetiological heterogeneity, being caused by mutations in either collagen, type 1, alpha 1 (*COL1A1*) or *COL1A2* (REFS 25,26). Although it was suspected that this disorder was recessive, it was subsequently shown that mosaicism was common in parents of the affected siblings[27].

Interestingly, the frequency of germline mosaicism is highly variable among various autosomal dominant disorders. For example, whereas pseudoachondroplasia is much less common than achondroplasia, it has more reported occurrences of germline mosaicism. This suggests that mosaicism for mutations in these genes has biological consequences for the germ line; these may be related to the effect that has been shown for *de novo* postzygotic mutations[28].

Detection of mosaicism

There is no reason to assume that mosaicism is limited either to disorders that have these easily observable clinical attributes or to those that have a known molecular aetiology. In fact, recent technological advances have provided us with tools to assess mosaicism on a much broader scale, in many disorders and in diverse tissues, with increasing resolution to detect smaller-scale mutations, and we predict that these tools will rapidly change our field.

Tissue type considerations. The detection of mosaicism in human disease has been challenging both because it requires analysis of ample cells within a given tissue and because mosaicism may be tissue-specific or tissue-limited. Therefore, the detection of the mosaicism in the tissue in which it occurs may require analysis of multiple tissues within an individual. In some cases, the choice of tissue is suggested by recognition of a suspected syndrome or phenotype, such as in the case of Pallister–Killian syndrome or when patchy pigmentation is detected. In the absence of phenotypic clues to trigger the search for mosaicism, its detection relies on using sensitive genotyping techniques — such as single-nucleotide polymorphism (SNP) microarrays or next-generation sequencing (NGS) — that can detect low-level mosaicism in a more routine fashion[29,30]. In some cases, a vigilant clinician will request analysis of multiple tissues to rule out low-level mosaicism: blood, skin, saliva and any particular affected tissues being the most common. Of course, not all tissues are amenable to analysis, and it is not always possible to predict which tissues might be affected.

Segmental mosaicism
This is a subtype of somatic mosaicism: an anatomically recognizable portion of the body has cells that have a mutation that is not present in other parts of the body.

Hereditary haemorrhagic telangiectasia
A disorder of vessel dysplasia that can be caused by mutations in a number of genes.

Darier–White disease
A disorder of heterogeneous skin lesions, which can include warty papules, plaques, and seborrhoea, caused by mutations in *ATP2A2*.

Penetrance
The proportion of individuals with a specific phenotype among carriers of a particular genotype.

Discordant monozygotic twins
Twins that result from the fission of a single fertilized inner cell mass but who have a distinct phenotypic difference between them.

Bony hyperostoses
Focal overgrowths of bone and osteoid (partially calcified bone matrix).

Café-au-lait spots
Light brown macules of the skin that are a common manifestation of neurofibromatosis, McCune–Albright syndrome and several of other disorders.

Proteus syndrome
A disorder of mosaic, progressive overgrowth caused by mutation in *AKT1*.

Nevus sebaceous
A skin lesion characterized by overgrowth of sebaceous glands.

Pallister–Killian syndrome
A disorder of dysmorphic features and intellectual disability caused by mosaic tetrasomy of chromosome 12p.

Osteogenesis imperfecta type II
A disorder of bone fragility and short stature caused by mutations in *COL1A1* or *COL1A2*.

Pseudoachondroplasia
A disorder of short stature and dysmorphic features, generally less severe than achondroplasia, caused by mutations in *COMP*.

Achondropasia
A disorder with severe short stature and dysmorphic features caused by mutations in *FGFR3*.

Nondisjunction
The failure of chromosomes to segregate normally during cell division, resulting in the mis-segregation of chromosomes into daughter cells. Nondisjunction at meiosis I results in products with additional or missing chromosomes that are genetically distinct (homologues), whereas nondisjunction at meiosis II results in missing or extra sister chromatids.

Cytogenetics. Cytogenetic analysis by study of banded metaphase chromosomes or by fluorescence *in situ* hybridization (FISH) is carried out in a cell-by-cell manner, and recognition of mosaicism is therefore fairly straightforward. Mosaicism is recognized when cells within an individual are found to have divergent chromosome contents, such as monosomic or trisomic chromosomes in the karyotype of one cell and a normal karyotype in another. Mosaicism has been recognized for cytogenetic abnormalities from the earliest usages of this technique[31]. Although mosaic aneuploidy was traditionally the most detected form of mosaicism, mosaicism for a wide variety of chromosome abnormalities has now been identified[32]. The cytogenetic detection of low-level mosaicism is challenging, as a sufficient number of cells must be counted[33].

Microarray-based techniques. Beginning in 2005, microarray-based techniques began to replace cytogenetic testing, with the introduction first of array-based comparative genomic hybridization (aCGH), which can analyse genomic copy number variants (CNVs), followed by genome-wide SNP arrays (FIG. 2A), which can analyse both SNPs and CNVs. The advantages of array-based testing (which typically analyses DNA extracted from whole blood) for mosaicism detection include: many cells are analysed simultaneously; different cell types are analysed; cells of all cell cycle phases are analysed; and samples do not require culturing, which might itself cause mutations. Several studies using aCGH tested the ability of this technique to identify mosaicism, and it was demonstrated that mosaicism could be identified when variant cells constituted >10% of the total cell population. SNP arrays are much more sensitive than aCGH for mosaicism detection, and mosaicism involving <5% of cells has been detected using these arrays[29,34]. In addition to detecting mosaicism at lower levels, SNP arrays are also able to analyse the zygosity of the SNPs, thus aiding the analysis of the genetic mechanism by which the mosaicism has occurred, which is discussed below[29].

DNA sequencing. Although Sanger-based sequencing can be extremely effective for many applications, including the positional cloning of disease genes, it has several limitations. These include its limited throughput and the fact that both alleles of an autosomal locus are sequenced concurrently and are displayed as analogue electropherograms, from which the often subtle contribution from mosaic alleles cannot be accurately determined (FIG. 2Ba–d).

NGS is a high-throughput technique that massively parallelizes genomic interrogation (for reviews on this technology, see REFS 35,36). In addition to the difference in throughput, NGS is inherently a digital assay that reports read counts for each allele as integer counts (FIG. 2Be). These digital data are more amenable to statistical approaches to distinguish mosaicism from sequencing errors. Furthermore, sequencing can potentially detect any *de novo* sequence variants, not just those that are represented on SNP arrays.

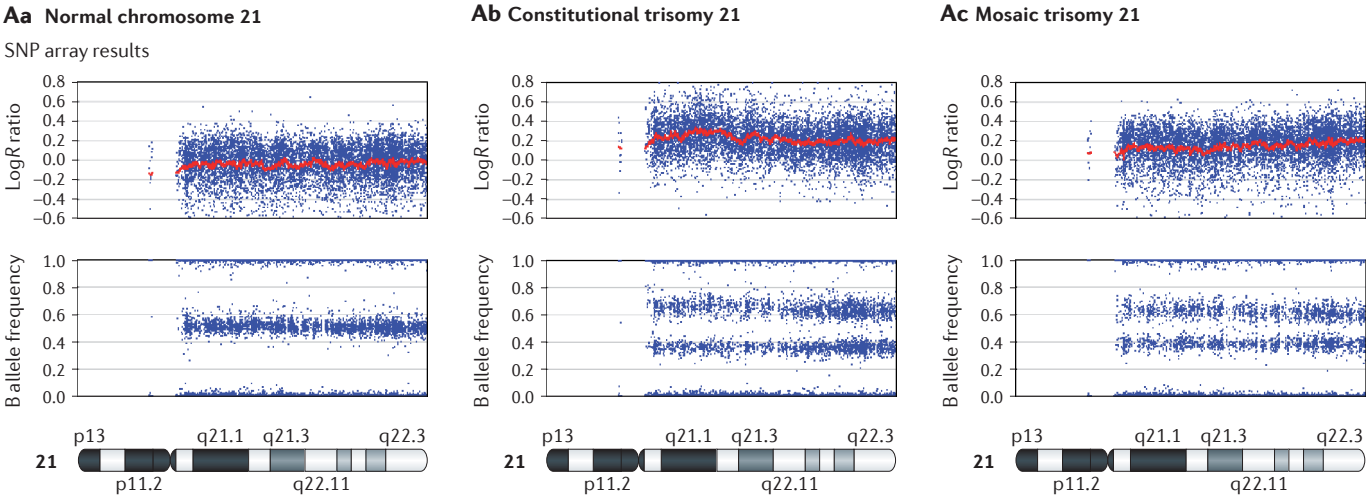
The massive throughput of NGS has another benefit, which is that it does not require genetic mapping data to identify causative genetic variants. For this reason, subtractive informatic approaches — in which two samples are informatically processed to identify only those positions in the genome that differ — can be coupled to NGS to identify mosaic alterations. Much as trio sequencing (that is, parents and child) has been used to identify *de novo* alterations as a cause of non-mosaic heritable disorders[2], intra-patient subtraction can be used to identify mosaic disorders, as has been done in cancer genomics, by comparing DNA samples from affected and unaffected tissues from the same patient. This approach has a measurable false-positive rate, which is due to a combination of molecular and informatic errors, yet has been successfully used to identify numerous mosaic disorders, as described below.

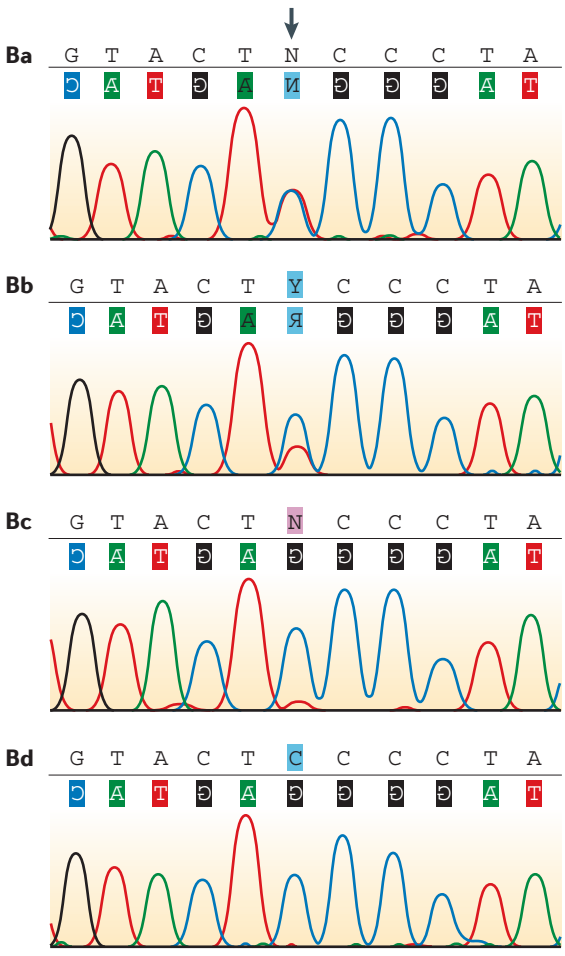
Molecular classes of mosaicism

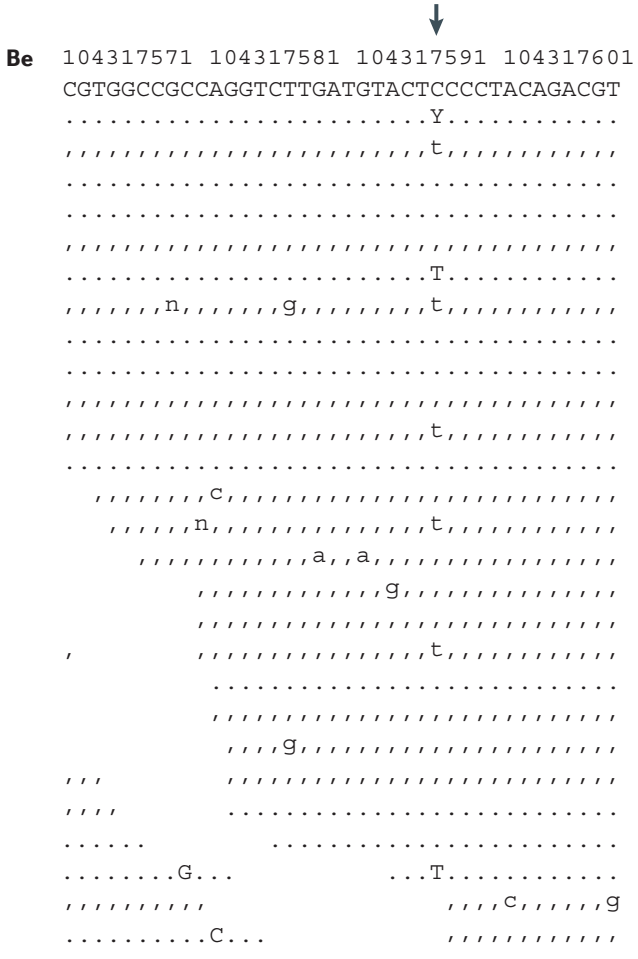
Large-scale chromosomal abnormalities. Chromosome abnormalities are a fairly common cause of developmental disorders, occurring in 1 in 200 liveborn individuals and in greater than 50% of spontaneous abortuses. Chromosome abnormalities cause disease because of missing or extra chromosomal material (either whole chromosome or segments of chromosomes (segmental)) or because a structural rearrangement interrupts a gene or separates an enhancer from its target gene. Mosaicism for many types of chromosome abnormalities is well known in both abortuses and liveborn individuals[37,38]. Recent analyses of embryos following *in vitro* fertilization have shown that 70% of embryos studied had mosaic segmental imbalances, which was higher than anticipated[39]. These studies reveal the high rate of mitotic errors that have potential to contribute to mosaic human disease.

Whole-chromosome aneuploidy is the most common type of chromosomal abnormality. Mosaicism can result from chromosomal nondisjunction during meiosis to generate germline mosaicism or in mitosis during development to result in somatic mosaicism. A constitutional gain or loss of only a few single chromosomes is compatible with viability: trisomy of chromosomes 13, 18, 21 and X, and monosomy of only chromosome X have been found in liveborn individuals. Consistent with mosaic aneuploidy being less serious than constitutional aneuploidy, all of these trisomies or monosomies have also been detected as mosaic abnormalities, usually with a milder phenotype than the constitutional aneuploidy, and an additional class of aneuploidies can be found only when they are mosaic, presumably owing to selection against the aneuploid cells in a specific tissue or at a specific stage of development. These include trisomy of chromosomes 8, 9, 14, 17 and 22 (REFS 40,41).

Mosaicism for structural chromosome abnormalities is less common than is seen for aneuploidy, but nevertheless mosaicism for many types of structural abnormalities has been identified, including balanced and unbalanced translocations, deletions, duplications, inversions, ring chromosomes and isochromosomes[42–46]. Errors in either mitosis or meiosis have been suggested







◀ Figure 2 | **Detection of mosaicism.** **A** | Single-nucleotide polymorphism (SNP) arrays are sensitive to the presence of copy number mosaicism. **a** | A view of chromosome 21 from a normal SNP array (Illumina Quad610 array). The upper plot shows the log*R* ratio, where *R* is a normalized intensity value that portrays the relative amount of each SNP across chromosome 21 compared to diploid individuals (ratio of 1; that is, a log*R* ratio of 0), whereas the lower plot shows the B allele frequency and indicates the genotypes. The B allele can have values of 1 (BB), 0.5 (AB) and 0 (AA) in a diploid individual. Schematic views of representative cell populations, including the ploidy of chromosome 21, are indicated below. **b** | A SNP array from an individual with constitutional trisomy 21. Here, the log*R* ratio is higher than for the normal disomic sample, mapping close to 0.2. On the B allele frequency plot, the additional bands are characteristic of the trisomic cell line, taking values of 1 (BBB), 0.67 (ABB), 0.33 (AAB) and 0 (AAA), which is expected if all cells have the same third copy of chromosome 21. **c** | The SNP array demonstrates mosaic trisomy and has a log*R* ratio (~0.1 in this example) and B allele frequencies (1 for BBB and BB; 0.6 for ABB and AB; 0.4 for AAB and AB; and 0 for AAA and AA) that are intermediate between the constitutional disomic and trisomic states, thus indicating mosaicism. **B** | Sequencing technologies (Sanger and next-generation) detect mosaicism for point mutations. **a** | A Sanger sequence trace can indicate (constitutional) heterozygosity by approximately equal peak heights of the two nucleotides at a single position (C and T in this example). **b** | A sample with 60–70% mosaicism for the mutation (60–70% heterozygous mutant cells and 30–40% homozygous wild-type cells) shows a reduced peak, representing ~35% of the normal peak height. **c** | This sample shows a lower level of mosaicism that is difficult to quantify. **d** | A sequence that is apparently negative for the variant (constitutional wild-type). **e** | By contrast, the next-generation sequencing data give a direct indication of mosaicism for a single-nucleotide variant (SNV). Dots and commas represent bases in agreement with the wild-type reference sequence (shown at the top), whereas letters represent bases that differ. The dots and upper-case letters indicate forward and commas and lower case letters denote reverse sequence read direction. Here, the sample has 17 wild-type reads (cytosine at the position indicated by the arrow) and 7 mutant reads (a C→T substitution), suggesting a ~40% mosaicism for the heterozygous mutation.

for the origins of the structural chromosome mosaics that have been studied, similarly to whole-chromosome aneuploidy.

Ring chromosomes are a unique type of structural abnormality formed by the fusion of two ends of the chromosome into a circular structure. Rings for every human chromosome have been identified, and each of these can occur as a mosaic or constitutionally in all cells studied. Analysis of a cohort of 28 patients with ring chromosome 20 demonstrated that mosaic rings are formed by a different mechanism than are constitutional rings. In every mosaic ring chromosome 20 studied (that is, in 21 out of 21 patients), SNP array analysis showed no deletions, which is consistent with formation via telomere fusion and was confirmed by FISH. In all of the patients with constitutional ring chromosomes (that is, in 7 out of 7 patients), there were deletions found at either or both chromosomal termini. These studies suggest that the mosaic ring chromosomes, which are presumably formed during mitosis, arise through a different mechanism than the constitutional rings, which are presumably formed during meiosis[42].

Isochromosomes are structurally abnormal chromosomes created by the presence of two copies of one of the arms of a chromosome; the other arm is missing. When complemented by a normal homologous chromosome, this creates trisomy for one chromosome arm and monosomy for the other. Several isochromosomes are common enough that recognizable syndromes have been described, including isochromosome 12p (Pallister–Killian syndrome) (FIG. 3), isochromosome 22q (cat eye syndrome), isochromosome 15q11 and isochromosome 18p. Isochromosomes are often supernumerary, presumably because the loss of even one copy of a chromosome arm can be lethal. The one fairly common exception is the isochromosome Xq, as loss of one copy of the short arm of the X chromosome is tolerated, and this isochromosome causes a variant of Turner's syndrome, which is most commonly caused by loss of one complete copy of the X chromosome[47]. The isochromosome 12p seen in the Pallister–Killian syndrome is always present in a mosaic form, demonstrating the example of an abnormality that is lethal when constitutional but tolerated if the abnormal chromosome can be eliminated from key tissues[48] (FIG. 3).

Copy number variants. The use of SNP arrays for genomic analysis has led to a greatly increased appreciation of the frequency of mosaicism for CNVs. Earlier chromosomal microarray studies using aCGH reported mosaic abnormalities in 8% of abnormal results in a diagnostic laboratory studying paediatric patients with a spectrum of developmental abnormalities[49]. However, this percentage has increased with the use of SNP arrays that are more easily able to detect several types of mosaicism, including monosomies and trisomies (by the characteristic changes in probe intensity in combination with the altered genotype frequencies across a whole chromosome) and mosaicism for some types of biparental and uniparental chromosomal regions (by the characteristic changes in genotype frequencies without an accompanying change in intensity, indicating a copy-number-neutral change, such as loss of heterozygosity (LOH))[29,34]. Not all individuals with uniparental disomy (UPD) can be identified by the SNP arrays, however. In patients who have constitutional heterodisomy, a normal genotype pattern will be seen, and the fact that the two chromosomes are inherited from one parent can be detected only if parental samples are analysed in conjunction with their child.

In the cytogenomics laboratory at the Children's Hospital of Philadelphia, analysis of paediatric individuals referred for genomic copy number analysis for various congenital and developmental anomalies has revealed a potentially pathogenic variant in 22% of patients; 17% of these were mosaic, and therefore 3.74% of patients showed a mosaic abnormality[29]. Other studies have reported that of individuals with paediatric disorders that warrant cytogenomic testing, mosaic abnormalities occur in ~0.5–2.0% of these cases[29,49,50]. Mosaicism has been observed both in individuals with paediatric presentation of clinical abnormalities as well as in adults who were studied for various diagnoses, most commonly cancer. The frequency of mosaic abnormalities was found to increase with age in a study of >50,000 individuals enrolled in the Gene–Environment Association Studies (GENEVA) consortium. The diagnoses under study included several types of cancer (including melanoma, lung and prostate) as well as other lung disease, cleft lip and palate, addiction, blood disorders, dental caries,

Cat eye syndrome
A syndrome of dysmorphic features and intellectual disability caused by duplication of a segment of chromosome 22q.

Supernumerary
Extra copies of either a whole chromosome or of a chromosome segment that contains a centromere.

Loss of heterozygosity
Describes that status of a cell or tissue that was originally heterozygous at a genetic locus but owing to somatic alterations is subsequently homozygous or hemizygous.

Uniparental disomy
(UPD). When both chromosomes of a homologous pair are inherited from the same parent. When these chromosomes are different, this is uniparental heterodisomy. When these chromosomes are identical through duplication, this is uniparental isodisomy.

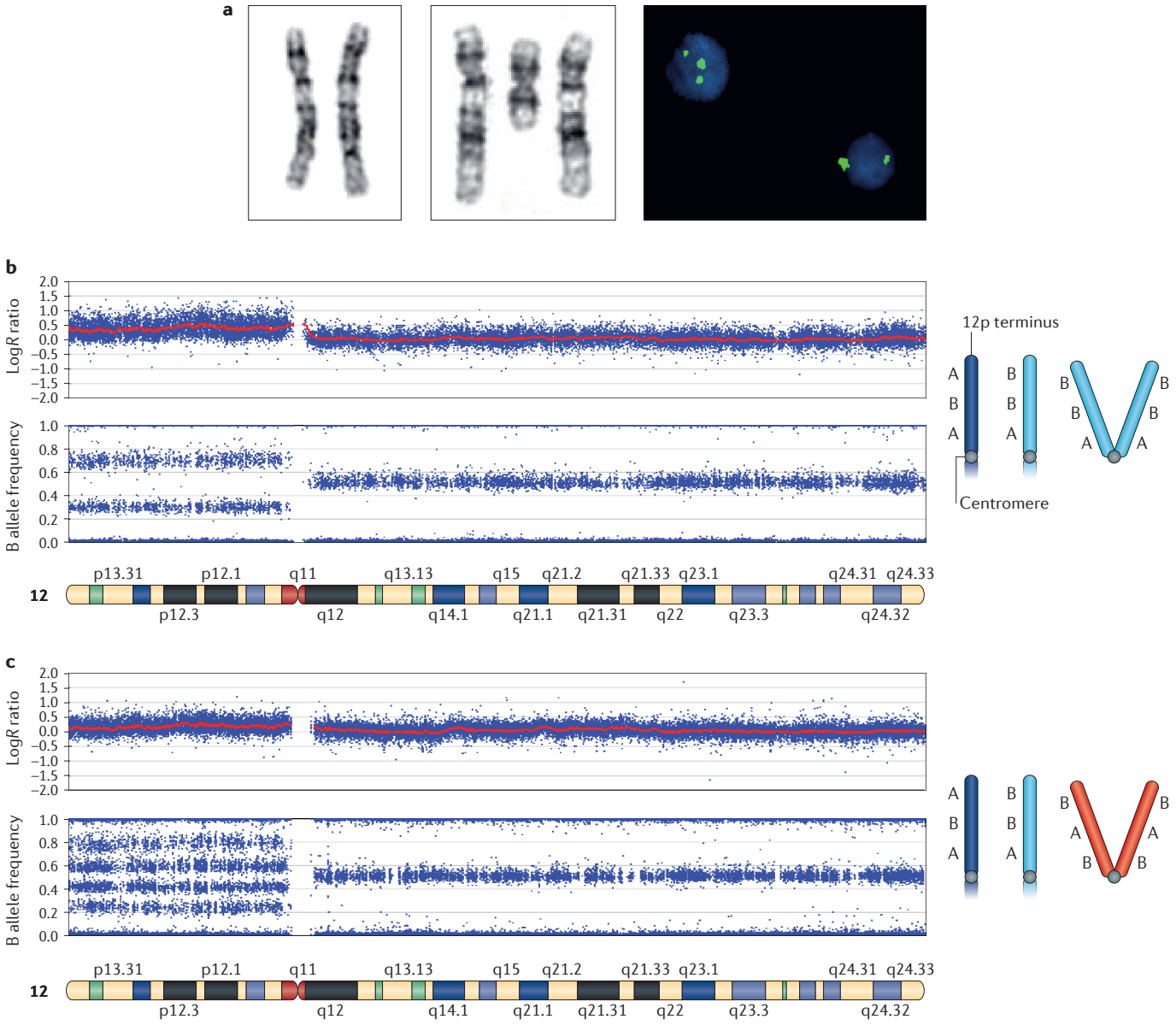


Figure 3 | **Mosaicism for isochromosome 12p in two patients with Pallister–Killian syndrome.** **a** | Both Giemsa (G)-banded partial cells and fluorescence *in situ* hybridization (FISH) show three signals for the centromere of chromosome 12 in one cell and only two in a second cell. **b**,**c** | The single-nucleotide polymorphism (SNP) array profiles both demonstrate the increased genotypes introduced by the isochromosome 12p, but **b** shows a result in which the two extra copies of 12p are the same and are identical to one of the normal 12p chromosome arms (schematically shown to the right of the SNP array). Panel **c** shows a result in which the isochromosome 12p has two arms that are identical, but they are different from either of the normal chromosome 12s, which we can identify by the additional B allele ratio frequencies. These data therefore provide information about mosaicism level and suggest mechanisms for the formation of the isochromosome 12p. See FIG. 2 for an explanation of log*R* ratio and B allele frequencies in the SNP array data. This figure is modified, with permission, from REF. 95 (2012) © Wiley.

prematurity and glaucoma. The frequency of individuals with detectable clonal mosaicism for genomic anomalies larger than 50 kb was less than 0.5% from birth to 50 years of age but rose quickly after age 50 to 2–3%[51,52]. In an independent study of 1,991 individuals with bladder cancer, mosaic genomic abnormalities were also found in 1.7% of samples and were present in both the blood and bladder tissue, suggesting an early origin of the genomic alterations rather than origination in the bladder tissue itself[34]. In another approach to detect mosaicism, induced pluripotent stem cells derived from skin fibroblasts were found to contain an average of two CNVs[53]. Although these CNVs were not initially detected in the parental fibroblasts, sensitive genomic analyses confirmed that at least half of these CNVs pre-existed at a low frequency in the parental fibroblasts[53].

Therefore, the mosaicism that is routinely detected is probably the tip of the iceberg, and between the challenges inherent in sampling adequate tissues across the body and in identifying genetic changes present at low levels, we may be underestimating the true frequency of mosaic alterations.

Copy number variation has been analysed across tissues from the same individual, confirming substantial variation across tissues[54]. This finding has clear relevance for disorders that might be caused by tissue-specific alterations, be they copy number or sequence alterations. Mutations limited to the affected tissue clearly pose diagnostic dilemmas, because they will not be identified from tests on other, more accessible tissues. As expected, mosaic variation has also been demonstrated between identical twins and may explain discordant phenotypes in monozygotic twins[55,56].

Point mutations and small insertions and deletions. Recently, a range of disorders has been shown to be caused by mosaicism for point mutations, primarily using NGS technologies. Non-overgrowth mosaic disorders include benign keratinocytic epidermal nevi, which have been shown to be caused by mutations in fibroblast growth factor receptor 3 (*FGFR3*), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*) and different RAS family members[57–59]. A series of mosaic overgrowth disorders was molecularly delineated, beginning with Proteus syndrome, which was shown to be due to *AKT1* mutations[60], and followed by several other disorders. These include asymmetrical neuronal migration abnormalities and hemimegencephaly caused by mutations in *PIK3CA*, *AKT3*, mammalian target of rapamycin (*MTOR*) and phosphoinositide-3-kinase, regulatory subunit 2 (*PIK3R2*)[61–63], and non-CNS fibroadipose overgrowth and CLOVES syndrome, which are both caused by *PIK3CA* mutations[64,65]. Different types of nevus sebaceous syndromes can be caused by mosaic *HRAS* and *KRAS* mutations[66], and Waldenström macroglobulinaemia can be caused by a mutation in myeloid differentiation primary response gene 88 (*MYD88*)[67].

Most of these overgrowth disorders are caused by mutations that are lethal in a constitutional state and, in addition, are found in tumours. These phenotypes have in common the attribute that they are hyperplastic or hypertrophic abnormalities that are related to the growth-promoting effects of these mutations, which can thus manifest macroscopically even when occurring late in development. It is hypothesized that these mutations are lethal when constitutional, because the aberration of growth regulation that they represent severely disrupts early embryonic development.

However, some growth-promoting mutations, such as activating mutations in *FGFR3* associated with achondroplasia, are compatible with viability when constitutional and can be inherited in an autosomal dominant pattern[68,69]. Furthermore, prezygotic *de novo FGFR3* mutations confer a substantial survival advantage to the male germ line, thus increasing the degree of germline mosaicism and also the frequency of transmission to affected offspring[28].

Other types of mosaicism

Reversion and rescue mosaicism. Mosaicism is not always detrimental. When a disease-causing mutation is present in the parental germ line, it can be inherited and thus be constitutional in the resultant embryo. However, mosaicism can result when that genetic lesion is completely or partially reverted, perhaps driven by selective pressure, in a subset of cells in the multicellular organism. For example, the phenomenon of revertant mosaicism has recently been described, in which a mutation was spontaneously directly corrected in a subset of cells in an affected organ[70,71]. This phenomenon has been demonstrated in blood, muscle, liver and skin, of which skin is most dramatic, as the normal patches can be directly visualized among the abnormal patches. Skin disorders that have demonstrated revertant mosaicism include the highly heterogeneous disorder epidermolysis bullosa and ichthyosis. For one form of epidermolysis bullosa, at least 35% of patients carried revertant patches, demonstrating the high frequency of this phenomenon. The reversion appears to occur through mitotic recombination, presumably with positive selection of cells with the normal allele[62,66].

In a related phenomenon involving aneuploidy, parental meiotic errors could result in a trisomy or monosomy constitutionally in the zygote, leading to subsequent reversion to mosaicism with a normal karyotype during embryonic development through the gain or loss of a chromosome (that is, trisomy or monosomy rescue) (FIG. 4). If mosaicism for a trisomic or monosomic karyotype is identified, it is possible using SNP arrays to determine whether the mosaicism arose from postzygotic *de novo* aneuploidy during mitosis in cells from a normal zygote or rather from postzygotic rescue in cells from a zygote that was aneuploid owing to prezygotic mistakes during meiosis I or meiosis II[29,72]. Monosomy or trisomy rescue can also result in mosaicism for UPD (FIG. 4): duplication of a monosomic chromosome will always result in UPD, whereas loss of a trisomic chromosome will leave either a biparental or uniparental chromosome pair, depending on the parent of origin of the chromosomes that undergo nondisjunction[29,34].

UPD is not limited to whole chromosomes, but UPD for chromosome segments has also been identified (FIG. 4). Segmental UPD appears as a regional LOH for which two copies of the chromosome region are derived from the same parent. Generation of segmental UPD seems to be a general somatic mutational mechanism, rather than being functionally linked to aneuploidy rescue but is discussed here because of its parallels with whole-chromosome UPD. Mosaicism for segmental UPD most probably arises through mitotic recombination or alternatively by chromosome breakage and repair, in which the DNA sequence from one allele is copied across to replace the other allele to generate homozygosity. In yeast, the emergence of LOH has been linked to an age-associated increase in homologous recombination[73].

LOH, including as a result of monosomy rescue or segmental UPD, can cause disease by revealing a mutation in a recessive gene. More generally, UPD (even

Hemimegencephaly
A descriptor for a brain that has substantial asymmetry, with one side being abnormally large and commonly malformed.

Fibroadipose overgrowth
A manifestation of overgrowth that includes excess fatty tissue with fibrous strands caused by somatic mutation of *PIK3CA*.

Waldenström macroglobulinaemia
A malignant B cell neoplasm with lymphoplasmacytic infiltration of bone marrow and excess monoclonal immunoglobulin M.

Hyperplastic
An enlarged tissue caused by an increased number of cells.

Hypertrophic
An enlarged tissue caused by enlarged cells.

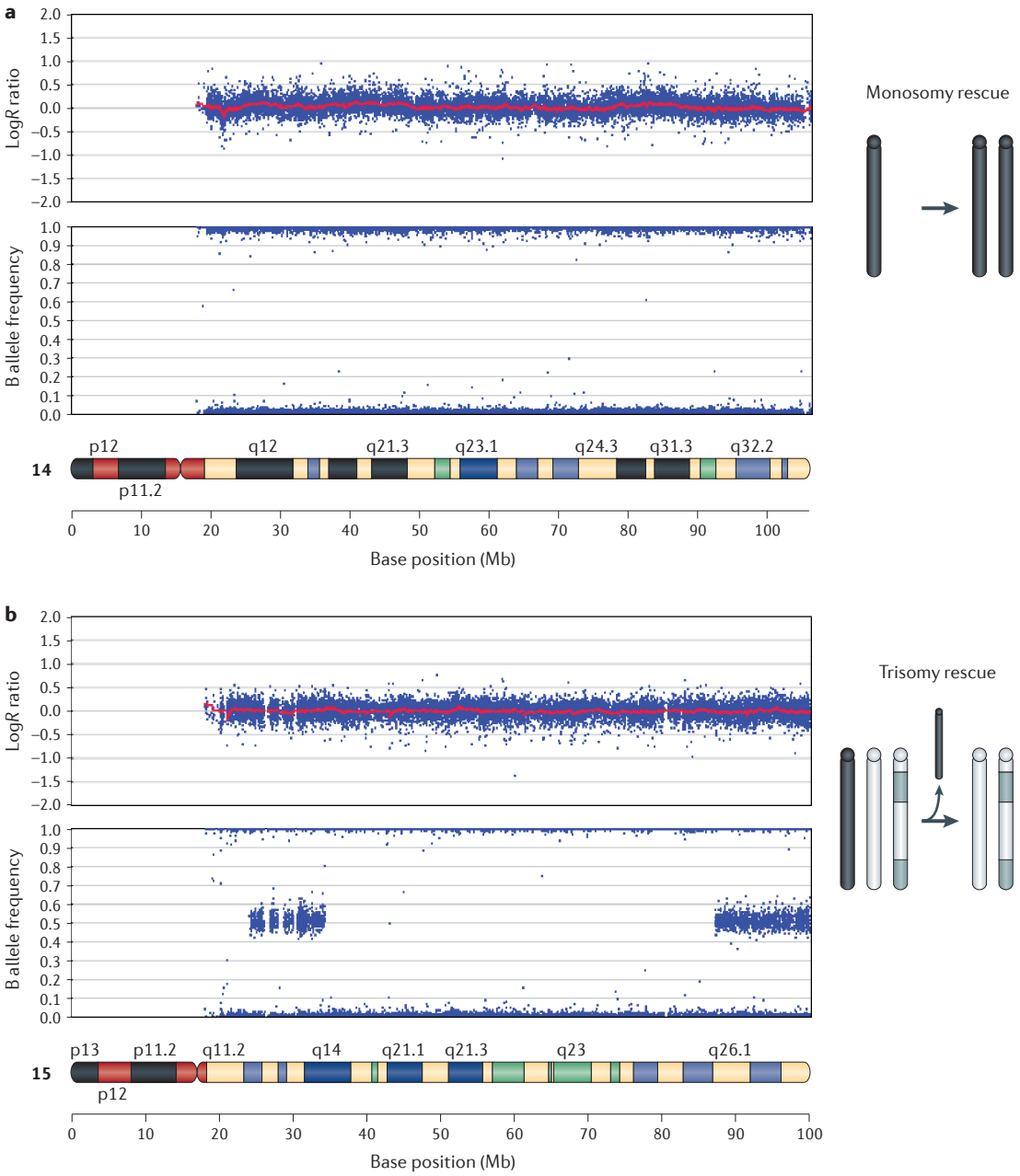


Figure 4 | **Mechanisms of whole-chromosome uniparental disomy.** Here we show two examples of uniparental disomy. **a** | Two chromosomes are present, as indicated by the normal log*R* ratio, but there is no heterozygosity, as shown by the B allele frequencies. This indicates that the two chromosomes are identical, presumably because first there was one chromosome and then it was duplicated. **b** | Again, two chromosomes are present, but they contain regions of homozygosity and regions of heterozygosity. The two chromosomes are from the same parent (as proved by analysis of molecular markers), thus the homozygous regions indicate uniparental isodisomy and the heterozygous regions indicate uniparental heterodisomy. In this patient, the two different chromosomes from one parent must have been present in the resulting trisomic zygote, which is resolved by the postzygotic loss of the one chromosome from the other parent. See FIG. 2 for an explanation of log*R* ratio and B allele frequencies in the single-nucleotide polymorphism (SNP) array data. The figure is modified, with permission, from REF. 29 © Oxford Univ. Press.

Beckwith–Wiedemann syndrome
An overgrowth and tumour susceptibility syndrome caused by imprinting defects of 11p15.

at heterozygous loci following trisomy rescue) can cause disease through the biallelic silencing or biallelic expression of an imprinted gene. Segmental paternal UPD for a portion of chromosome 11p15.5 is a cause of Beckwith–Wiedemann syndrome (BWS) in 10–20% of patients[74]. BWS is characterized by somatic overgrowth, macroglossia and abdominal wall defects and includes other features, such as hemihyperplasia, neonatal hypoglycaemia, renal anomalies and embryonal tumours. The growth promotion is thought to result in part from the biallelic expression of the usually maternally imprinted insulin-like growth factor 2 (*IGF2*) gene in

this region. The 11p UPD is always mosaic, and it has been hypothesized that UPD for this region may be lethal early in development[75].

Confined placental mosaicism. The mammalian zygote gives rise to both the placenta and the embryo proper, and it has been shown in mice that the embryo is derived from only three cells of the inner cell mass of the blastocyst: the remainder goes to form the placenta[76]. This separation of the embryonic and placental tissues very early in development sets the stage for mosaicism arising in the placenta that is not found in the embryo itself (that is, confined placental mosaicism (CPM)). This has implications for prenatal testing, especially in the case of chorionic villous sampling (CVS), in which the placenta is sampled. In ~1% of patients, CVS will identify an abnormality in the placenta that may not be in the fetus, and follow-up studies are required to determine the distribution of the chromosome abnormality. CPM can cause abnormalities in the fetus by two different mechanisms. The first is placental dysfunction caused by the genomic abnormality. The second occurs when a zygote is trisomic, and chromosomal nondisjunction leads to trisomy rescue within a few cell divisions. If only the normal karyotype cell derivatives go on to form the fetus, but both normal and abnormal karyotype cells form the placenta, then CPM will result. Owing to the trisomy rescue, the karyotypically normal fetus might have constitutional UPD, which has disease implications[77].

Implications for counselling

The component of genetic counselling that addresses recurrence risks is challenging for patients with mosaic disorders. As noted above, in the case of a couple who have an offspring with an apparently constitutional, *de novo* occurrence of a disorder that can also be inherited in an autosomal dominant pattern, one of the parents must be considered to be at risk for having germline mosaicism. Therefore, the couple is at risk of passing on the same mutation to additional offspring. The level of this risk is known for a few disorders (for example, osteogenesis imperfecta) but is difficult to estimate for most disorders.

When a child has a mosaic disorder that is not due to a reversion or rescue mutation, by definition this mutation was not inherited from either parent and occurred postzygotically; thus the parents are only at the population risk for having an additional affected child. However, the mosaic, affected child is at risk for transmission to his or her offspring if the disorder is viable in the constitutional state. Their risk is dependent on whether that mosaic mutation is present in his or her germ line and, if so, on the proportion of the germ cell progenitors that harbour the mutation. A notable exception is in some patients with supernumerary chromosomes whose mosaicism resulted from the creation of a population of karyotypically normal cells through the postzygotic loss of the unstable supernumerary chromosome. The germ cells of the parents of these patients are likely to include the original supernumerary chromosome, and thus there may be a risk of transmission to additional offspring.

For disorders caused by mutations that are lethal when constitutional, germline transmission from an affected mosaic person should be impossible. However, the truth of the assumption that the disorder is always lethal when the mutation is constitutive is impossible to prove, raising the possibility of germline transmission of the mutation. Should it come to pass, as we predict here, that several more common phenotypes (such as schizophrenia and autism) are due to mosaicism, it will be challenging to assess the risk of recurrence in the siblings and children of the affected individuals.

Mosaic disorders pose a new challenge for genotype–phenotype correlations and prediction of disease manifestations and severity. When mosaicism is identified early prenatally or early in life, it is difficult to predict which tissues are affected and to what extent, confounding the clinician's ability to prognosticate. Mosaicism can result in a less severe phenotype compared to the same mutation present in a constitutional state[24], implying that more widespread mosaicism is likely to have a more severe phenotype. Even more challenging is that for some mutations, the germline phenotypes can be qualitatively distinct from the mosaic phenotypes. For example, mosaic mutation of the Gly12 residue of HRAS is associated with benign keratinocytic epidermal nevi[57] and also cancer (particularly bladder cancer), but when the mutation is constitutional, it is associated with Costello syndrome[78], which has distinct and pleiotropic clinical manifestations from the mosaic forms. The timing and location of the somatic mutation may explain these distinct phenotypes[57]. Thus, studying both the mosaic and constitutional manifestations of genomic mutations can shed light on biological mechanisms of disease that neither alone can fully illuminate.

Conclusions and future perspectives

The recognition of mosaic disorders is rapidly increasing. It can be evenly distributed throughout an organism, segmental or tissue-specific (FIGS 1,5), and it can affect somatic tissues, the germ line or both. It can arise at various stages of development or adult life and can be caused by mutation from a normal genotype to a variant genotype or from a variant genotype to a normal genotype. Furthermore, recent advances in genomic technologies have enhanced our ability to detect and to characterize diverse molecular types of mosaicism with ever-increasing sensitivity, which has demonstrated the widespread nature of mosaicism in both healthy individuals and in patients with a wide range of disorders.

Recent insights include: identification of disease genes for disorders that are seen only as mosaics as they are lethal when constitutional; the recognition of the frequency of mosaicism in the early embryo (made possible by the ability to analyse single cells); the recognition of the importance of tissue-specific mosaicism in disease (made possible by the ability to recognize low levels of mosaicism in non-dividing cells); and the identification of chimerism by whole-genome tools. Furthermore, recognition of the changing rates of mosaicism with increasing age represents a window on the ageing process.

Costello syndrome
A syndrome of dysmorphic features, intellectual disability and tumour predisposition caused by mutations in the *HRAS* gene. A member of the rasopathy family of phenotypes.

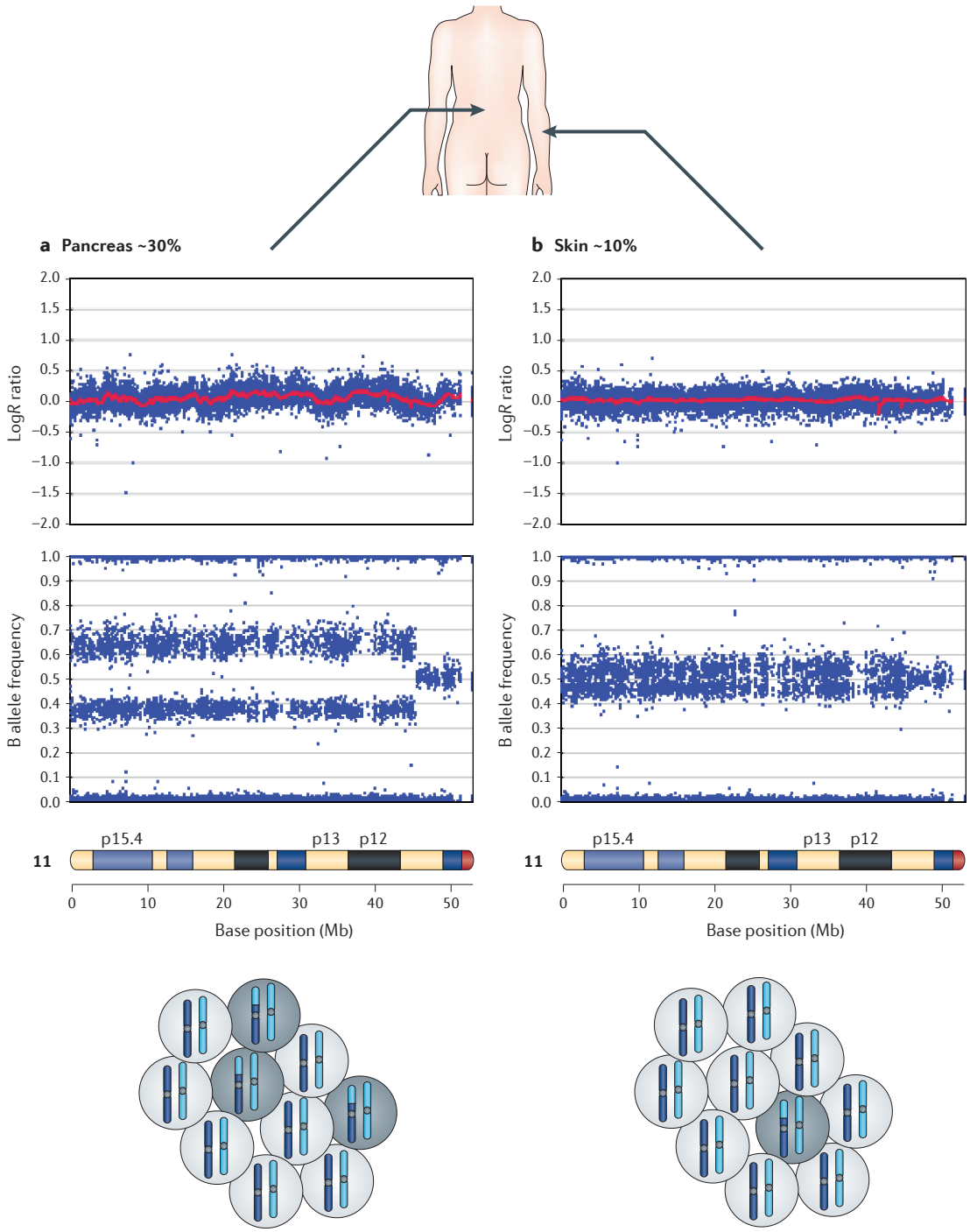


Figure 5 | **Segmental uniparental disomy for 11p in two tissues from one patient.** This figure shows a single-nucleotide polymorphism (SNP) array result for a portion of chromosome 11 showing mosaic segmental uniparental disomy (UPD). This is evident because the log*R* ratio is normal, plotting at 0, whereas the B allele frequency shows a split. See FIG. 2 for an explanation of log*R* ratio and B allele frequencies in the SNP array data. **a** | DNA purified from the pancreas of a patient with hyperinsulism, showing values at 0.65 and 0.35. We estimate that 30% of cells in this tissue demonstrate the segmental UPD. **b** | Analysis of DNA from fibroblasts, which also demonstrate mosaic UPD, but at a lower level, estimated at 10%. The figure is modified, with permission, from REF. 29 © Oxford Univ. Press.

Such advances are facilitating broad studies of mosaicism in disease beyond those disorders in which the mosaicism generates a phenotypic change that is grossly observable — disorders that include cancer, dermatological and overgrowth phenotypes. For the myriad other disorders that do not have this attribute, we suggest that mosaicism is just as common. Diagnosis of tissue-limited mosaicism is clearly challenging when the affected tissue is not blood or skin, which are the two tissues that are most frequently analysed in clinical

laboratories. Nevertheless, there are reports of tissue-limited mutations that may cause organ-specific disease (FIG. 5), as has been reported for schizophrenia and Alzheimer's disease[79], autism[80–82] and cardiac disease[41,83].

Technological progress is also allowing the aetiological analysis of uncharacterized disorders with unusual clinical features and/or inheritance patterns that might be best explained by mosaicism. The ideal attributes of disorders that should be studied with genomic and imaging methodologies to look for mosaicism would be those that have organ-specific manifestations, that occur in patients with negative family histories for those disorders and that have negative results for putative causative mutations in genomic analyses of peripheral blood.

These discoveries lead us to hypothesize that somatic mutations are more representative than germline mutations of the true diversity and range of mutations in humans and human disease. This is because many mutations are lethal when constitutional and thus will be present only in a mosaic state. The powerful genomic tools at our disposal will allow this hypothesis to be tested in an effective and expeditious manner and will allow us to expand our understanding of the pathophysiology of disease-causing mutations well beyond the fraction that can survive early development.

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Competing interests statement
The authors declare competing financial interests: see Web version for details.