

Human inversions and their functional consequences

Marta Puig, Sònia Casillas, Sergi Villatoro and Mario Cáceres

Corresponding author: Mario Cáceres, ICREA Research Professor, Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain. Tel.: +34 93 586 8726; Fax: +34 93 581 2011; E-mail: mcaceres@icrea.cat

Abstract

Polymorphic inversions are a type of structural variants that are difficult to analyze owing to their balanced nature and the location of breakpoints within complex repeated regions. So far, only a handful of inversions have been studied in detail in humans and current knowledge about their possible functional effects is still limited. However, inversions have been related to phenotypic changes and adaptation in multiple species. In this review, we summarize the evidences of the functional impact of inversions in the human genome. First, given that inversions have been shown to inhibit recombination in heterokaryotes, chromosomes displaying different orientation are expected to evolve independently and this may lead to distinct gene-expression patterns. Second, inversions have a role as disease-causing mutations both by directly affecting gene structure or regulation in different ways, and by predisposing to other secondary arrangements in the offspring of inversion carriers. Finally, several inversions show signals of being selected during human evolution. These findings illustrate the potential of inversions to have phenotypic consequences also in humans and emphasize the importance of their inclusion in genome-wide association studies.

Key words: inversions; human genome; phenotypic effects; disease; gene expression; evolution

Introduction

Chromosomal inversions were first discovered in *Drosophila* almost a century ago and they were the first type of genetic variants to be studied [1–3]. An inversion involves a change in orientation of a segment of DNA within a chromosome, but this balanced nature together with the fact that many of them are mediated by repeats complicate their analysis. Therefore, for a long time inversions have been largely overlooked, especially in humans. Recent genomic information, however, has revealed that structural variation is more common than previously thought, increasing the interest in this type of variants [4–7].

Inversions are often generated by non-allelic homologous recombination (NAHR) between inverted repeats, but they can

also be originated by double-strand break repair mechanisms, like non-homologous end joining, or replication-based mechanisms mediated by microhomology, like fork stalling and template switching [8–11] (see also the accompanying review by Escaramís *et al.* [12] for a detailed explanation of each mechanism). Thus, in most cases no significant DNA gain or loss is produced. However, inversions have been demonstrated to affect phenotype in multiple species: from size or developmental time in *Drosophila* [13], to flowering time in plants [14], or adaptation to freshwater in sticklebacks [15], although the mechanisms by which they are able to do so are far from clear. Accordingly, depending on their phenotypic consequences, evolutionary forces such as natural selection or random drift will determine the

Marta Puig is a postdoctoral researcher at the Institut de Biotecnologia i de Biomedicina of the Universitat Autònoma de Barcelona (Spain) working in the functional effects of inversions in humans.

Sònia Casillas is a postdoctoral researcher at the Institut de Biotecnologia i de Biomedicina and the Departament de Genètica i de Microbiologia of the Universitat Autònoma de Barcelona (Spain) with a long trajectory in bioinformatics and molecular evolution.

Sergi Villatoro is a PhD student at the Institut de Biotecnologia i de Biomedicina of the Universitat Autònoma de Barcelona (Spain) studying human genetic diseases related to structural variants.

Mario Cáceres is an ICREA Research Professor and leads the Comparative and Functional Genomics group at the Institut de Biotecnologia i de Biomedicina of the Universitat Autònoma de Barcelona (Spain) focused on the study of human inversions.

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fate of inversions (fixation or elimination), shape their geographical distributions and determine their frequencies in populations [16].

Inversions are considered to have both direct and indirect effects at the molecular level [3, 13, 16]. Indirect effects involve the effective suppression of recombination within the inverted sequence in heterozygotes. This can be caused either by an inhibition of crossovers in this region or by selection against the unbalanced gametes generated by single crossovers that take place within oppositely oriented segments. As a consequence, standard (*Std*) and inverted (*Inv*) chromosomes evolve separately and diverge, accumulating changes that remain associated with the arrangement in which they first appeared. Over time, this leads to the generation of distinct haplotypes in linkage disequilibrium (LD) with each arrangement that maintain together combinations of alleles that may be favorable under certain environmental conditions. This in turn could provide an advantage to one of the arrangements and cause its increase in frequency in the population [13, 17–19]. Alternatively, inversion breakpoints can directly disrupt coding sequences or alter gene expression of adjacent genes by separating regulatory elements from the corresponding coding sequences, providing new regulatory sequences, or moving the genes to different regulatory domains [20, 21]. Finally, for some inversions an additional direct consequence has been observed: the predisposition to other rearrangements, mainly copy number alterations and translocations [22].

In this review, we will focus on the current knowledge about the functional consequences of human polymorphic inversions, ranging from their role in disease to their effects on gene expression, as well as any possible signals of natural selection.

Human polymorphic inversions

Even though the existence of inversions during primate evolution and speciation has been long known [23, 24], we still lack a complete picture of how many polymorphic inversions exist in the human genome and their association with changes in gene expression, adaptation or disease. Human inversions have been typically studied by cytogenetic techniques such as G-banded karyotypes [25] that only allowed the identification of variants of several megabases. Other traditional molecular approaches that have been applied to the targeted detection of inversions are fluorescence in situ hybridization [26, 27], southern blot hybridization [28] or pulsed-field gel electrophoresis [29]. This resulted in the initial identification of a handful of human inversions, most of which were relatively large [30, 31].

In the past years, sequencing techniques have provided valuable applications to the global discovery of inversions (and other structural variants). First, whole-genome sequence comparison allowed us to identify inverted segments between independently assembled individuals [6, 32, 33]. Second, paired-end sequencing and mapping (PEM) supposed a major breakthrough in the field, and most predicted inversions so far have been detected using this strategy [4, 5, 7, 11, 34–36]. The method consists in sequencing the two ends of DNA segments of known size from a given individual and mapping the resulting reads to a reference genome (see the accompanying review by Escaramís et al. [12]). In the case of inversions, abnormal orientation mappings of the read pairs are indicative of the presence of a breakpoint. Both approaches should work well on simple, non-repetitive genomes, but they have important limitations when applied to complex genomes such as that of humans. Because they rely on a reference genome, miss-assembled regions in the

reference are a source of spurious structural variation in the testing genome [33]. In addition, PEM usually fails to detect inversions flanked by long inverted repeats of high identity, such as repetitive elements and segmental duplications (SDs), owing to problems in identifying unique placements for the sequence reads close to the breakpoints [37, 38], and it also generates a high (and yet unknown) rate of false positives [39].

Given the limitations and the relatively low-throughput of the PEM strategy, computational algorithms have been developed that benefit from the interplay between structural and nucleotide variation to predict inversions in multiple individuals and/or estimate inversion genotypes [40–45]. These methods rely on two main assumptions: (i) inversions lie in regions of strong LD and generate specific LD patterns across the breakpoints, and (ii) SNP haplotypes are indicative of the inversion status. It is still unclear how accurate these predictions are, but a high rate of false positives (i.e. regions of high LD owing to low recombination or recent selective sweeps) and false negatives (i.e. small, rare, recent or recurrent inversions) is expected. Therefore, it is always necessary to perform targeted validations and population-wide genotyping using independent methods. PCR-based techniques are among the most widely used experimental methods to validate and genotype inversions, including regular or long-range PCR [5, 11, 24], haplotype-fusion PCR [46, 47] and inverse PCR [48, 49]. Other promising methodologies that could be applied to study inversions include optical mapping, which generates high-resolution restriction maps from single DNA molecules [50], and BioNano technology, which allows the construction of genomic maps by imaging linearized DNA molecules with fluorescent marks introduced at specific sites [51], although in both cases samples have to be processed one by one and the ability of these techniques to detect different types of inversions has to be assessed yet. In addition, we are currently developing a new method to genotype simultaneously multiple inversions with known breakpoints based on probe hybridization, which could be very useful to analyze a large number of individuals [52].

Over the past decade, the Database of Genomic Variants (<http://dgv.tcag.ca>) [53] has provided a publicly accessible, comprehensive catalogue of all types of structural variation in the human genome, including inversions. More recently, the new InvFEST database (<http://invfestdb.uab.cat/>) [39] represents an effort to generate a curated, non-redundant catalogue of human polymorphic inversions, which provides refined breakpoint locations, associations with genes and SDs, plus data on experimental validation, population frequency, known functional effects and evolutionary history (for easier access, InvFEST names will be provided for all polymorphic inversions mentioned in the review). In its current version, it contains 1092 candidate inversions, of which only 18% are reported by multiple studies and 85 have been validated experimentally (Figure 1). Interestingly, for those inversions with no experimental data (either validated or false), InvFEST provides information on their reliability according to different bioinformatic internal scores based on the PEM support of each prediction and the mapping to simple repetitive sequences (see [39] for further details). This categorization suggests that more than half of the predictions might be artifacts, supporting the suspicion that large-scale detection methods (which account for 98% of InvFEST inversions) have a high false-positive discovery rate. Still, an analysis of the inversions in InvFEST database reveals some features of human polymorphic inversions. First, although the size distribution of inversions is similar to that reported 5 years ago considering the set of inversions included in the Database of Genomic

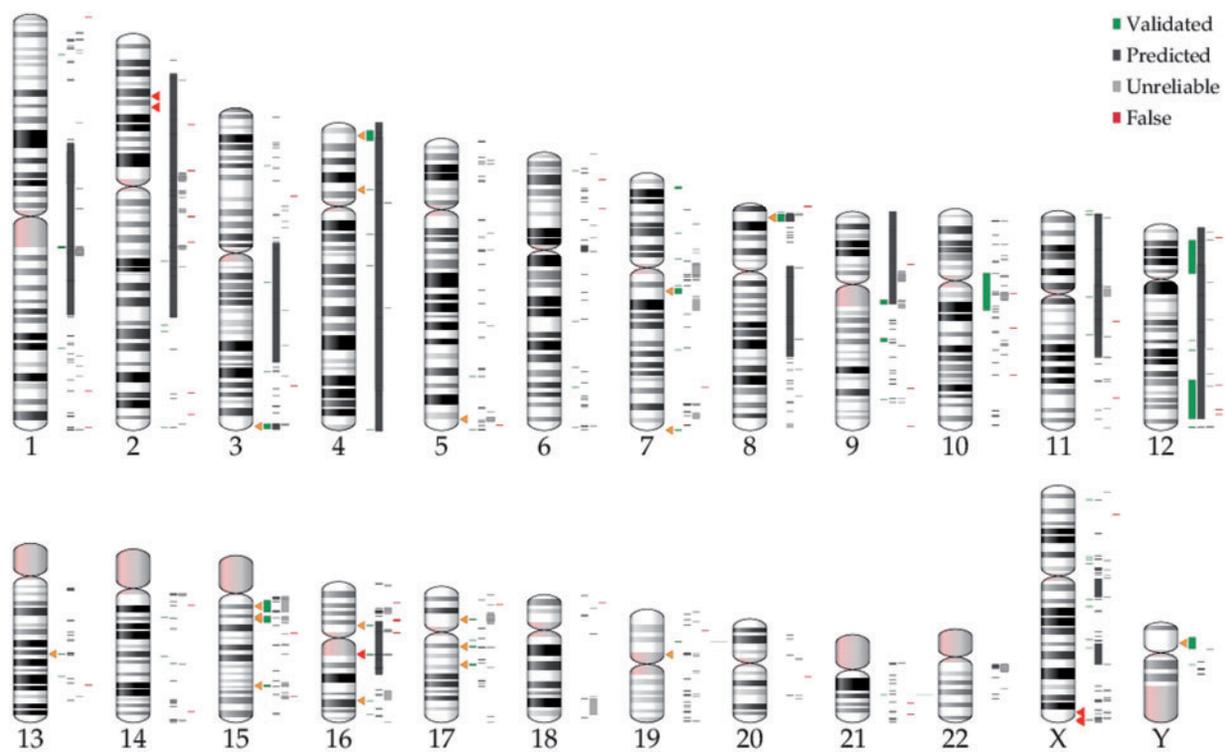


Figure 1. Distribution of inversion polymorphisms in the human genome. The chromosomal positions of the 1092 inversions reported in InvFEST [39] are indicated to the right of each ideogram (with the status of inversions according to InvFEST represented in the same order as shown in the legend; see main text for details). Triangles mark the position of individual inversions discussed in the main text, with 5 pathogenic inversions in dark and 21 polymorphic inversions in light color. Please note that while triangle marks indicate the center of the inversion, some big inversions span several megabases, specially the pathogenic inversion in chromosome 16, which inverts almost the whole chromosome. (A colour version of this figure is available online at: <http://bfg.oxfordjournals.org>)

variants [30], most inversions >10 kb could be false positives (Figure 2A). Second, as suggested by Pang *et al.* [11], when only validated and predicted inversions are considered, their size distribution according to the association with SDs (Figure 2B) shows that the longer the inversion, the more probable it is flanked by SDs. Nevertheless, the uncertainty about the final set of human polymorphic inversions complicates making reliable conclusions about the observed patterns.

Inversions as simple mutations causing disease

Many inversions traditionally detected in human karyotypes do not appear to have any phenotypic effects of clinical significance. This is the case of pericentric inversions (the inverted sequence includes the centromere) in chromosomes 1, 2, 3, 5, 9, 10 and 16, which mainly invert heterochromatic sequences and are frequently observed in cytogenetic analysis [25]. For example, the pericentric inversion in chromosome 9 shows variable frequencies in different human populations ranging from 0.26% in Asians to 3.57% in individuals from African origin [54]. In addition, despite the possible effects on fertility owing to the unbalanced chromosomes resulting from a crossover within the inverted region in heterozygotes, for some of these inversions no increased risk for miscarriage or problems in chromosome segregation during meiosis have been observed [55, 56]. Not all inversions are harmless, however, and several diseases have been found to be occasionally caused by inversions, mostly by direct disruption of one gene [57, 58] or by altering its gene expression [59, 60]. These inversions appear *de novo* in patients or

are inherited mutations restricted to a given family, and thus they do not represent polymorphic variants segregating in human populations. Nevertheless, they have clinical importance and can contribute to the identification of genes underlying certain rare disorders [61, 62]. In this sense, it is possible that the contribution of structural variants to disease is currently underestimated owing to the frequent use of exome sequencing to identify the causal genes, a strategy that fails to detect genes truncated by a breakpoint.

Besides, there are a few inversions that have been demonstrated to produce some diseases in a recurrent manner, such as in hemophilia A and Hunter syndrome (a metabolic disorder called mucopolysaccharidosis type II). In hemophilia A, an inversion is generated by recombination between a 9.5 kb repeat in intron 22 of the F8 gene and one of two other copies located ~565 kb away on the X chromosome [46, 63, 64]. This gene encoding coagulation factor VIII is disrupted by these inversions in ~45% of severe hemophilia A patients [65, 66]. Another ~140 kb inversion mediated by repeats, one of which is located in F8 intron 1, accounts for 4.8% of hemophilia A cases [66]. Apart from disrupting the gene, this second inversion also generates hybrid transcripts including the first F8 exon and exons of the VBP1 gene. The two types of inversions have been detected in familial hemophilia in which the mutation was inherited by the male patients from their unaffected mothers and in isolated cases where the inversion has been generated *de novo* [65]. In Hunter syndrome, an inversion truncates the X-linked *IDS* gene, which encodes the iduronate 2-sulfatase enzyme, and occurs by recombination with the homologous sequences of an adjacent partial pseudogene (*IDS2*) in 13% of patients [67]. Similarly, in some cancers, inversions disrupting

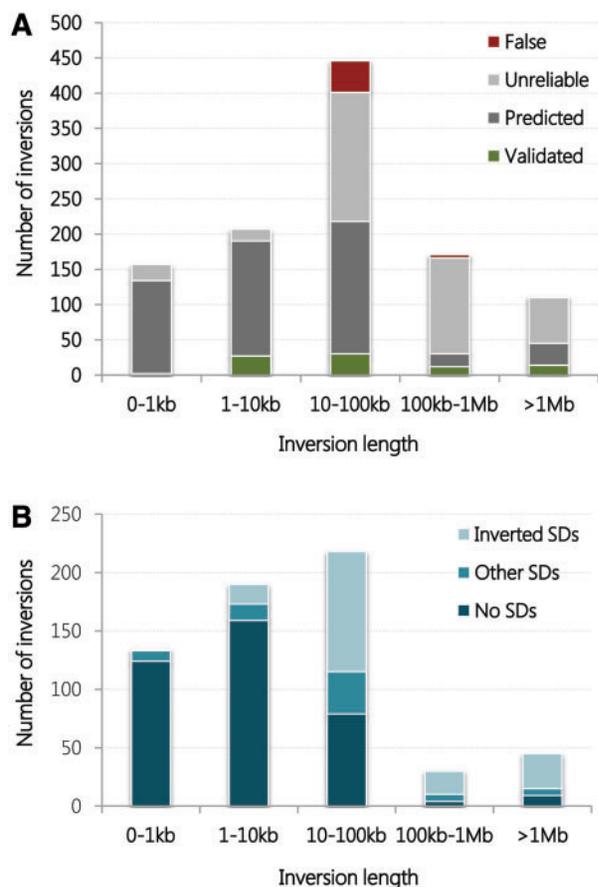


Figure 2. Size distribution of human polymorphic inversions. (A) The size distribution of inversions in InvFEST [39] shows that the majority of inversions reported to date are <100 kb long, and that while inversions <10 kb long are mostly reliable, more than half of >10 kb inversions might be false positives. (B) If unreliable or false predictions are discarded, the majority of <10 kb inversions have simple breakpoints, while the majority of the remaining inversions are flanked and appear to be mediated by SDs. (A colour version of this figure is available online at: <http://bfg.oxfordjournals.org>)

coding regions [68] or creating fusion genes that can be directly related to the prognosis of the patient [69, 70], have been found in several unrelated individuals.

Polymorphic inversions associated to phenotypes or diseases

One of the main limitations in the study of the functional effects of human polymorphic inversions is the lack of genotyping information for most of them. However, although the underlying molecular mechanisms remain unclear, phenotypic effects have been associated to the two orientations of a few human inversion polymorphisms. The most striking and well-studied case is that of inversion 17q21.31 (HsInv0573) that inverts ~970 kb of single-copy sequence containing the gene *MAPT* among others, and maintains two separated haplotypes: H1 and H2 (inverted with respect to the reference genome) [71]. The two haplotypes have an estimated divergence of 0.476% [72] and include different combinations of duplicated sequences at the breakpoints [73, 74]. H1 haplotype has been associated with several neurodegenerative diseases [75], including progressive supranuclear palsy [76], corticobasal degeneration [77], Alzheimer's disease [78] and Parkinson's disease [79–81], which exhibit aggregation

of hyperphosphorylated Tau protein (encoded by gene *MAPT*) in neuronal cell bodies [77]. On the other hand, H2 haplotype has been associated with increased mean rates of recombination in females [71, 82]. Women with high recombination rates tend to have more children [83] and both traits were detected in H2 female carriers in the Icelandic population [71]. Similarly, in the 8p23.1 inversion (HsInv0501), the longest inversion polymorphism (~4.5 Mb) described in humans, which is mediated by large complex repeats [26], risk alleles for systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), are apparently transmitted only on one of the *Std* haplotypes [44, 84], so the *Inv* haplotypes would protect against these diseases.

The recently developed computational methods to predict inversions based in nucleotide variation data [42–45] have opened a new way to investigate potential phenotypic effects of inversions from available genome wide association studies (GWAS). In the first successful application of this strategy, a previously identified inversion in 16p11 (HsInv0786), a 171 kb inverted region flanked by 134 kb inverted SDs [32], was genotyped from SNP data by the *inveR*sion [42] and *PFIDO* [44] algorithms, and association analysis revealed that the *Inv* allele protects against asthma and, more significantly, against the co-occurrence of asthma and obesity [85]. This association between inversion genotypes and the asthma-obesity phenotype was replicated in five independent studies and was not found when independent SNPs were considered, indicating that the observed effect is most likely owing to the whole haplotype [85]. In addition, Ma et al. [86] incorporated the inversions predicted with their principal components analysis-based approach from SNP data [43] in a GWAS analysis to find association of these regions with psoriasis, a chronic, inflammatory skin disease affecting 2–3% of the world population. They found significant associations of this disease with several candidate inversions, of which only two replicated in two different data sets. It turns out that neither of the two candidate inversions overlaps those reported in the InvFEST database [39]. Therefore, these results should be taken cautiously given that the SNP signatures found might not be owing to an inversion, although the regions detected by the association test may still shed light on the genetic architecture of the disease, as the authors point out [86].

Inversions predisposing to other rearrangements

While some inversions apparently do not have phenotypic consequences by themselves, carrying a certain allele may predispose to other rearrangements in the same genomic region, which in turn leads to a disease. In these cases, the parents of affected individuals show an increased frequency of one of the alleles of the inversion compared with the general population. One of the first described examples was that of the Williams-Beuren syndrome. This disease is caused by a 1.5 Mb deletion in chromosome 7 and an inversion involving the same region has been detected with a frequency of 12.4% in the transmitting parents of patients, although it has a frequency of 2.9% in control individuals [29, 87, 88]. Similar results have been observed for deletions causing Angelman syndrome, where the inversion has a frequency of ~33% in mothers of patients with the class II deletion but only 4.5% in controls [89], and Sotos [90] or 17q21.31 microdeletion [91] syndromes, among others (Table 1). Most of these diseases are thought to be caused by haploinsufficiency of one of the deleted genes, since affected individuals usually carry one normal chromosome (the exceptions being

Table 1. Human polymorphic inversions predisposing to other rearrangements

Cytogenetic band	Inversion (InvFEST)	Inversion length	Secondary arrangement	Disorder	References
3q29	HsInv0264	1.9 Mb	Deletion	3q29 microdeletion syndrome	[27]
4p16	HsInv0472	6 Mb	t(4;8)(p16;p23)	Wolf-Hirschhorn syndrome	[26]
5q35	HsInv0687	1.9 Mb	Deletion	Sotos syndrome ^a	[90]
7q11.23	HsInv0301	1.5 Mb	Deletion	Williams-Beuren syndrome (WBS) ^a	[29, 88]
8p23.1	HsInv0501	4.5 Mb	Deletions/Duplications t(4;8)(p16;p23)	Mental retardation and other problems Wolf-Hirschhorn syndrome	[92] [26]
15q11-q13	HsInv0549	4 Mb	Deletion	Angelman syndrome ^a	[89]
15q13.3	HsInv1049	1.8 Mb	Deletion	15q13 microdeletion syndrome	[93]
15q24	HsInv0544	1.2 Mb	Deletion	15q24 microdeletion syndrome	[27]
17q12	HsInv1048	1.5 Mb	Deletion	RCAD syndrome	[27]
17q21.31	HsInv0573	970 kb	Deletion	17q21.31 microdeletion syndrome ^a	[91]
Xq28	HsInv0389	48 kb	Deletion	Emery-Dreifuss muscular dystrophy (EMD)	[28]
Yp11.2	HsInv0415	4 Mb	PRKX/PRKY translocation	Sex reversal/male infertility	[94]

^aDisorders in which a higher frequency of the inversion has been found in the transmitting parents of the affected individuals compared with the general population.

Emery-Dreifuss muscular dystrophy, which is inherited in an X-linked recessive manner, and Angelman syndrome, which is caused by the loss of maternal expression of the imprinted *UBE3A* gene). In addition, while the causal gene is known in some cases (e.g. *EMD* for Emery-Dreifuss muscular dystrophy [95] and *NSD1* for Sotos syndrome [96]), in others, like Williams-Beuren or 17q21.31 microdeletion syndromes, it has not yet been identified because the deletions are large and eliminate several genes [29, 91].

Apart from deletions, inversions could also promote more complex intrachromosomal arrangements and translocations (Table 1). This is what happens in region 8p23.1, where the 4.5 Mb inversion could predispose to the formation of a dicentric chromosome carrying a single copy of the inverted region but duplications or deletions of a substantial fraction of chromosome 8 that have been associated with developmental delay and mental retardation, among other problems [92]. In mothers that are doubly heterozygous for the 8p23.1 inversion and another common inversion in chromosome 4 mediated by similar complex repeats, this same inverted region is involved in the generation of the recurrent unbalanced t(4;8)(p16;p23) translocation, which is one of the causes of Wolf-Hirschhorn syndrome [26]. Also, sex reversal caused by a translocation between homologous genes *PRKX* and *PRKY* (located on chromosomes X and Y, respectively) seems to occur preferentially on Y chromosomes carrying a ~4 Mb inversion [46, 94].

The main mechanism by which one of the inversion alleles is thought to preferentially predispose to deletion rearrangements is through specific repetitive sequences in direct orientation. This can be achieved either by the switch of the relative orientation of pre-existing repetitive sequences by the inversion, or by accumulation of structural changes that increase the direct repeat content exclusively in one haplotype. The repeats could then recombine to generate structural variants that were not possible from the original arrangement. This is the situation found in inversion 17q21.31, where both H1 and H2 haplotypes have separately experienced independent duplications at the inverted repeats that mediated the inversion, which resulted in diverse complex structures ranging between 518 kb and 1 Mb of duplicated sequence associated to different sub-haplotypes [73, 74]. One of these sub-haplotypes, H2D, which is found at a higher frequency in European populations, predisposes to the 17q21.31 microdeletion syndrome [91] caused by the deletion of the inverted region by NAHR between 155 kb directly oriented

repeats found only in this particular H2 haplotype [74]. Accordingly, the 17q21.31 microdeletion syndrome has been reported almost exclusively in individuals of European descent [97]. Furthermore, in the Emery-Dreifuss muscular dystrophy, the *EMD* gene deletion may be facilitated by recombination between two *Alu* elements located respectively in a *Std* and an *Inv* chromosome [28]. However, other factors may also play a role in the production of secondary rearrangements. For example, heterozygosity for a polymorphic inversion has been proposed to increase susceptibility to unequal recombination because it may lead to abnormal meiotic pairing [29, 98]. This is the case of the unstable dicentric chromosomes that are generated by recombination between the two arrangements within the 8p23.1 inverted region [92].

Despite the above evidences, we have to take into account that the predisposition to other rearrangements is weak given the low incidence of these diseases and the relatively high frequency of some inversions. Thus, the probability for an inversion carrier to have an affected child, although slightly higher than for a non-carrier, is still extremely low [88, 92]. Besides, not all patients for the diseases carry a deletion, and for those with other types of mutations, the presence of the inversion in their parents might be totally irrelevant. Even in some cases like the Williams-Beuren syndrome, recent studies have not found significant evidence of the association between inversion and deletion [99], suggesting that the increased susceptibility may not be completely clear or that it may be restricted to certain haplotypes. Similarly, an increase in the direct repeat length not always predisposes to deletion. For example, a 130 kb 15q13.3 inversion, which generates ~188 kb of directly oriented SDs with 99.4% identity at deletion breakpoints (compared with 58 kb in the *Std* haplotypes), shows the same frequency among microdeletion cases than in the general population [100]. Therefore, further studies are necessary to quantify better the role of inversions in other rearrangements.

Effects of inversions on genes and gene expression

As mentioned in the introduction, inversions can directly affect genes and their regulation at different levels, with consequences that range from drastic to more subtle, and not all have been studied at the same detail. While it is relatively simple to

determine if an inversion disrupts a gene (assuming that the exact position of the breakpoints is known), predicting the effects on gene regulation is much more difficult. Considering the most reliable inversions in the InvFEST database (i.e. those with a validated or predicted status), the majority of them are either intergenic or intronic, but ~12% might break genes or at least some alternative gene transcripts (Figure 3). In several cases, two gene copies located within highly similar SDs at inversion breakpoints are exchanged in the inverted arrangement and no significant expression changes are expected [49]. For example, in HsInv0030 the first exon and promoter of the two chymotrypsinogen precursor genes *CTRB1* and *CTRB2* are exchanged in one of the inversion alleles [11, 36] (Figure 4). In addition, hybrid transcripts including exons from both genes have been detected [11], confirming the effect of the inversion in the transcript sequence. Other validated polymorphic inversions disrupt genes completely and move part of them several kb away (Figure 4), as

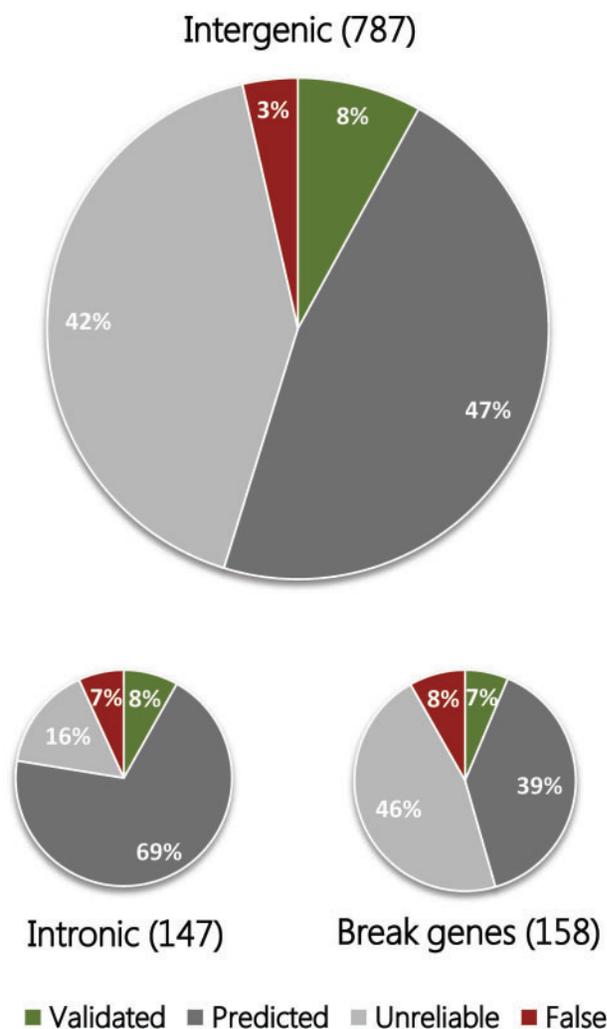


Figure 3. Classification of inversions according to their overlap with gene regions. The 1092 inversions reported in InvFEST [39] are classified into three main categories: (i) *Intergenic*, which do not disrupt any genic sequence, even though they might invert complete genes; (ii) *Intronic*, which are completely included within the intron of a gene; and (iii) *Break genes*, which are inversions that disrupt genes, either at their ends or inverting any internal exons. Colors indicate the status of inversions as shown in the legend, and illustrate that more than half of inversions that disrupt genic sequences might be false positives. (A colour version of this figure is available online at: <http://bfg.oxfordjournals.org>)

happens for long non-coding RNA *LINC00395* in HsInv0340 [49], gene *ZNF257* in HsInv0379 [39], gene *VIPR2* in HsInv0626 [101] and the putative pseudogene *CCDC144B* in HsInv1051 [49]. Inversions can also affect gene structure with unknown consequences to gene expression, like in HsInv0102 that inverts an alternative non-coding exon of gene *RHOH* (S. Villatoro and M. Cáceres, unpublished results) (Figure 4). For these cases, detailed analyses are needed to determine how the inversion affects the expression of both the broken genes and more distant genes. Another possibility is that an inversion relocates a gene to a region close to heterochromatin, thus suppressing its expression, as it occurs in position-effect variegation in *Drosophila* [102]. Although this phenomenon has not been reported in humans, topological domains associated with coordinately regulated gene clusters have been described in mammalian chromatin [103, 104] and the disruption of such domains by inversions might influence gene regulation.

To date, associations between inversion alleles and gene expression have been performed only for a few of the most studied human polymorphic inversions for which it was possible to predict the genotypes in many individuals [44, 85, 105]. All studies have focused on total expression levels with only a few tissues analyzed in each case, so differences relative to tissues and times of expression remain largely unexplored. Lymphoblastoid cell lines (LCLs) are the most used tissue because they are a common source for DNA and RNA, and several established collections like HapMap samples have available expression data. Also, the analysis has been usually limited to genes located within and around the inversion.

For inversions 8p23.1, 17q21.31 and 16p11, several genes located within the inverted region have been detected as differentially expressed between *Std* and *Inv* chromosomes [44, 85, 105]. These expression differences could be caused either by inversion breakpoints separating coding regions from regulatory elements, or by functional SNPs fixed in one of the divergent haplotypes maintained by the inversion, with the last option being generally favored [44, 85, 105]. Of the observed differences, some persist across tissues and populations, indicating a strong association with inversion alleles. This is the case for gene *PPP1R3B* in inversion 8p23.1, which is downregulated in *Inv* chromosomes in three different populations both in blood and LCLs [44], or for gene *MAPT* in inversion 17p21.31, which shows a higher expression in H1 chromosomes in frontal cortex and cerebellum [105, 106]. Some other genes show consistent differences across diverse data sets of the same origin. For example, *BLK* expression exhibits a positive correlation with the number of 8p23.1 *Inv* alleles in European populations that is not found in Asians or Africans [44]. Finally, the expression levels of several genes have been associated with the inversion only in a given data set, like genes *XKR6*, *CTSB*, *NEIL2* and *MSRA* in LCLs for inversion 8p23.1 [44, 107], *PLEKHM1* and *CRHR1* in cerebellum for inversion 17q21.31 [105] or genes *CCDC101* (blood) and *IL27* (LCLs) for inversion 16p11 [85], which illustrates the complexity of determining the effects of inversions in gene expression. In inversion 16p11, other genes outside the inverted region but adjacent to the inversion breakpoints appear to be differentially expressed between arrangements as well (genes *TUFM* and *SPNS1* show a higher expression in *Inv* alleles in two different tissues), suggesting that the inversion effects extend beyond the inverted region [85]. In addition, most of these expression differences follow an additive model in which the heterozygote shows an intermediate expression compared with both homozygotes, although there are also genes with higher expression in heterozygotes, like *IL27* in inversion 16p11 [85].

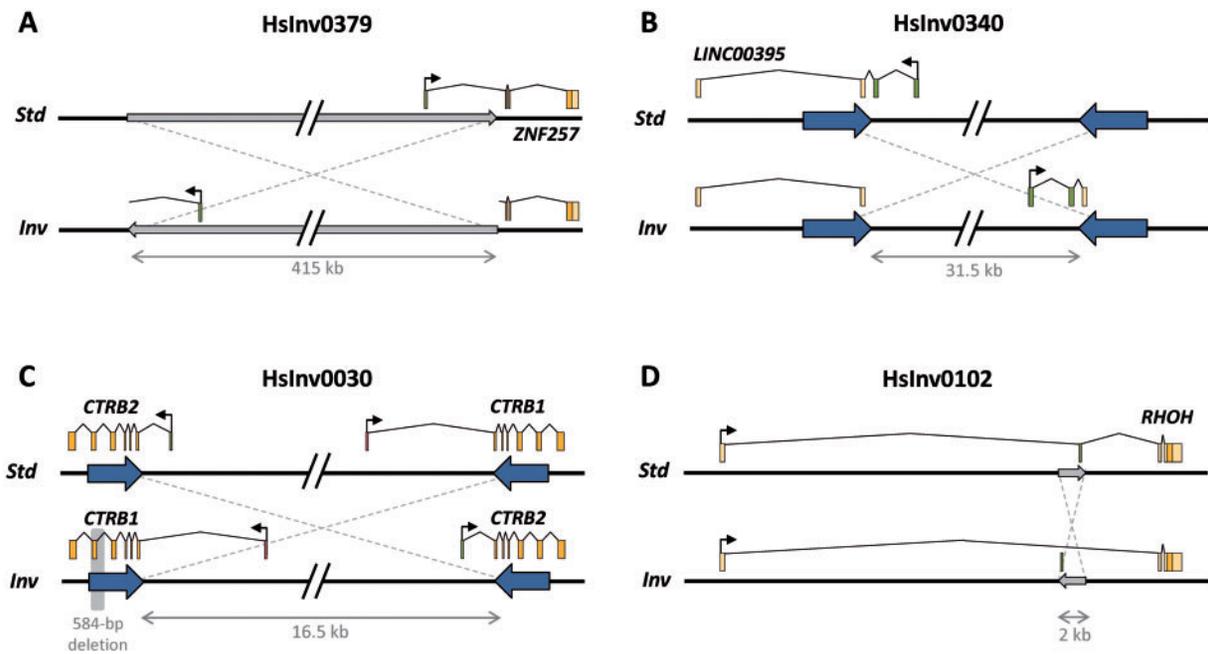


Figure 4. Examples of genes affected by polymorphic inversion breakpoints. (A) Disruption of the protein-coding sequence of gene *ZNF257* by one inversion breakpoint of *HsInv0379* located in the first intron of the gene [39]. (B) Disruption of a long non-coding RNA gene of unknown function by *HsInv0340* [49]. (C) Exchange of *CTR1B1* and *CTR2B2* first exons by inversion *HsInv0030* generated between inverted repeats overlapping the two genes [11, 36]. (D) Change of orientation of an alternatively spliced exon of gene *RHOH* by inversion *HsInv0102*, which no longer can be included in the transcript in the inverted orientation (S. Villatoro and M. Cáceres, unpublished results). Exons are depicted as light boxes with different shades indicating coding and non-coding parts of the transcripts and an arrow showing the direction of transcription. Exons affected by the inversion are represented as dark boxes. The minimum size of the inverted region is indicated below and in those inversions without inverted repeats at the breakpoints (large arrows); it is represented as a narrow gray arrow. In *HsInv0030*, a polymorphic deletion that removes one exon of *CTR1B1* in some inverted chromosomes is shaded in gray. (A colour version of this figure is available online at: <http://bfg.oxfordjournals.org>)

On the other hand, expression differences have been detected in multiple-copy genes located within the SDs at the inversion breakpoints (e.g. *LRRC37A* and *A4* in 17q21.31, or *EIF3C* and *EIF3CL* in 16p11) or in gene families with copies located both within and outside the inverted region (e.g. genes *SULTA1*, *SULTA2* and *SULTA4* in 16p11) [85, 105]. However, these differences should be taken with caution because it is difficult to quantify accurately gene expression from each individual copy either by microarrays or RNA-Seq without specific probes or specific mappings. This is exemplified by *LRRC37A*, whose expression shows highly significant differences between the 17q21.31 inversion alleles in several brain tissues, but the probes used are able to bind more than one target gene in the family [105]. In inversion 17q21.31 the situation is further complicated by the existence of H1 and H2 chromosomes with different numbers of partial copies of genes *KANSL1* and *NSF* at the inversion breakpoints [73, 74], which might result in expression differences owing to the extra copies rather than to the inversion itself. In fact, new *KANSL1* transcripts that may have an impact on female fertility are produced from both H1 and H2 haplotypes with partial *KANSL1* duplications [73].

Remarkably, a few of the detected gene expression differences have been linked to phenotype, providing a mechanism by which the inversion could have functional consequences. *BLK* expression might mediate the increased risk for SLE and RA associated with one of the 8p23.1 inversion *Std* haplotypes [44], and overproduction of Tau protein encoded by *MAPT* (increased in inversion 17q21.31 H1 haplotypes) in neuronal cell bodies has been linked to several neurodegenerative disorders [77]. In inversion 16p11, expression changes in genes involved in energy balance and immunity like *TUFM* and *IL27* might explain the protective effect of the *Inv* allele against co-occurrence of

asthma and obesity [85]. However, in none of these cases a genome-wide differential expression analysis has been performed to identify possible downstream expression changes due to those generated within and around the inverted region. Such analysis, together with a more complete screening of human tissues, could provide a better understanding of the functional consequences of inversions at the organism level.

Inversions and natural selection

An alternative way to identify the functional impact of inversions is through the detection of the action of natural selection. Although inversions may have played an important role in primate speciation [108, 109], their role in shaping human populations is still uncertain. Basically, we lack tests to study the selective advantage or disadvantage of inversions, even at a small scale, and thus for just a handful of inversions their selective effects have been reported in terms of the frequency and geographic distribution of the alleles.

The 17q21.31 inversion is the most intensively studied inversion also in this sense. The inversion has been reported to be under positive selection in Europeans [71], where the *Inv* haplotype (H2) reaches a relatively high frequency (~10–35%) [74]. Specifically, as mentioned before, women carrying either one or two copies of the inversion tend to have more children [71], but at the same time, H2D, the most common H2 haplotype in European populations, has been associated to disease-causing microdeletions [91]. Therefore, the haplotype associated to the detrimental duplication architecture predisposing to intellectual disability is also the one conferring advantage to female carriers, and is protective against neurodegenerative diseases [75–81]. These results, together with the global distribution of

the inversion 17q21.31 haplotypes, led some authors to hypothesize that the high frequency of H2 in Europeans would be the result of founder effects instead of selection [72, 110]. However, the finding that H2 seems to be the ancestral haplotype [72], together with the analysis of nucleotide variation in the region and the dating of the different characterized haplotypes has raised the alternative hypothesis that the original H1 haplotypes first appeared and increased in frequency in Africa, and the complex duplicated haplotypes were independently generated later on, being maintained predominantly in populations that migrated out of Africa [74]. Advances in population genetics inference might help better explain the nucleotide variation patterns found in this region and differentiate between selection and demographic processes in the expansion of the H2D haplotype in Europe [31, 74].

For other inversions like 8p23.1, no clear signals of positive selection have been found in spite of multiple loci within the inverted region being putative targets of natural selection, including loci associated with autoimmune and cardiovascular disease [44]. In this case, the *Inv* allele is the ancestral but no SNPs are in perfect LD with the inversion haplotype, which suggests some level of gene flow between *Std* and *Inv* haplotypes, or that the inversion has appeared recurrently throughout evolution [27, 107]. Even though these observations hinder the study of the inversion, its worldwide clinal distribution is consistent with neutral demographic models of the human expansion out of Africa [44].

Finally, inversions HsInv0030 and 16p11 share some characteristics with the two inversions above [11, 85]. In HsInv0030, the *Inv* orientation is the ancestral one and is the major allele [11]. Furthermore, ~6% of inverted alleles are associated to a 585 bp deletion that eliminates exon 6 of *CTRB2*. The authors found evidence of population differentiation in the haplotype frequencies, and together with the possible functional effect of the inversion and deletion alleles on the genes, they hypothesized that the observed differences could be the result of adaptation to different diets across populations [11]. Estimated inversion 16p11 allele frequencies also show remarkable worldwide population stratification, with the derived allele frequency (*Std* orientation) ranging from 90% in East Africa to 51% in Northern Europe, which has led to suggest the existence of selective sweeps for the inverted allele after human migration out of Africa [85]. Nevertheless, for none of the two inversions formal tests of selection have been performed.

Concluding remarks

As we have seen, different types of evidence indicate that inversions could have important consequences in the human genome. However, for most human inversions this has not been investigated yet and more work needs to be done to determine their effects in humans. In this sense, it is important to note that a high proportion of the inversions mediated by inverted repeats have apparently occurred multiple times in the human lineage [27, 49, 98], and the number of recurrent inversions could increase considerably as more individuals and more populations are studied. As a result, in contrast to copy number variants (CNVs), in which most of the disease associations found are mimicked by SNPs and have been already indirectly explored [111], recurrent inversions that are not tagged by SNPs have likely been missed from current GWAS studies and their potential effects remain hidden. Therefore, high-throughput methods are needed to directly genotype inversions in a high number of individuals and should contribute

to unveil their real functional and evolutionary impact in the near future.

Key points

- While there are multiple examples of particular inversions leading to disease by disruption of coding regions or by affecting gene expression, only a few recurrent inversions have been described that repeatedly cause disease by these mechanisms.
- Some polymorphic inversions segregating in human populations with no apparent phenotype predispose to pathogenic microdeletions in the offspring of carriers.
- Diverse studies demonstrate that several inversions are linked to haplotypes that carry alleles with different expression between inverted and non-inverted chromosomes.
- Although they are difficult to analyze, phenotypic effects have been associated to certain inversions that might be evolving under positive selection.

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