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Genome destabilization by homologous recombination in the germline

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Abstract

Meiotic recombination, which promotes proper homologous chromosome segregation at the first meiotic division, normally occurs between allelic sequences on homologues. However, recombination can also take place between non-allelic DNA segments that share high sequence identity. Such non-allelic homologous recombination (NAHR) can markedly alter genome architecture during gametogenesis by generating chromosomal rearrangements. Indeed, NAHR-mediated deletions, duplications, inversions and other alterations have been implicated in numerous human genetic disorders. Studies in yeast have revealed insights into the molecular mechanisms of meiotic NAHR as well as the cellular strategies that limit NAHR.

Gametes are the products of a meiotic programme in which diploid germ cells undergo one round of DNA replication followed by two successive rounds of cell division. During this process, gametes acquire chromosomes comprising new assortments of parental alleles. Subsequent fusion of two gametes during sexual reproduction results in the reconstitution of a diploid genome, yielding offspring that are genetically distinct from their parents.

In most sexually reproducing organisms, homologous recombination lies at the heart of meiosis by promoting proper segregation of HOMOLOGOUS CHROMOSOMES (also referred to as homologues) (reviewed in ^{REF. 1}). Prior to the first meiotic division, recombination begins when DNA double-strand breaks (DSBs) are deliberately introduced into each chromosome. The DSBs are then repaired by genetic exchange with allelic sequences, resulting in physical linkages between pairs of homologues. These connections ensure that homologues orient correctly on the meiotic spindle and migrate to opposite spindle poles. Meiotic recombination thus promotes genetic stability and faithful transmission of the genome by limiting the repair of each DSB primarily to DNA sequences at the allelic position on the homologue and ensuring the accurate distribution of chromosomes to gametes.

Errors in meiotic recombination can affect genome stability during gametogenesis. Research spanning several decades has shown that chromosome non-disjunction (that is, missegregation) in meiosis results in constitutive ANEUPLOIDY, which can lead to spontaneous abortion or congenital birth defects (reviewed in ^{REF. 2}). More recent work has uncovered a second process that influences genome stability in the germline: aberrant meiotic

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recombination between non-allelic DNA segments that share high sequence similarity. This process is known as non-allelic homologous recombination (NAHR); the synonymous term 'ectopic recombination' is more prevalent in older literature and in yeast studies. NAHR is a more recent, mechanistically evocative term and is used in this Review. Because eukaryotic genomes from yeasts to human harbour repeated blocks of DNA^{3, 4}, a meiotic DSB formed within a repeat has the potential to induce genomic rearrangement through repair with non-allelic sequences. In humans, NAHR-mediated events leading to chromosomal alterations have been implicated in numerous disorders. It is thus important to understand the mechanisms behind NAHR in the germline.

This Review focuses on genome instability induced by meiotic NAHR. We begin with an overview of the mechanism of meiotic recombination and then discuss the types of chromosomal rearrangement and human disorder that are associated with NAHR between duplicated regions of the genome. We next examine insights into NAHR mechanisms obtained from studies in other organisms. Finally, we review cellular strategies that prevent meiotic NAHR and favour recombination between allelic sequences, and consider some of the future challenges in the field.

Meiotic recombination

Meiotic recombination is inherently hazardous because it is initiated by developmentally programmed DSBs at multiple sites in the genome (FIG. 1) (reviewed in REF. 5). A cascade of homologous recombination events, normally templated by DNA at the allelic position on the homologue, results in DSB repair either with reciprocal exchange of chromosome arms that flank the DSB site (a crossover) or without exchange of flanking arms (a noncrossover).

Mechanisms of meiotic recombination

Much of what is known about the molecular events of meiotic recombination comes from studies in the budding yeast *Saccharomyces cerevisiae* (FIG.1) (reviewed in REFS. 5, 6). Each initiating DSB is formed by a homodimer of the enzyme Spo11, which mediates a topoisomerase-like transesterification reaction. Each Spo11 monomer remains covalently attached to the 5' terminus of a DNA strand at each DSB and is released following endonucleolytic cleavage of each strand at sites 3' to the DSB. The two 5' ends of the DSB are further resected to expose 3' single-stranded DNA (ssDNA) tails. The strand exchange proteins Rad51 and Dmc1 bind these tails, search for homologous DNA sequences and then catalyse invasion by one of the two 3' ssDNA tails into the homologous sequence of an intact, double-stranded homologue (that is, not the sister chromatid), thereby generating HETERO DUPLEX DNA. This results in separation of the strands of the invaded DNA duplex and the formation of an intermediate known as a displacement loop (D-LOOP). Re-synthesis of DNA strands destroyed by resection is primed by each of the 3' ssDNA tails, using the strands of the invaded homologue as templates. For some recombination events, branched DNA intermediates containing pairs of HOLLIDAY JUNCTIONS are formed and subsequently resolved, yielding mature recombinant DNA molecules that are primarily in a crossover configuration. Other recombination events are thought to proceed through a mechanism termed synthesis-dependent strand annealing (SDSA) (FIG. 1), in which the invading DNA strand is displaced after DNA synthesis, and then anneals to the complementary ssDNA tail on the other side of the DSB. This mechanism leads primarily to a noncrossover outcome. The choice between crossover and noncrossover outcomes is highly regulated (reviewed in REF. 7).

In both recombination pathways, heteroduplex DNA forms between strands of the two parental alleles, and, importantly, GENE CONVERSION can occur when heterologies that are caused by sequence differences between the two strands are corrected by MISMATCH REPAIR (MMR). Gene conversion can occur with both crossover and noncrossover modes of recombination. The

direction of genetic information transfer caused by gene conversion is most often such that the site where the DSB was originally formed is converted to a copy of the sequence of the unbroken homologue. As a consequence of this genetic information transfer, recombination results in sequence homogenization between the participating alleles (reviewed in REFS. 5, 6) (FIG. 1).

Many of the molecular players involved in meiotic recombination in yeast are conserved in larger eukaryotes (reviewed in REFS. 1, 6). For example, disruption of *SPO11* homologues in *Caenorhabditis elegans*⁸, in *Drosophila melanogaster*⁹ and in *Mus musculus*^{10, 11} eliminates meiotic recombination. Furthermore, orthologues of Dmc1 and Rad51, as well as proteins acting later in the crossover and noncrossover pathways, have roles in recombination in larger eukaryotes^{12, 13}. This high degree of conservation means that studies in experimentally tractable organisms provide insight into mechanisms of allelic recombination and NAHR in humans.

Meiotic recombination hotspots

The distribution of meiotic recombination in genomes from yeasts to mammals is not random: most recombination occurs in highly localized 1- to 2-kilobase (kb) genomic domains termed hotspots, which are preferential sites of DSB formation by Spo11 (reviewed in REF. 14). Humans and mice display sex-specific variation of recombination^{15, 16}.

Individual human crossover hotspots were first identified at high resolution by sperm typing, a PCR-based approach for recovering crossover DNA molecules directly from sperm DNA¹⁴. Subsequently, a genome-wide map of crossover hotspots inferred from population genetic methods was generated, leading to an estimate of 25,000–50,000 crossover hotspots in the human genome¹⁷. Genome-wide meiotic DSB and/or recombination maps are also available in budding and fission yeasts^{18–24}. However, despite the hotspot catalogues available, little is known about the factors that govern where DSBs are formed (BOX 1). In perhaps the most significant association in humans to date, a single sequence feature — the degenerate 13-nucleotide motif CCNCCNTNNCCNC — is present in >40% of the meiotic crossover hotspots inferred from population genetic analyses^{17, 25}. The fact that recombination is not distributed randomly across the genome is important for understanding the mechanisms behind NAHR because NAHR events are, by definition, highly dependent on the sequence context in which DSBs form (that is, whether DSBs occur in unique sequences or in repeated DNA).

NAHR in the human genome

The human genome is replete with sequences that are present in a high number of copies, such as transposable elements (for example, Alu elements and long interspersed repetitive elements (LINEs)), or in low number of copies²⁶. Roughly 5% of the human genome comprises low copy repeats (LCRs; also known as segmental duplications), which are blocks of DNA that share ≥90% sequence identity over ≥1 kb (REF. 4). Interest in LCRs has intensified as their roles in human disease, genome architecture and genome evolution have emerged (reviewed in REF. 27).

Genomic disorders: outcomes of NAHR

The high sequence identity over large stretches of DNA renders LCRs susceptible to germline NAHR events that result in deletion, duplication, inversion or ISODICENTRIC CHROMOSOME formation (FIG. 2). Structural alterations that change the constitutional copy number of dosage-sensitive genes, that disrupt genes or that generate gene chimeras result in conditions collectively termed GENOMIC DISORDERS (reviewed in REF. 28). Much of the work on these disorders was pioneered by Lupski and colleagues, who first recognized the role of LCRs in the

aetiology of two inherited neurological disorders: *CHARCOT-MARIE-TOOTH DISEASE TYPE 1A* (CMT1A) and *HEREDITARY NEUROPATHY WITH LIABILITY TO PRESSURE PALSIES* (HNPP)^{29, 30}. Unequal crossing over between two LCRs on chromosome 17p results in the duplication (causing CMT1A) or deletion (causing HNPP) of genes located in the intervening 1.4-megabase (Mb) region. NAHR-mediated events occurring in the germline are now known to account for over 30 genomic disorders, with phenotypes ranging from mental retardation to blood diseases or male infertility (TABLE 1). NAHR also contributes to copy-number and structural variation in the human genome through LCR-mediated duplications, deletions and inversions that are not pathogenic^{31–33}.

The study of genomic disorders has uncovered several parallels between meiotic allelic homologous recombination and NAHR, which suggest that they share common mechanisms and that, by inference, NAHR events occur during prophase of the first meiotic division. First, for some rearrangements caused by NAHR, recombination breakpoints cluster within narrow, 1- to 2-kb hotspots in LCRs, which are reminiscent of allelic recombination hotspots^{34–40}. Interestingly, the allelic recombination hotspot motif CCNCCNTNNCCNC is found in many of these NAHR hotspots²⁵.

Second, allelic crossover hotspots identified by population genetic analysis in the LCRs on chromosome 17p coincide with the hotspots targeted in duplications and deletions that give rise to CMT1A and HNPP, consistent with the hypothesis that allelic homologous recombination and NAHR at these hotspots are both initiated by similarly programmed DSBs⁴¹. Hotspots of both allelic and non-allelic recombination also coincide within a set of LCRs on chromosome 17q that undergo recurrent NAHR associated with neurofibromatosis 1, a pleiotropic disorder that includes the development of cutaneous neurofibromas⁴². However, more general tests of whether allelic recombination and NAHR share crossover hotspots in LCRs is hampered by the difficulty in identifying crossover hotspots in LCRs by population genetic methods because scoring of allelic polymorphisms in one LCR can be confounded by identical sequences in its non-allelic counterpart⁴³.

Third, several studies directly tested the rates of recurrent, reciprocal NAHR-mediated duplications and deletions in DNA isolated from sperm and blood of healthy males (for example, reciprocal duplication and deletion associated with CMT1A and HNPP). One study found that each of the events tested was specific to the germline and that sperm-based frequency estimates of *de novo* rearrangements encompassed the disease-based estimates. (For example, for the CMT1A duplication, a frequency of 1/23,000–1/79,000 was estimated from sperm analysis and a frequency of 1/23,000–1/41,000 was estimated in the population⁴⁴.) Further studies showed that LCR-sponsored deletions and duplications at the α -globin gene locus are significantly more common in sperm than in blood^{45, 46}. These findings establish that the NAHR-mediated deletions and duplications under study are detected primarily in cells that have gone through meiosis, which is consistent with the hypothesis that meiotic allelic homologous recombination and NAHR share common mechanisms.

Fourth, for many disorders, the causal NAHR events are predominantly either paternal or maternal. This aspect is congruent with the fact that sex-specific differences in allelic recombination rates at loci throughout the genome are a known feature of allelic meiotic recombination (for example, see REFS. 36, 47) (BOX 1).

Fifth, statistical analysis of the sequence divergence between the LCRs targeted in CMT1A and HNPP uncovered evidence that the repeat sequences have been homogenized by gene conversion. This would be predicted if the repeats were undergoing repeated noncrossover recombination⁴⁸.

Last, in several HNPP patients, the breakpoint junction in the recombinant LCR remaining after deletion of the intervening sequence displays interspersed patches of sequences from each of the two LCRs. This finding provides evidence of crossover-associated gene conversion, similar to what would occur during allelic recombination³⁵.

Taken together, these results build a strong case that pathological NAHR events in humans are initiated during meiosis by the same type of SPO11-dependent DSBs that initiate normal allelic recombination, although it is possible that at least some NAHR events occur in mitotically dividing cells in the germline. Studies in mice, in which meiotic recombination mutants such as *Spo11*^{-/-} knockouts are available^{10, 11}, may help resolve this issue.

The Y chromosome: stability and instability by NAHR

Ninety-five percent of the sequence of the human Y chromosome is restricted to males⁴⁹ (The remaining 5% of its sequence participates in recombination with the X chromosome and is therefore shared with females.). The male-specific region of the Y chromosome (MSY) is unique compared with all other nuclear DNA in that it is haploid and passed on clonally. Until recently, the MSY was thought to not recombine. Because meiotic recombination can purge the genome of deleterious mutations by breaking up linkage groups and segregating otherwise linked loci away from one another, this assumption led some to speculate that, in the absence of recombination, the Y chromosome will disappear in 15 million years^{50, 51}.

At the expense of this 'imminent demise' hypothesis, recent sequencing of the human Y chromosome and comparative analyses with primate Y chromosomes have overturned the notion that the MSY does not participate in recombination^{49, 52, 53}. Roughly one third of the MSY is composed of LCRs in direct or inverted orientation⁴⁹. The most conspicuous of these LCRs are eight palindromes (inverted repeat pairs) that harbour mirror-imaged gene pairs with crucial roles in spermatogenesis⁴⁹. Ranging in size from 30 kb to 2.9 Mb, the palindromes show >99.9% arm-to-arm sequence identity; such high similarity would normally suggest that they arose on the MSY roughly 200,000 years ago⁵⁴. However, Y-linked orthologues of these palindromes are found (with equally high arm-to-arm sequence identity) in chimpanzees, indicating that the palindromes predate the human-chimp divergence over 6 millions years ago. Because the arm-to-arm identity of these palindromes in each species is higher than the identity of the palindromes between species (98.6%), it can be inferred that the palindrome arms have been undergoing sequence homogenization since the human-chimp divergence, which would be expected if intrapalindrome NAHR occurred repeatedly⁵². Such concerted evolution, along with evidence in modern human lineages of recent gene conversion in MSY palindromes, led to the hypothesis that the palindromes have been maintained by intrapalindrome arm-to-arm recombination and that such recombination provides a mechanism to preserve the testis-specific genes located there⁵².

One group⁵⁵ proposed that this mechanism would come at a cost. They predicted that, if noncrossover resolution of recombination arising from a DSB in a palindrome accounts for intrapalindrome sequence homogenization, then crossover resolution of such a DSB by unequal sister-chromatid exchange should generate an isodicentric Y chromosome (FIG. 2). Indeed, from a group of >2,300 patients, they identified 51 individuals with isodicentric Y chromosomes that had evidently formed by this mechanism. These chromosomes were associated with a wide range of sex-linked reproductive disorders, including failure to produce sperm, anatomic feminization of individuals bearing Y chromosomes and Turner syndrome in women⁵⁵. Sex reversal is likely caused by the loss of the mitotically unstable isodicentric Y chromosome in the cells of the fetal gonad that determine sex. This model could also account for the features of Turner syndrome observed in some of the individuals.

NAHR in the human Y chromosome is not limited to LCRs in inverted orientation. Several recurrent interstitial deletions ranging in size from 0.8 Mb to 7.7 Mb and associated with reduced spermatogenesis arise by NAHR between LCRs in direct orientation^{56–61}. As predicted by the model of meiotic recombination, reciprocal duplications of these deletions^{33, 62}, evidence of gene conversion⁶³ and hotspots of NAHR^{56–59} are all observed for Y chromosome-linked LCRs in direct orientation. For at least one pair of LCRs, recurrent reciprocal deletion and duplication events have been shown to be specific to the germline⁴⁴.

The results of studies of recurrent NAHR events in the human Y chromosome are consistent with the hypothesis that these events are meiotic in origin and are initiated by SPO11-generated DSBs. Furthermore, chromosome-associated complexes of MSH4 (a protein that is required for meiotic recombination in mice and yeast) have been observed on the MSY in cytological studies of spread human spermatocytes⁶⁴. These findings support the conclusion that meiotic DSBs form on the MSY, although it is not possible from available studies to determine whether DSBs localize to LCRs.

Meiotic NAHR in yeast

The consequences of NAHR in humans emphasize the importance of understanding the molecular mechanisms of this process. Studies in experimentally tractable organisms such as budding yeast have played a key part in addressing this issue. The ease of genetic manipulation of this organism enables facile analysis of genes that are involved in NAHR. Moreover, the ability to recover all four meiotic products (spores) through TETRAD ANALYSIS enables the comprehensive investigation of NAHR-mediated products. Finally, the availability of physical assays to detect directly the DNA intermediates of recombination provides highly detailed views of the molecular steps in this pathway.

Mechanistic parallels to allelic recombination

Meiotic NAHR has been studied by artificially dispersing HETEROALLELES of a gene in various non-allelic positions in the budding yeast genome. Recombination between mutant heteroalleles can restore a wild-type sequence in one of the gene copies, allowing rapid, quantitative analysis of even infrequent recombination events. NAHR between artificially dispersed genes occurs frequently during meiosis (often at 10–100% of allelic frequencies)^{65–67}. Moreover, NAHR in yeast is mechanistically similar to allelic recombination by several criteria. First, NAHR is initiated by Spo11-dependent DSBs⁶⁸. Second, heteroduplex DNA is formed as an intermediate in NAHR, as assessed by post-meiotic segregation patterns in tetrad analysis^{69, 70} and by physical assays that directly detect heteroduplexes in genomic DNA extracted from meiotic cells⁷⁰. Last, the ratio of crossover- to noncrossover-associated gene conversions is similar for allelic recombination and NAHR in at least some instances⁶⁶.

Nuclear organization of repeat sequences

The frequency of NAHR between artificial repeats is strongly influenced by the spatial organization of the repeated loci in the nucleus^{65, 67}. For example, if repeats are at interstitial locations (≥ 60 kb away from the closest TELOMERE), on HETEROLOGOUS CHROMOSOMES (HETEROLOGUES) they recombine approximately 10-fold less efficiently than the same sequences in allelic positions (FIG. 3a). By contrast, repeats dispersed in non-allelic positions on homologues recombine essentially as efficiently as allelic sequences^{65–67}. One interpretation of these findings is that the progressive pairing of homologues during meiosis (discussed below) favours recombination between repeated sequences on homologues by

increasing their proximity in the nucleus^{65, 71}. If so, then repeats on heterologues would not benefit from this proximity advantage.

However, the frequency of NAHR is complicated further by the proximity of the artificial repeats to telomeres^{65, 67}. Heteroallelic markers integrated near chromosome ends (<17 kb from each telomere) recombine only two–six-fold less efficiently than allelic sequences, regardless of whether they are on homologues (at opposite chromosome ends) or on heterologues^{65, 67} (FIG. 3b). During early meiotic prophase, telomeres become physically attached to the inner surface of the nuclear envelope, which is a conserved phenomenon in many organisms, including mammals^{1, 72}. Telomeres then begin rapid, irregular movements and undergo clustering within a small region of the nuclear envelope, in a process known as bouquet formation (reviewed in REFS. 1, 72). Increased proximity driven by this clustering may underlie the increased recombination frequency between non-allelic sequences located near telomeres.

NAHR between natural repeats

The frequency of NAHR between natural repetitive elements is generally lower than NAHR between artificial repeats. The budding yeast genome contains dispersed repetitive elements such as T_Y ELEMENTS, which are present in ~30–40 copies per haploid genome⁷³. If Ty elements behave similarly to artificial repeats, they could have a marked impact on cell viability. NAHR has been studied between Ty elements engineered to contain different selectable markers and located in allelic or non-allelic positions, as well as between marked Ty elements and endogenous ones^{74, 75}. Allelic Ty elements recombined less frequently than allelic non-Ty sequences, and the frequency of NAHR was dramatically reduced – about 100-fold – compared with allelic recombination^{73–75}. Moreover, recombination products were predominantly noncrossover gene conversions; crossover-associated gene conversions were extremely rare (<1% of recombinants)⁷⁴.

Similar observations were made in the fission yeast *Schizosaccharomyces pombe*. tRNA genes, which are another example of dispersed repeat sequences, recombined infrequently and gene conversions were rarely associated with crossovers⁶⁶. The reasons for the reduced probability of a crossover outcome for NAHR in these two different systems remain to be determined, but possible mechanisms are discussed below.

Insights into meiotic NAHR in mammals

Studies in yeast have shown that meiotic NAHR is mechanistically similar to allelic recombination. The recombination pathways and the players involved are conserved in mammals, reinforcing the view that human germline NAHR is an outcome of aberrant meiotic recombination.

Yeast studies have also shown that the spatial organization of repeats in a nucleus influences NAHR. Non-random nuclear organization of chromosomes has been observed in both somatic and germ cells of mammals, and its importance in the maintenance of genome integrity has begun to be appreciated⁷⁶. For example, in somatic cells, the frequency of recombinational repair of targeted DSBs varies depending on the location of the homologous sequences that will be used in the recombination reaction: allelic sequences recombine more frequently than nonallelic ones⁷⁶. Similarly, in mouse germ cells gene conversion frequency between transgenic *lacZ* heteroalleles varies over a seven-fold range for *lacZ* transgenes integrated at different positions, indicating that chromosomal location may be a contributing factor to NAHR⁷⁷. These findings suggest that nuclear organization of repeats may affect the frequency of NAHR in mammals.

Yeast studies have also shown that many naturally occurring repeats show low levels of meiotic NAHR and that crossovers are particularly suppressed. There are as yet no genetic data that directly address this question in mammals. Alu elements are one of the most abundant repetitive elements in the mammalian genome²⁶. Genetic disorders mediated by recombination between non-allelic Alu elements have been identified (reviewed in ^{REF. 78}), although it remains unknown whether such recombination occurred during meiosis. In both human and mouse somatic cells, a targeted DSB in a LINE inserted into the genome could be repaired by gene conversion using endogenous copies of the repeat^{79, 80}. Given the prevalence of these multicopy elements in the human genome, understanding their meiotic behaviours is an important challenge.

Restraining NAHR

Because of the substantial impact of NAHR on genome stability, it is not surprising that cells have evolved multiple strategies to suppress or control NAHR. There are several general mechanisms through which NAHR can be discouraged: suppressing Spo11-dependent DSB formation in or near DNA repeats; inhibiting the use of non-allelic homologous templates for recombination and/or favouring the use of allelic templates; and channelling recombination intermediates into pathways that minimize the likelihood of deleterious outcomes. Examples of each type of mechanism have been elucidated and are described below.

Preventing breaks in repeats

The most straightforward way to prevent NAHR is not to form DSBs in susceptible DNA sequences in the first place, and this strategy does indeed seem to operate in many contexts. In budding yeast, the ribosomal DNA (rDNA) contains ~140 tandem repeats of a 9-kb region that encodes ribosomal RNA genes and constitutes almost 10% of the genome⁸¹. Despite the large physical distance occupied by this region, interallelic recombination — at least crossover events — occurs 100-fold less frequently than in other regions⁸¹, and this low recombination level is largely due to suppression of meiotic DSB formation⁸². Suppression of DSBs and recombination in the rDNA depends strongly on *SIR2*^{82, 83}, which encodes a histone deacetylase that promotes the formation of a closed, compact chromatin structure in the rDNA and other regions^{84, 85}. DSB formation in budding yeast is favoured in regions with an open (that is, nucleosome free) chromatin structure (BOX 1). Therefore, Sir2 may suppress DSBs in the rDNA in part through the formation of a nucleosomal conformation that is not permissive for Spo11 activity. Other features probably contribute as well. For example, Sir2 mediates the exclusion of Hop1, a chromosome structure protein that promotes DSB formation, from the rDNA^{86, 87}.

Budding yeast chromosome ends contain ~300 base pairs of telomeric repeats and 10–30-kb subtelomeric regions that are enriched in several different repetitive elements^{88, 89}. Subtelomeric regions are evolutionarily dynamic with respect to the composition of these repetitive elements and sequence divergence between them⁹⁰. For example, the Y' element, which is adjacent to telomeric repeats on some chromosomes, exhibits only 1% sequence divergence between copies (other subtelomeric repeats show 10–20% divergence on average)^{88, 91}. Mitotic NAHR between Y' elements has been observed⁹¹, suggesting that it contributes to sequence homogenization of Y' elements^{88, 90}. It remains unknown to what extent Y' elements undergo meiotic NAHR and whether meiotic telomere clustering (as discussed above) influences the frequency. However, the distal 20 kb of most chromosomes exhibit significantly lower meiotic DSB levels^{20, 21} and undergo few crossovers^{23, 89}. Similarly to what is observed in the rDNA, DSBs in subtelomeric regions are increased in the absence of Sir2, suggesting that there are active mechanisms to suppress DSBs in this part of the genome^{82, 92}.

As noted above, meiotic NAHR is suppressed between Ty elements. It is possible that, by chance, the regions near the analyzed Ty elements do not undergo frequent DSB formation. However, a more likely explanation is that an active mechanism suppresses DSB formation near Ty elements, because insertion of a Ty element close to a recombination hotspot reduced the frequency of both DSB formation and recombination in this otherwise recombination-active region⁹³. Moreover, insertion of the Ty element converted the chromatin at the hotspot from an open configuration (nuclease hypersensitive) to a closed one (nuclease resistant)⁹³. Although the molecular mechanisms behind these effects are not fully understood, these findings are consistent with the view that recombination of DNA repeats is controlled, at least in part, by establishment of chromatin structures that are not permissive for Spo11-dependent DSB formation.

DSB suppression is likely to be a conserved strategy for minimizing meiotic NAHR for some natural repetitive elements, as DSBs are also rare in the large blocks of repetitive DNA in pericentromeric regions in *S. pombe*²⁴. Moreover, cytological analyses have shown that recombination-associated protein complexes are rare or absent from the highly repetitive pericentromeric heterochromatin in mice⁹⁴ and humans⁶⁴. Although there are no genetic data in mammals to verify that DSB formation is actively inhibited in these regions, it seems likely that DSB suppression in repetitive sequences is a general phenomenon.

Constraining recombination partner choice

Once a DSB is formed in a repetitive sequence, the choice of homologous template for repair becomes an important factor in determining the likelihood that NAHR will occur. In such circumstances, it seems that the presence of additional recombination events at other locations on the same pair of homologues may help to enforce 'good behaviour' of a DSB formed in or near repetitive DNA by promoting the use of an allelic template and/or restricting the use of non-allelic sequences. During early meiotic prophase, homologues find their partners and pair along their entire lengths (FIG. 4a). In many organisms, including fungi (budding yeast and *Sordaria*), the plant *Arabidopsis thaliana* and mice, stable homologue pairing depends on DSB formation and early processes of recombination (reviewed in REFS. 1, 72). As an early step in pairing, homologues become aligned at multiple sites. In organisms in which the chromosomes can be easily visualized and studied, alignment is accompanied by the appearance of roughly 400-nm-long bridges between chromosome axes. These bridges are thought to be sites where recombination reactions are in progress (reviewed in REF. 95). As prophase continues, these interactions become increasingly stable, and homologues pair along their entire lengths. In many organisms, homologue pairing culminates in the formation of the SYNAPTONEMAL COMPLEX, a proteinaceous structure that holds homologues together in close juxtaposition (see REFS. 96, 97 for details of synaptonemal complex formation and functions of its components). In many fungi, in plants and in mice, neither the initial alignment of homologues nor the subsequent stable pairing interactions are observed in mutants that are defective in DSB formation and strand invasion of DSB ends (for example, *spo11Δ* and *dmc1Δ* mutants in yeast)^{72, 98}, emphasizing the importance of early recombination activities in homologue pairing. Note that *C. elegans* and *D. melanogaster* use DSB-independent mechanisms for homologue pairing (see REFS. 1, 72).

In principle, the formation of progressively more stable pairing interactions between homologues could inhibit NAHR through two complementary effects: pairing could favour allelic recombination by placing the allelic copy in close proximity, and pairing may place constraints on the ability of broken DNA segments to find and engage non-allelic sequences that are themselves constrained elsewhere in the nucleus. Elegant experiments in yeast tested this scenario using artificial repeats located on two different chromosomes⁷¹ (FIG. 4b). When the homologue of a chromosome carrying a recombination reporter was present (but did not itself carry the recombination reporter), NAHR occurred at a low level. However,

when the ability of the homologues to engage in normal pairing was disrupted by substituting their pairing partners with chromosomes from a different yeast strain ($\geq 15\%$ sequence divergence), the frequency of NAHR increased ~seven-fold (FIG. 4b). These findings indicate that homologous pairing in meiosis can contribute substantially to the suppression of NAHR.

There is also evidence that similar effects occur in mammals. An interesting example is provided by mice that carry two different reciprocal translocations between chromosomes 1 and 13 that have slightly different translocation breakpoints⁹⁹. During meiosis in mice heterozygous for these semi-identical translocations, the two short translocation chromosomes and the two long translocation chromosomes each undergo a separate pairing reaction that culminates in the formation of synaptonemal complexes along most of their lengths. However, at the positions of the translocation breakpoints, the synapsing chromosomes differ from one another and the non-homologous portions form loops that bulge out of the synaptonemal complexes (FIG. 4c). The loop on the larger chromosome pair is homologous to the loop on the smaller chromosome pair. Nevertheless, markers for the presence of unrepaired DSBs were often seen to persist⁹⁹. A plausible interpretation of this finding is that the pairing and synapsis of homologues imposes a physical constraint that impedes the ability of broken DNA sequences to find homologous templates elsewhere in the genome⁹⁹.

Controlling the outcome of recombination between divergent sequences

If a DSB forms in a repetitive sequence and a non-allelic homologous partner is then used as template for recombination repair, opportunities still remain to avoid deleterious consequences from NAHR. Specifically, this can be achieved by channelling the recombination events into pathways that give primarily noncrossover outcomes, which do not result in gross chromosomal rearrangements.

One way this may be facilitated is through action of MMR proteins (for example, Pms1 and Msh2 in budding yeast). MMR proteins are conserved among bacteria and eukaryotes and have important roles in mismatch correction during DNA replication and recombination (reviewed in REFS 100, 101). MMR proteins also impede the strand exchange reaction during homologous recombination if a high level of mismatches is present on the heteroduplex DNA (known as heteroduplex rejection)¹⁰², which probably promotes noncrossover recombination pathways such as SDSA^{103–106}. Natural repeats (such as retrotransposons) are often more divergent than allelic sequences, thus recombination between non-allelic sequences is expected to generate heteroduplex DNA with more mismatches on average. Although direct evidence is thus far lacking to implicate MMR proteins in crossover suppression during NAHR between natural repeats in yeast and mammals, this is a plausible strategy for stabilizing the genome.

Another means to antagonize NAHR is through the action of any of several helicases that exert anti-recombination activity by disrupting Rad51 or Dmc1 protein-DNA complexes, by dissociating strand exchange intermediates and/or by taking apart Holliday junction recombination intermediates in a manner that disfavours crossover formation. Examples of helicases that can carry one or more of these functions include the RecQ family helicases Sgs1 in yeast and BLM in humans, and the unrelated Srs2 helicase in yeast (reviewed in REFS. 101, 107). Each of these anti-recombinational activities could potentially be involved in preventing deleterious outcomes of meiotic NAHR, although this has yet to be tested.

Perspectives

Recent work has clearly established that homologous recombination between non-allelic repeated sequences in the human genome influences sequence diversity at both the nucleotide and structural levels. It can result in deletions, duplications, inversions and other rearrangements that can lead to human disorders or contribute to architectural polymorphism in the human genome. Paradoxically, NAHR seems to promote both stability and instability of large repeats on the Y chromosome.

Many questions remain to be examined to understand the molecular mechanisms of NAHR. To what extent are the mechanisms of allelic homologous recombination and NAHR similar? Are the DSBs that initiate NAHR part of the cohort of developmentally programmed DSBs intended for resolution by allelic homologous recombination or do they consist of additional, non-programmed DSBs that must nonetheless be repaired? To begin to answer these questions, a high-resolution map of the human meiotic DSB landscape is required. A caveat to the catalogue of 25,000–50,000 human crossover hotspots identified by population genetic analysis is that it fails to capture new hotspots or to mask hotspots that have recently disappeared. Indeed, sperm typing has uncovered emerging crossover hotspots concealed in regions of low historical recombination^{108, 109}, and comparative analysis of the human and chimpanzee genomes has shown that hotspots evolve rapidly^{110, 111}. Therefore historical crossover analysis reveals an incomplete picture of the contemporary recombination map. Maps of meiotic DSB distributions in the human genome will inform us of how DSBs are distributed in repeats such as LCRs and provide further insight into the similarities and differences in the mechanisms of allelic homologous recombination and NAHR.

In addition, studies in experimentally tractable organisms, particularly budding yeast, have begun to flesh out the broad principles behind cellular strategies that have evolved to prevent NAHR, but there is a need to translate experimental findings from yeast to humans. Studies in mice will likely help to bridge this gap but the present inability to study mammalian meiosis in cell culture is a crucial impediment to progress in this area. It will also be interesting to examine, in humans, whether mutants and/or sequence variants in various factors involved in recombination pathways or in the prevention of NAHR prove to be NAHR susceptibility loci. Continuing to examine these mechanisms will provide insights into the events that lead to the genomic disorders and conditions that arise by NAHR in the human genome.

Box 1 | Determinants of meiotic break formation and recombination

Budding yeast (reviewed in ^{REF. 112})

- Meiotic double-strand breaks (DSBs) occur preferentially in intergenic promoter-containing regions rather than in genes.
- An open chromatin environment, as assayed by hypersensitivity to nucleases, correlates with increased frequency of recombination, and, at some sites, DSB formation depends on the binding of specific transcription factors. Transcription does not seem to be required for hotspot activity, but there is a weak correlation between the expression level of promoters and their activity as recombination hotspots²⁰.
- Although crossovers are suppressed near centromeres, DSBs do form there, indicating that pericentric DSBs may be resolved preferentially by intersister-chromatid recombination^{20, 23}.

- DSBs occur infrequently in telomeric regions and retrotransposons.
- Trimethylation of lysine 4 of histone H3 is constitutively higher in regions close to DSB sites, and deletion of the Set1 methyltransferase (which generates this methyl mark) dramatically reduces DSB frequency in most hotspots¹¹³.

Larger eukaryotes

- In humans, crossover rates are lower in genes than in non-coding sequences, based on analysis of a genome-wide catalogue of crossover hotspots inferred from population genetic data¹⁷.
- The degenerate thirteen-nucleotide motif CCNCCNTNNCCNC accounts for >40% of human hotspots²⁵. This finding was supported by detailed sperm typing studies at one hotspot that contains the motif: a polymorphism that disrupts the consensus sequence was associated with decreased crossover frequencies¹¹⁴.
- In the genomes of flies, birds and mammals, there are fewer interspersed repetitive elements in regions of higher recombination^{115, 116}. This may reflect the effects of selection against initiating recombination in such elements because of the potential for NAHR.
- A study of recombination across mouse chromosome 1 identified a positive but non-uniform correlation between gene density and crossover rate¹¹⁷.
- Several histone modifications are enriched at the known mouse hotspot *Psm9*: di- and trimethylation of lysine 4 of histone H3 and acetylation of lysine 9 of histone H3 are thought to be part of the substrate for the initiation of recombination, and H4 hyperacetylation increases after DSB formation by Spo11¹¹⁸.
- PRDM9 has recently been identified as an important determinant of meiotic hotspots in humans and mice^{119, 120}. In mouse, PRDM9 has been shown to trimethylate lysine 4 of histone H3 and is expressed specifically in meiotic germ cells¹²¹. Both human and mouse PRDM9 have an array of zinc fingers, and human PRDM9 binds specifically to the thirteen-nucleotide consensus motif *in vitro*. In addition, allelic variation in human *PRDM9* correlates with differences in hotspot usage. These findings suggest that the binding of PRDM9 to specific DNA sequences could target recombination to those sites and that allelic variation in PRDM9 could account for variability in hotspot usage between and within species.

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Glossary

HOMOLOGOUS
CHROMOSOMES
(HOMOLOGUES)

In a diploid individual, chromosome pairs that share the same genes, but not necessarily the same alleles, at loci along their lengths

ANEUPLOIDY	The condition of a cell or organism that is associated with having one or more extra or missing chromosomes, caused by inaccurate chromosome segregation during cell division
HETERODUPLEX DNA	Hybrid double-stranded DNA that consists of one strand from each homologue; formed by strand invasion of one DSB end into a homologous DNA duplex during recombination
D-LOOP	A single DNA strand displaced when strand invasion induces the dissociation of a DNA duplex
HOLLIDAY JUNCTION	Four-stranded, branched DNA structure formed as an intermediate in homologous recombination
GENE CONVERSION	Transfer of DNA sequence information that “overwrites” the sequence of one allele with the sequence of the other allele; carried out by mismatch repair on heteroduplex DNA
MISMATCH REPAIR	The repair system that recognizes and corrects mismatches that form during DNA replication and recombination
ISODICENTRIC CHROMOSOME	A mirror-imaged duplication of part of a chromosome, including the centromere
GENOMIC DISORDERS	Conditions arising in the germline by submicroscopic, regional chromosomal rearrangements that alter the copy number of dosage-sensitive genes, disrupt genes or generate fusion genes
CHARCOT-MARIE-TOOTH DISEASE TYPE 1A	Inherited disorder of the nerves that results in weakness and atrophy of muscles of the lower legs and hands
HEREDITARY NEUROPATHY WITH LIABILITY TO PRESSURE PALSIES	Inherited neuropathy characterized by recurrent episodes of numbness, tingling and/or weakening and atrophy of muscles, most commonly in wrists, elbows and knees
TETRAD ANALYSIS	Tetrads refer to all four products (spores in yeast) of a single meiosis, which are grouped together in an ascus. Spores in a tetrad be individually isolated by micromanipulation and can be grown in culture for further analysis
HETEROALLELES	Two different alleles of a gene, each carrying a mutation(s) at a different position within the coding sequence
TELOMERE	segment at the end of each chromosome arm that consists of repetitive sequences and that prevents the normal chromosome ends from being recognized as a double-strand break
HETEROLOGOUS CHROMOSOMES (HETEROLOGUES)	Chromosomes that are neither homologues nor sister chromatids
TY ELEMENT	Retrotransposon in yeast, about 5–6 kb in length, comprising a central domain that is flanked by ~330 bp of long terminal repeats (LTRs) The yeast genome contains full Ty elements as well as solo LTRs and LTR fragments
SYNAPTONEMAL COMPLEX	A proteinaceous structure that is formed between homologues during meiosis Synaptonemal complexes are composed of lateral elements

that are assembled between the entire length of sister chromatids, and transverse filaments that connect lateral elements to the central element. Within the context of synaptonemal complexes, homologues are intimately connected

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Figure 1. Meiotic recombination pathway

A double-strand break (DSB) introduced by Spo11 is resected to expose 3' ssDNA tails. Mediated by Rad51 and Dmc1, one of the 3' ssDNA tails first invades the homologous chromosome to form a displacement loop (D-loop) and heteroduplex DNA (highlighted), and then initiates DNA synthesis. In the canonical pathway, capture of the second end, DNA synthesis and ligation generate Holliday junctions flanking the DSB site. The Holliday junctions can be resolved by cutting the outside strands (filled arrowheads) or crossed strands (open arrowheads) of each junction (two of the four possible resolution configurations are shown here). In principle, resolution can lead to either a crossover or a noncrossover configuration of the DNA flanking the recombination site. *In vivo*, however, most resolution products are crossovers. An alternative recombination pathway depicted on the right (known as synthesis-dependent strand annealing (SDSA)) leads primarily to noncrossovers. In both types of pathway, gene conversion can occur by mismatch repair if DNA sequence polymorphisms are incorporated into the heteroduplex DNA that forms as part of the recombination process.

Figure 2. Genome rearrangement by non-allelic homologous recombination

Crossover recombination between repeated DNA sequences at non-allelic positions can generate a deletion, a duplication, an inversion or an isodicentric chromosome. Depicted here are six chromosomal outcomes of non-allelic homologous recombination (NAHR) between repeats located on the same chromosome: two orientations of repeats relative to one another (direct or inverted) for each of three types of interactions (between homologues, between sister chromatids or in the same chromatid). Homologous chromosomes are shown in blue and red, and sister chromatids are depicted in the same colour (homologous chromosomes are not shown in the schematics depicting inter-sister chromatid or intrachromatid exchanges for simplicity). Low-copy repeats (LCRs) are shown as white and black arrowheads. Figure modified, with permission, from REF. 55.

Figure 3. Non-allelic homologous recombination between artificially duplicated repeats in yeast
 a | Non-allelic homologous recombination (NAHR) between interstitial repeats. Artificial repeats (depicted as arrowheads) located interstitially in allelic positions (a) and artificial repeats in non-allelic positions (b) on homologous chromosomes (blue and red) recombine with similar efficiency. By contrast, repeats located on heterologous chromosomes (blue and yellow) recombine ~10-fold less efficiently (c) than allelic sequences. These findings suggest that, compared with repeats on heterologues, repeats on homologues are in a closer proximity, probably through recombination-dependent homologue pairing. b | NAHR between subtelomeric repeats. An artificial repeat located in a subtelomeric region recombines only slightly less efficiently with another subtelomeric repeat (two-six fold less than between allelic repeats on homologues (a)), whether on the opposite chromosome arm (b) of the homologue or on a heterologue (c). The dynamic organization of telomeres during prophase of the first meiotic division (that is, attachment to the nuclear envelope and bouquet formation) might increase the proximity of telomeric regions of different chromosomes and thus influence the frequency of NAHR.

Figure 4. The influence of homologue pairing on recombination

a | Double-strand break (DSB)-dependent homologue pairing in budding yeast. After pre-meiotic DNA replication, homologous chromosomes are dispersed in the nucleus. DSBs are formed early in meiotic prophase, providing substrates for recombination proteins to carry out homology search and strand invasion, and resulting in the alignment of homologous chromosome axes at multiple sites (axial interactions)¹. As recombination continues, homologue interactions are progressively stabilized, culminating in the formation of the synaptonemal complex⁹⁶. The development of progressively more stable pairing interactions may inhibit non-allelic homologous recombination (NAHR) by juxtaposing allelic sequences in close proximity. b | The influence of homologue pairing on restraining NAHR. Under normal homologue pairing conditions, NAHR between artificial repeats (indicated by arrowheads) located on one of the paired homologues and on a heterologue occurs at a low level. When pairing between the homologues is disrupted because of sequence divergence between them, the frequency of NAHR between the artificial repeats on heterologues increases ~seven-fold. c | Persistence of recombinational DSBs in unpaired regions of the genome. Mouse chromosomes with semi-identical reciprocal translocations (shown schematically in right panel) undergo homologous pairing and synapsis along most of their lengths and DSBs are repaired in synapsed regions (left panel). However, DSBs in sequences that are not shared between homologues persist into pachytene despite the presence of identical sequences on heterologues.

Figure 5. Suppression of recombination between divergent sequences

When a double-strand [see cover letter comment 1] break (DSB) undergoes strand invasion, heteroduplex DNA is formed. When there are few or no mismatched bases, subsequent recombination processes continue. However, when there are many mismatches, mismatch repair (MMR) proteins (such as Pms1 and Msh2) impede strand invasion steps that could lead to crossover formation. Subsequently, heteroduplex DNA might be destabilized (known as heteroduplex rejection) and possibly channelled into the synthesis-dependent strand annealing (SDSA) pathway, which leads to a noncrossover outcome.

Table 1

Recurrent human genomic disorders likely due to meiotic NAHR

Disorders	NAHR events	Causal genotypes and phenotypes	References
Neurological disorders			
Charcot-Marie-Tooth disease type 1A [‡]	1.4 Mb duplication on 17p	<ul style="list-style-type: none"> Duplication of <i>PMP22</i> Weakness and atrophy of muscles of the lower legs and hands 	29
Hereditary neuropathy with liability to pressure palsies [‡]	1.4 Mb deletion on 17p	<ul style="list-style-type: none"> Deletion of <i>PMP22</i> Episodes of numbness, tingling and/or weakening and atrophy of muscles, most commonly in wrists, elbows and knees 	30
Developmental disorders			
1q21 deletion syndrome [‡]	1.35 deletion on 1q	<ul style="list-style-type: none"> Deletion of many genes Moderate mental retardation, facial features and cardiac abnormalities 	122
Sotos syndrome [‡]	1.9 Mb deletion on 5q	<ul style="list-style-type: none"> Deletion of <i>NSD1</i> Facial dysmorphism, mental retardation and childhood overgrowth 	40
Congenital adrenal hyperplasia [§]	30 kb deletion or gene conversion on 6p	<ul style="list-style-type: none"> Deletion of <i>CYP21</i> by unequal crossing over between <i>CYP21</i> and pseudogene <i>CYP21P</i> or non-functional <i>CYP21</i> by gene conversion from <i>CYP21P</i> Abnormal sexual development and growth defects; severe form can include dehydration 	123, 124
Williams-Beuren syndrome [‡]	1.5 Mb deletion on 7q	<ul style="list-style-type: none"> Deletion of many genes Developmental abnormalities, including cardiac, neurodevelopmental and facial phenotypes 	125
7q11.23 duplication syndrome [‡]	1.5 Mb duplication on 7q	<ul style="list-style-type: none"> Duplication of Williams-Beuren syndrome region Delay in expressive speech 	126
Prader-Willi syndrome [‡]	Paternal 4 Mb deletion on 15q	<ul style="list-style-type: none"> Deletion of paternally imprinted genes Neurobehavioural disorder with some congenital abnormalities 	127
Angelman syndrome [‡]	Maternal 4 Mb deletion on 15q	<ul style="list-style-type: none"> Deletion of maternally imprinted genes Neurobehavioural disorder with some congenital abnormalities 	127
15q13.3 deletion syndrome [‡]	680 kb, 1.5 Mb or 3.95 Mb deletion on 15q	<ul style="list-style-type: none"> Deletion of <i>CHRNA 7</i> Developmental delay, mental retardation and seizures 	128, 129

Disorders	NAHR events	Causal genotypes and phenotypes	References
15q24 deletion syndrome [‡]	3.9 Mb deletion on 15q	<ul style="list-style-type: none"> Deletion of many genes Developmental abnormalities, including mental retardation, growth retardation and facial features 	130
Smith-Magenis syndrome [‡]	3.7 Mb deletion on 17p	<ul style="list-style-type: none"> Deletion of many genes Congenital anomalies, neurodevelopmental and behavioural phenotypes 	131
Potocki-Lupski syndrome [‡]	3.7 Mb duplication on 17p	<ul style="list-style-type: none"> Duplication of many genes Congenital anomalies, neurodevelopmental and behavioural phenotypes 	47
17q21.31 deletion syndrome [‡]	500–650 kb deletion on 17q	<ul style="list-style-type: none"> Deletion of <i>MAPT</i> Severe hypotonia, moderate mental retardation and facial features 	132 – 134
Familial isolated growth hormone deficiency, type 1A [§]	6.7 kb deletion on 17q	<ul style="list-style-type: none"> Deletion of <i>GHI</i> Severe growth retardation 	135
DiGeorge syndrome/velocardiofacial syndrome [‡]	1.5 Mb or 3 Mb deletion on 22q	<ul style="list-style-type: none"> Deletion of <i>TBX</i> Hypocalcemia, cardiac malformations and facial features 	136
Xp11.22–p11.23 duplication syndrome [‡]	Variable-sized duplications on Xp	<ul style="list-style-type: none"> Deletion of many genes Mental retardation and speech delay 	137
Hunter syndrome [*]	20 kb inversion or 30 kb deletion on Xq	<ul style="list-style-type: none"> Disruption of <i>IDS</i> by inversion of portion of <i>IDS</i> via intrachromosomal crossing over with <i>IDS</i> pseudogene or by deletion that includes <i>IDS</i> Lysosomal storage disease resulting in progressive damage of various tissues and organs, leading to numerous phenotypes, including distinct facial features, enlarged organs, cardiovascular disorders and deafness 	138, 139
Metabolic and tissue function disorders			
Bartter syndrome type 3 [‡]	11 kb deletion on 1p	<ul style="list-style-type: none"> Deletion of <i>CLCNKB</i> Defective renal reabsorption of sodium chloride and abnormal kidney function, leading to polyuria, polydipsia and a tendency to dehydration 	140
Gaucher disease [§]	16 kb deletion on 1q	<ul style="list-style-type: none"> Deletion of <i>GBA</i> encoding a lysosomal enzyme by unequal crossing over between <i>GBA</i> and <i>GBA</i> pseudogene Can affect many organs (spleen, liver, kidney and bone marrow) and can include neurological phenotypes (muscle twitches and dementia) 	141

Disorders	NAHR events	Causal genotypes and phenotypes	References
Familial juvenile nephronophthisis 1 [§]	290 kb deletion on 2q	<ul style="list-style-type: none"> Deletion of <i>NPHP1</i> Juvenile kidney disorder: polyuria, polydipsia 	142
Facioscapulohumeral muscular dystrophy, type 1A [‡]	Variable-sized deletions on 4q	<ul style="list-style-type: none"> Causal gene deletion not known Progressive skeletal muscle weakness of facial and shoulder muscles, followed by other muscles and arms 	143
Glucocorticoid-remediable aldosteronism [‡]	45 kb duplication on 8q	<ul style="list-style-type: none"> Fusion gene comprising 5' regulatory region of 11β-hydroxylase gene and coding sequence of aldosterone synthase gene Hypertension, variable hyperaldosteronism and abnormal adrenal steroid production 	144
Polycystic kidney disease 1 [‡]	Gene conversion on 16p	<ul style="list-style-type: none"> Non-conservative amino acid substitutions in <i>PKD1</i> by gene conversion with nearby duplicated copies Bilateral renal cyst formation resulting in enlargement and impairment of renal functions 	145
Neurofibromatosis type 1 [‡]	1.5 Mb deletion on 17q	<ul style="list-style-type: none"> Deletion of <i>NF1</i> Cafe-au-lait spots and fibromatous tumours of the skin 	146
17q12 deletion syndrome [‡]	1.5 Mb deletion on 17q	<ul style="list-style-type: none"> Deletion of <i>TCF2</i> Congenital renal abnormalities and/or maturity-onset diabetes 	147
Ichthyosis [*]	1.5 Mb deletion on Xp	<ul style="list-style-type: none"> Deletion of <i>STS</i> Dry scaly skin 	148
Incontinentia pigmenti [*]	10 kb deletion on Xq	<ul style="list-style-type: none"> Deletion of <i>NEMO</i> Highly variable abnormalities of the skin, hair, nails, teeth, eyes and central nervous system 	149, 150
Red-green colour blindness [*]	Variable-sized deletions and duplications or gene conversion on Xq	<ul style="list-style-type: none"> Deletion within array of red and green pigment genes, duplication that creates a hybrid gene or gene conversion between genes Inability to discriminate between red and green 	151
Blood diseases			
β -thalassaemia [§]	7 kb deletion on 11p	<ul style="list-style-type: none"> Deletion of β-globin gene by unequal crossing over between repeated sequences in δ-/β-globin gene cluster Anaemia 	152
α -thalassaemia [□]	3.7 kb or 4.2 kb deletion on 16p	<ul style="list-style-type: none"> Deletion of α-globin gene(s) by unequal crossing over between repeated sequences in α-globin locus 	153

Disorders	NAHR events	Causal genotypes and phenotypes	References
		<ul style="list-style-type: none"> Anemia 	
Haemophilia A [*]	500 kb or 600 kb inversion on Xq	<ul style="list-style-type: none"> Disruption of factor VIII gene by intrachromosomal inversion between inverted repeats within and outside of gene Impaired blood coagulation 	154
Sex disorders			
Isodicentric Y chromosome formation [‡]	Variable-sized isodicentric Y chromosomes	<ul style="list-style-type: none"> Intersister-chromatid exchange at inverted repeats Infertility, sex reversal or Turner syndrome owing to loss of spermatogenesis genes and/or to instability of dicentric chromosome 	55
Male infertility or subfertility [‡]	Variable-sized deletions on Yq	<ul style="list-style-type: none"> Deletion of genes critical for spermatogenesis by unequal exchange between direct repeats No, or severely reduced, production of sperm 	56 – 61

[‡]Mode of inheritance is autosomal dominant,

[§]Mode of inheritance is autosomal recessive,

^{*}Mode of inheritance is X-linked

[‡]Mode of inheritance is Y-linked.

[□]There are two α globin genes at the α -globin locus. The severity of the thalassaemia is correlated with the number of functional α globin genes. $-/-$ α $-/\alpha$ or $-/-$ α $/\alpha$: mild anaemia; $-/-$ $-/\alpha$: severe anaemia; $-/-$ $-/-$: death *in utero* or shortly after birth. *CHRNA7*, neuronal nicotinic acetylcholine receptor; *CLCNKB*, chloride channel Kb; *GBA*, glucocerebrosidase; *MAPT*, microtubule associated protein tau; *GHI*, growth hormone 1; *NF1*, neurofibromatosis type 1; *PMP22*, peripheral myelin protein 22; *STS*, steroid sulfatase.