

Gene conversion: mechanisms, evolution and human disease

Jian-Min Chen^{*†}, David N. Cooper[§], Nadia Chuzhanova^{||}, Claude Férec^{**†#} and George P. Patrinos^{**}

Abstract | Gene conversion, one of the two mechanisms of homologous recombination, involves the **unidirectional transfer of genetic material from a 'donor' sequence** to a highly **homologous 'acceptor'**. Considerable progress has been made in understanding the molecular mechanisms that underlie gene conversion, its formative **role in human genome evolution** and its implications for human inherited disease. Here we assess current thinking about how gene conversion occurs, explore the key part it has played in fashioning extant human genes, and carry out a meta-analysis of gene-conversion events that are known to have caused human genetic disease.

Unequal crossover

A recombination event between non-allelic sequences on non-sister chromatids of a pair of homologous chromosomes.

Homologous recombination

The process by which segments of DNA are exchanged between two DNA duplexes that share high sequence similarity.

^{*}INSERM, U613, 29220 Brest, France.
[†]Etablissement Français du Sang — Bretagne, 46 rue Félix Le Dantec, 29220 Brest, France.
^{**}Erasmus University Medical Center, Faculty of Medicine and Health Sciences, MGC-Department of Cell Biology and Genetics, PO BOX 2040, 3000 CA, Rotterdam, The Netherlands.
 Correspondence to J.M.C. or G.P.P.
 e-mails: Jian-Min.Chen@univ-brest.fr; g.patrinos@erasmusmc.nl

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Gene duplication and amplification have contributed to the **marked expansion of vertebrate genomes**, which has in turn led to the proliferation of regulatory and developmental processes and enzymatic reactions. Following gene duplication, the newly generated gene copies are **prone to reciprocal unequal crossover** or unidirectional **gene-conversion** events by virtue of the high degree of homology between them. As a consequence of the latter process, the 'acceptor' sequence is replaced, wholly or partly, by a sequence that is copied from the 'donor', whereas the sequence of the **donor remains unaltered**. A common phenomenon in fungi, gene conversion has also long been noted in mammalian cells, with the human haemoglobin genes *HBG1* and *HBG2* genes being the first characterized examples¹.

The past few years have witnessed significant progress towards an understanding of the mechanism of gene conversion and its impact on human genome evolution. This has been made possible by advances in several fields: the burgeoning characterization of **homologous recombination-associated factors**, sperm typing, and the availability of large-scale human population genetic variation data and complete genome sequences from multiple species. Gene conversion has also increasingly been found to **underlie human inherited disease**. Here we bring together the many recent developments in this rapidly evolving field. Although current models of gene conversion are largely derived from work carried out in model systems (particularly yeast), our discussion, whether in an evolutionary or a pathological context, focuses almost exclusively on human data. In this context, it is important to bear in

mind an important caveat: direct evidence for gene conversion in humans is always lacking because it is impossible to analyse both products of a single recombination event in humans.

The mechanistic basis of gene conversion

In eukaryotes, **gene conversion constitutes the main form of homologous recombination** that is initiated by **DNA double-strand breaks (DSBs)**. During meiosis, DSBs are **created by a topoisomerase-like enzyme (SPO11)**, whereas during mitosis they can be induced by radiation, **stalled replication forks** or specialized endonucleases (for example, the site-specific HO endonuclease in the switching of yeast mating-type (*MAT*) genes; reviewed in REFS 2,3). Gene conversion mediates the transfer of genetic information from intact homologous sequences to the region that contains the DSB, and it can **occur between sister chromatids, homologous chromosomes or homologous sequences on either the same chromatid or different chromosomes**.

Current models of gene conversion. Our current understanding of how gene conversion occurs is summarized in FIG. 1. According to the seminal double-strand break repair (DSBR) model of Szostak and colleagues⁴, the **ends of the DSB are resected by 5'→3' exonucleases**, resulting in the **formation of two 3' ssDNA tails**. These tails actively **'scan' the genome for homologous sequences**; one of them **invades the homologous DNA duplex to form a displacement (D)-loop**, which is then **extended** by DNA synthesis, having been primed from this single-end invasion. The extended D-loop then

Double-strand break

Breaks in opposite DNA strands that lie within ~10–20 bp of each other.

Holliday junction

A point at which the strands of two dsDNA molecules exchange partners, an event that occurs as an intermediate in crossing over or gene conversion.

Mismatch repair

A natural enzymatic process that replaces a mispaired nucleotide within a DNA duplex to yield perfect Watson–Crick base pairing.

Orthologue

A homologous gene that is derived from a speciation event or by vertical descent.

pairs with the other 3' ssDNA tail (second-end capture), while DNA synthesis at the newly captured strand followed by ligation of the nicks results in the formation of an intermediate with two Holliday junctions (HJs). Random cleavage of the two HJs by an HJ resolvase yields either a non-crossover (that is, gene conversion) or a crossover product (reviewed in REFS 2,3). During this process, most gene conversion is derived from the mismatch repair of the heteroduplex DNA that is formed between the donor and acceptor DNA sequences, rather than from double-strand gap repair, as was originally thought⁴. The mismatch correction probably occurs before the resolution of the double HJs. It is the broken strand that is usually corrected using the intact strand as a template (reviewed in REF. 5).

The DSB model has provided a satisfactory explanation for several features of meiotic recombination, and HJs have been physically identified as intermediates in meiotic recombination (for example, REF. 6). However, because the DSB model predicts that an equal number of crossover and non-crossover outcomes are generated, it fails to account for the extremely low occurrence of crossover events (<8%) in mitotic DSB-induced recombination in several model systems⁷. To account for this, the synthesis-dependent strand-annealing (SDSA) model was put forward (FIG. 1): after strand invasion and D-loop extension, the newly synthesized strand is displaced from the template and anneals to the other 3' ssDNA tail; this is followed by DNA synthesis and ligation of nicks. The SDSA model has two characteristic features: it generally yields only non-crossover products, and all DNA synthesis occurs on the receiving strand^{5,7}. In yeast, the SDSA pathway may be promoted by the Srs2 helicase, which is thought to facilitate displacement of the invaded strand and the newly synthesized DNA from the template by removing Rad51 (see BOX 1 for information about the proteins involved in gene conversion) during the strand-exchange process^{8,9}, a view that is supported by many studies^{10–13}. In addition, the *Drosophila melanogaster* orthologue of the Bloom syndrome helicase (BLM), a member of the highly conserved RecQ family, promotes SDSA by unwinding a D-loop intermediate^{14–16}; in support of this, BLM has also been shown to efficiently displace the invading strand of mobile D-loops¹⁷.

Rad54 has a novel DNA branch-migration activity¹⁸, and might therefore be able to promote branch migration of DNA junctions at the D-loop in either direction, away from or towards the DSB. The former would stabilize the D-loop, thereby facilitating the capture of the second 3' ssDNA tail and subsequent

formation of double HJs; the latter would destabilize the D-loop, thereby facilitating the displacement of the invaded strand and the newly synthesized DNA from the template¹⁸.

Another mechanism, known as double-HJ dissolution^{8,19}, shares the two defining characteristic features of SDSA in terms of outcome. However, it generates its non-crossover product from the convergent migration of the two HJs towards each other, leading to the collapse of the double HJs (FIG. 1). This mechanism is promoted by the coordinated action of BLM and topoisomerase III α (by BLM in humans¹⁹, Sgs1 in yeast^{8,9,20} and the fly homologue of BLM in *D. melanogaster*^{21,22}). More recently, the BLM-associated protein BLAP75 (also known as RMI1) has been identified as a third component of the double HJ 'dissolvosome'^{23,24}.

At least in yeast, the control of crossover and non-crossover recombination is differentially timed during meiosis^{25,26}. It would appear that "...once the DSB-repair reaction has committed to a resolution pathway that involves double Holliday junctions, the intermediate is already destined to be resolved in a specific orientation that leads to crossover."²⁷ This, when considered together with the double-HJ dissolution and SDSA models, may pose a serious challenge to the DSB model: the non-crossover events that are presumed to result from random resolution of double HJs (FIG. 1) might in fact come from either the HJ dissolution or SDSA pathways.

Our current thinking points to the existence of several mutually exclusive pathways leading to the occurrence of gene conversion (FIG. 1). It is apparent that these pathways are finely regulated, as evidenced by the observations that SDSA can be promoted by several different proteins (that is, Srs2, BLM and Rad54), the same protein can act in different pathways (that is, BLM in both SDSA and double-HJ dissolution) and the same protein (that is, Rad54) can promote bidirectional branch migration of HJs, thereby facilitating either SDSA or the formation of double HJs.

Characteristics of gene conversion. Efficient gene conversion generally requires homology between interacting sequences. Our meta-analysis of the pathogenic gene-conversion events (see below) revealed that, with one exception (88% between the folate receptor gene *FOLR1* and its pseudogene), the homology between the interacting sequences is always >92% and usually >95% (data not shown). In addition, the frequency of gene conversion is inversely proportional to the distance between the interacting sequences *in cis* (for example, REF. 28), and a ~15-fold higher frequency has been observed between linked as opposed to unlinked gene pairs in mice and rats²⁹. Furthermore, the rate of gene conversion is directly proportional to the length of the uninterrupted-sequence tract in the putatively converted region: the minimal efficient processing segment (MEPS) for efficient meiotic homologous recombination in mouse cells is >200 bp (REFS 30,31), whereas in humans it is estimated to be in the range of 337–456 bp (REF. 32).

Author addresses

[§]Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff, CF14 4XN, UK.

^{||}Department of Biological Sciences, University of Central Lancashire, Preston PR1 2HE, UK.

^{*}Faculté de Médecine de Brest et des Sciences de la Santé, Université de Bretagne Occidentale, 29238 Brest, France.

[#]Laboratoire de Génétique Moléculaire et d'Histocompatibilité, Centre Hospitalier Universitaire de Brest, Hôpital Morvan, 29220 Brest, France.

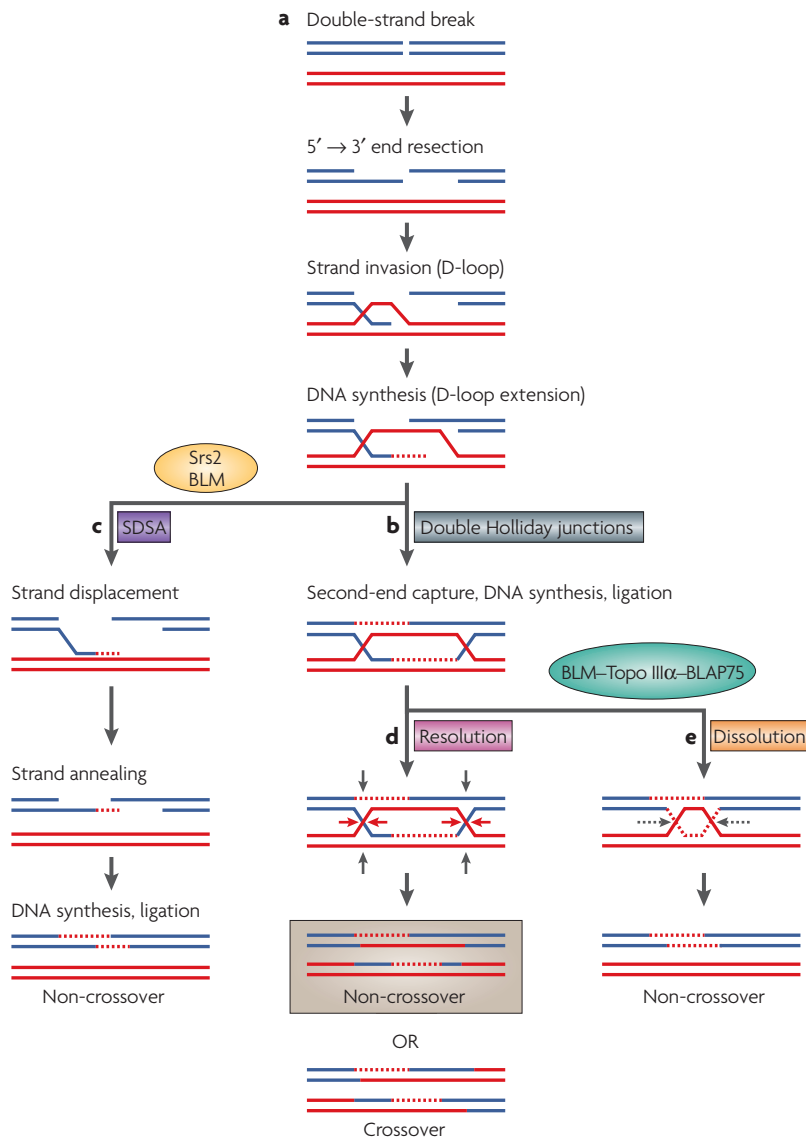


Figure 1 | Mechanisms of gene conversion. The double-strand break repair (DSBR; **a–b–d**), synthesis-dependent strand-annealing (SDSA; **a–c**; refer to REF. 5 for other possible SDSA pathways) and double Holliday junction (HJ) dissolution (**a–b–e**) models are illustrated. All models share a common initiating step: the 5' ends of the double-strand break (DSB) are resected to form 3' ssDNA tails; the tails actively 'scan' the genome for homologous sequences, and one tail invades the homologous DNA duplex forming a displacement (D)-loop, which is then extended by DNA synthesis. SDSA diverges from the other two pathways after D-loop extension: the invading strand and the newly synthesized DNA are displaced from the template (through the action of the Srs2 helicase in yeast, or the Bloom syndrome protein (BLM) in humans and flies) and anneal to the other 3' end of the DSB, leading to the formation of only gene-conversion events. Otherwise, the other 3' end of the DSB is captured, and DNA synthesis and ligation of nicks lead to the formation of double HJs. According to the dissolution model, BLM, topoisomerase IIIα (Topo IIIα) and the BLM-associated protein BLAP75 (also known as RMI1) act together to remove the double HJs via convergent branch migration (indicated by dotted arrows at both HJs) leading exclusively to gene conversion. In DSBR, the resolution of the double HJs by an HJ resolvase is predicted to generate an equal number of non-crossover (indicated by red arrows at both HJs) and crossover (indicated by black arrows at one HJ and red arrows at the other HJ) events. Identification of the distinct SDSA and double-HJ dissolution pathways that result in gene conversion challenges the concept of DSBR: the non-crossover events in DSBR (highlighted within the coloured box) might in fact come either from SDSA or double-HJ dissolution. See BOX 1 for more information on the proteins that are involved in gene conversion.

In yeast, **mitotic gene-conversion tracts** (often larger than 4 kb) are generally **much longer than meiotic ones** (typically 1–2 kb; for example, REF. 33). In mammals, **gene-conversion tracts are usually short**, of the order of 200 bp to 1 kb in length. For example, estimates range from 113–2,266 bp for the human globin genes³⁴, to 1–1,365 bp for two Yq-located human endogenous retroviral (HERV) sequences³⁵, to 54–132 bp in single-sperm analysis of the human leukocyte antigen *HLA-DPB1* locus³⁶ and 55–290 bp in various gene-conversion hotspots³⁷. Our own review of **pathological gene-conversion events** has revealed that the lengths of the maximally **converted tracts rarely exceed 1 kb** (TABLE 1; see **Supplementary Information S1** (box) for details of the meta-analysis). In practice, the **length of the converted tract has been used to distinguish a gene-conversion event from a double-crossover event** (BOX 2).

It has long been assumed that **specific motifs** surrounding DNA sequences involved in gene conversion can either **promote or inhibit** gene-conversion and branch-migration events (for example, REF. 34). Such motifs include alternating purine and pyrimidine or polypurine and polypyrimidine tracts, palindrome-like sequences, minisatellite sequences, *chi* (χ)-like sequences and sequences that can adopt tetraplex, Z-DNA, slipped and triplex structures, although many examples of gene-conversion events are not associated with any of the above sequence motifs³⁵. Our analysis of gene-conversion tracts that have been reported in the context of human disease reveals that short (5 bp) and long (≥ 10 bp) polypurine and polypyrimidine tracts are over-represented at the 1% level of significance within the minimal and maximal converted tracts, in comparison with a simulated data set that matched the corresponding original minimal and maximal converted tracts in terms of their length and nucleotide composition (N.C., unpublished observations). Several sequences that can form **tetraplex, triplex, Z-DNA and slipped structures** were **overrepresented** ($p \leq 0.01$) in the regions flanking the minimal but not the maximal converted tracts (FIG. 2d). Bearing in mind the spatial coincidence of **non-B DNA structures with deletion breakpoints**³⁸, it is tempting to speculate that the DSBs that initiated gene conversion occurred either within or very close to the regions flanking the minimal converted tracts. Neither the χ nor the human minisatellite or χ -like elements were overrepresented, even at the 5% level of significance, within converted tracts (N.C., unpublished observations).

Gene-conversion events can be non-allelic (also known as interlocus) or interallelic (FIG. 2a–c). **Non-allelic gene conversion often shows biased directionality**. For example, whereas **proximal-to-distal** (relative to the centromere) gene conversion between two directly repeated HERV elements on the long arm of the human Y chromosome occurs at a rate of between 2.4×10^{-4} and 1.2×10^{-3} events per generation, the rate of **distal-to-proximal gene conversion is some 20-fold lower**³⁵. In some cases (for example, in human globin genes³⁴), the **directionality of gene conversion correlates with the relative levels of expression** of the participating genes,

Box 1 | Proteins involved in gene conversion

Gene conversion is initiated by the processing of DNA ends to yield molecules with 3' single-stranded tails that are subsequently coated with replication protein A (RPA), an ssDNA binding protein. Rad51, which shares sequence similarity with the *Escherichia coli* RecA strand-exchange protein¹⁰⁵, has a key role in recombination. The Rad51 paralogues (XRCC2, XRCC3, Rad51B, Rad51C and Rad51D) are present in two distinct complexes in which Rad51C is the common component. The Rad51B–Rad51C–Rad51D–XRCC2 complex binds ssDNA, single-stranded gaps in dsDNA and nicks in duplex DNA¹⁰⁶; the Rad51C–XRCC3 complex also has a DNA binding activity and associates with a Holliday junction resolvase¹⁰⁷.

Other key components of the gene-conversion machinery are Rad52 and the dsDNA-dependent ATPase Rad54. Both proteins are accessory partners of Rad51, but they operate at different stages; Rad52 functions early on to assist loading of Rad51 onto resected DNA ends, whereas Rad54 functions later when the intact double-stranded template has become engaged in the gene-conversion process¹⁰⁸.

The meiotic recombination protein 11 (Mre11)–Rad50–Nijmegen breakage syndrome protein 1 (Nbs1) complex, or MRN complex, is a central player in homologous recombination. The existence of Mre11 nuclease and the Rad50 ATPase homologues in different organisms (from yeast to humans) suggests that this complex is fundamental for genomic stability. An essential biological function of the complex in the context of double-strand break (DSB) repair is to tether DNA ends through a DNA-driven conformational change^{109,110}.

The breast cancer susceptibility proteins BRCA1 and BRCA2 also participate in gene conversion. BRCA1 interacts with Rad51 and the MRN complex¹¹¹, whereas BRCA2 interacts with both Rad51 (REF. 112) and BRCA1 (REF. 113). Biochemical evidence implies that BRCA2 is likely to act preferentially at the interface between dsDNA and ssDNA to displace RPA from the overhang and assist with loading of Rad51. Following DNA damage and initial DSB processing, BRCA2 is required for the formation of Rad51 foci¹¹⁴. Recent data suggest that the C-terminal region of BRCA2 specifically interacts with multimeric Rad51 and binds an intersubunit interface of Rad51 that is present in the Rad51 multimer and in the nucleoprotein filament¹¹⁵. This interaction is crucial for the protection of Rad51–DNA filaments from disassembly during recombination-mediated DNA repair¹¹⁶.

Gene-conversion tract

In theory, this is the portion of the 'acceptor' sequence that is copied from the 'donor'. Because in practice the length of the tract cannot be precisely known, it must be expressed in terms of the lengths of the minimal and maximal converted tracts: the former refers to the entire region spanned by converted discriminant nucleotides and the latter refers to the region that is delimited by the two nearest unconverted discriminant nucleotides between the donor and acceptor sequences.

Double crossover

Two crossovers that occur in a chromosomal region between highly homologous genes, resulting in reciprocal sequence exchange between them.

Chi (χ) sequence

An 8-bp sequence (5'-GCTGGTG-3') that acts as a recombination hotspot in *Escherichia coli*.

with the gene that is expressed at a higher level (the 'master' gene) converting the gene that has the lower expression level (the 'slave' gene).

The master and slave gene rule should be interpreted with caution because it was originally formulated in an evolutionary context. Indeed, increased transcription of a gene seems to increase the likelihood of its use not only as a donor (for example, REF. 39), but also as an acceptor (for example, REF. 40). Moreover, multiple pseudogene-mediated conversion events that self-evidently do not follow this rule have been shown to cause human inherited disease (TABLE 1). Thus, although it seems clear that gene conversion can occur in the opposite direction (from slave to master), such events are much less likely to be observed in the human genome because they would probably generate defective alleles that would tend to become extinct.

Evolutionary consequences of gene conversion

Since the first reported characterization of gene-conversion events in the human globin genes¹, interlocus gene conversion has been implicated in the concerted evolution of many human gene families including the Rh blood group antigen genes *RHD* and *RHCE*⁴¹, gonadotropin hormone β -subunit gene (*CGB*)⁴², red (*OPN1LW*) and green (*OPN1MW*) opsin genes⁴³, olfactory receptor genes⁴⁴, α -interferon gene⁴⁵, γ -crystallin gene⁴⁶ and the chemokine receptor genes *CCR2* and *CCR5* (REF. 47).

The key role of gene conversion in maintaining the high level of sequence homogeneity between the tandemly repeated ribosomal RNA (rRNA) genes in eukaryotes has been reviewed elsewhere⁴⁸. Below we discuss gene conversion in the context of some well-studied examples. We then discuss how recent population genetic studies have provided new insights into how gene conversion is shaping fine-scale structural variation in the human genome, and how these findings promise to improve the design of disease association studies.

The role of interlocus gene conversion in concerted evolution.

Interlocus gene conversion has an important role in the concerted evolution of multigene families and highly repeated DNA sequences (see also BOX 3). A hallmark of its action is that paralogous gene sequences become more closely related to each other than they are to their orthologous counterparts in a closely related species⁴⁹. The dramatic impact of this process is perhaps best exemplified by the multi-copy testis-expressed gene families that lie within the eight large palindromes that comprise 25% of the euchromatic portion of the male-specific region (MSY) on the human Y chromosome⁵⁰. All of the known genes within these palindromes have an identical or near-identical copy (with a typical sequence similarity of 99.97%) on the opposite arms of the palindrome. Such a high degree of similarity would normally be indicative of a recent duplication event; however, human–chimpanzee sequence comparison revealed that the MSY palindromes pre-dated the split of these two lineages (~5 million years ago)⁵⁰. Furthermore, analysis of single-nucleotide differences between extant MSY palindromes provided evidence of recurrent arm-to-arm gene conversion in humans: as many as 600 bp (from the 5.4 Mb contained within MSY palindromes) in each newborn male have been estimated to undergo Y–Y gene conversion⁵⁰.

The arm-to-arm sequence divergence values that have been calculated for the human and chimpanzee palindromes are 0.028% and 0.021%, respectively. By contrast, an average sequence divergence of 1.44% was observed between the orthologous palindrome arms in the two species⁵⁰. Interlocus gene conversion has homogenized the paralogous palindrome sequences within each species while diversifying the orthologous palindrome sequences between the two species. So, how precisely does gene conversion influence sequence similarity or divergence between paralogues and orthologues? On the basis of simulations involving two paralogous HERV15 proviral sequences that flank the Y-chromosomal azoospermia factor A (*AZFA*, also known as *USP9Y*) locus in three primate species (that is, humans, chimpanzees and gorillas)⁵¹, it seems that the higher the conversion rate, the higher the degree of homogenization between paralogous sequences and the greater the extent of the divergence between orthologous sequences. Moreover, the higher the degree of sequence divergence before speciation, the greater the subsequent divergence between both the paralogous and the orthologous sequences. Finally, although the biased directionality of interlocus gene conversion does

Table 1 | Interlocus gene-conversion events that cause human inherited disease* (part 1)

Disease/phenotype	Donor gene [†]	Acceptor gene	Chromosomal localization	Directionality [§]	Mutation	Converted tract length (bp)	Refs
Atypical haemolytic uraemic syndrome	<i>CFHR1</i> ^{§§}	<i>CFH</i>	1q32	3'>5'	c.[3572C>T;3590T>C] [¶]	19–331	131
Congenital adrenal hyperplasia	<i>CYP21A1P</i>	<i>CYP21A2</i>	6p21.3	5'>3'	[-209T>C;-198C>T;-189/-188insT]	21–155	132
					[-4C>T;92C>T;118T>C;138A>C]	142–523	133
					[1380T>A;1383T>A;1389T>A; IVS6+12_13AC>GT]	42–210	134
					[1688G>T;1767_1768insT] [¶]	80–202	133
Syndrome of corticosterone methyloxidase II deficiency	<i>CYP11B1</i> ^{§§}	<i>CYP11B2</i>	8q21–q22	3'>5'	Conversion of exons 3 and 4	446–626	135
Increased 18-hydroxycortisol production	<i>CYP11B1</i> ^{§§}	<i>CYP11B2</i>	8q21–q22	3'>5'	Conversion of two nucleotides separated by two bases in exon 8	4–56	136
Autosomal dominant cataract	<i>CRYBP1</i>	<i>CRYBB2</i>	22q11.2–q12.1	3'>5'	c.[475C>T;483C>T]	9–104	137
Neural tube defects	<i>FOLR1P</i>	<i>FOLR1</i>	11q13.3–q14.1	5'>3'	7497_7662 including 13 discriminant nucleotides [¶]	166–215	138
					[7539T>C;7541G>A] [¶]	3–65	138
Gaucher disease	<i>GBAP</i>	<i>GBA</i>	1q21	3'>5'	[del55bp;D409H;L444P;A456P; V460V]	604–974	139
					[D409H;L444P;A456P;V460V]	525–848	140;141
					[L444P;A456P]	36–175	139
					[L444P;A456P;V460V]	50–475	141;142
Short stature	<i>GH2</i> ^{§§}	<i>GH1</i>	17q22–q24	3'>5'	Conversion involving 12 discriminant nucleotides in the promoter region	40–218	143
Mild microcytosis	<i>HBB</i> ^{§§}	<i>HBD</i>	11p15.5	3'>5'	Conversion involving exons 1 and 2	≥212–≤348 [#]	144
Hereditary persistence of fetal haemoglobin	<i>HBG2</i> ^{§§}	<i>HBG1</i>	11p15.5	5'>3'	Conversion involving 9 discriminant nucleotides in the promoter region [¶]	423–1554	145
Agammaglobulinaemia	<i>IGLL3</i>	<i>IGLL1</i>	22q11.23	Inverted ^{**}	c.[393T>C;420T>C;425C>T]	33–152	146
Chronic granulomatous disease	<i>NCF1B</i> or <i>NCF1C</i>	<i>NCF1</i>	7q11.23	5' or inverted ^{**}	[C>T;ΔGT]	124–1474	147
					[C>T;ΔGT;ins20bp]	369–1529	147
					[ΔGT;ins20bp]	247–423	148
Blue cone monochromacy	<i>OPN1MW</i> ^{§§}	<i>OPN1LW</i>	Xq28	3'>5'	3' limit of the maximal converted region cannot be defined owing to the lack of information on an intervening discriminant nucleotide	≥636–≤3676 [#]	149
Autosomal dominant polycystic kidney disease	?††	<i>PKD1</i>	16p13.3	?††	[8446T>G;8490T>C;8493G>C; 8502T>C]	57–126	150
					[8446T>G;8490T>C;8493G>C; 8498C>G;8502T>C]	57–126	150
					[8639G>T;8651G>A;8658T>C; 8662C>T]	24–230	151
Chronic pancreatitis	<i>PRSS2</i> ^{§§}	<i>PRSS1</i>	7q35	3'>5'	Conversion involving 22 discriminant nucleotides	289–457	88

* Only interlocus events that were informative with respect to the converted tracts, and that comprised at least two neighbouring but non-consecutive markers, were collected. Some mutations were re-named or re-interpreted. In mutation nomenclature, 'c.' refers to coding nucleotides. The full selection criteria used in the collation of these mutations and the details of how they were annotated are provided in the [Supplementary Information S1](#) (box) online. See the main text for pathogenic interallelic gene-conversion events and gene-conversion mutations in cancer. [†]Functional donor genes are indicated by ^{§§}. The solitary case of a 'partially functional' donor gene, *SMN2*, is indicated by ^{|||}. Other donor genes are pseudogenes. [§]The directionality of sequence transfer from the donor gene to the acceptor gene (in the context of the sense strand) is specified whenever the donor and acceptor pairs represent tandem duplications. ^{||}Limits to the length of the converted tract, minimal to maximal (see also FIG. 2d). [¶]De novo mutations. ^{¶¶}Lengths of the minimal and maximal converted tracts cannot be unequivocally assigned owing to the lack of information on certain internal marker(s); see [Supplementary Information S1](#) (box) for details. ^{**}Gene-conversion events between gene copies that are not tandem duplications. ^{††}Donors cannot be assigned to current human genome sequence assemblies, because of either copy-number variation or the presence of a 'gap' (there are at least six *PKD1* pseudogenes at 16p13).

Table 1 | Interlocus gene-conversion events that cause human inherited disease* (part 2)

Disease/phenotype	Donor gene [‡]	Acceptor gene	Chromosomal localization	Directionality [§]	Mutation	Converted tract length (bp)	Refs
Shwachman–Bodian–Diamond syndrome	SBDSP	SBDS	7q11.22	Inverted**	c.[129-443A>G;129-433G>A]	11–104	152
					c.[141C>T;183_184TA>CT]	44–217	152
					c.[141C>T;183_184TA>CT;201A>G]	61–276	153
					c.[141C>T;183_184TA>CT;201A>G;258+2T>C] [¶]	120–398	152
					c.[183_184TA>CT;201A>G]	19–118	153
					c.[183_184TA>CT;201A>G;258+2T>C]	78–240	154
					c.[201A>G;258+2T>C]	60–197	152
Spinal muscular atrophy	SMN2	SMN1	5q13.2	5'>3'	Conversion of exon 7 and intron 7	264–776	155
von Willebrand disease	VWFP	VWF	22q11.22–q11.23 (VWFP)/12p13.3 (VWF)	Not applicable	[IVS27-45C>T;IVS27-36C>T;3686T>G;3692A>C]	63–131	156
					c.[3686T>G;3692A>C]	7–95	157
					c.[3686T>G;3692A>C;3735G>A;3789G>A;3797C>T]	112–195	90
					c.[3789G>A;3797C>T]	9–99	158;159
					c.[3789G>A;3797C>T;3835G>A]	47–195	157
					c.[3789G>A;3797C>T;3835G>A;3931C>T;3951C>T]	163–291	90
					c.[3835G>A;3931C>T;3951C>T]	117–229	90
					c.[3835G>A;3931C>T;3951C>T;4027A>G;4079T>C;4105T>A]	271–335	90
					c.[3931C>T;3951C>T]	21–191	90
					c.[3931C>T;3951C>T;4027A>G;4079T>C;4105T>A]	175–297	160

*Only interlocus events that were informative with respect to the converted tracts, and that comprised at least two neighbouring but non-consecutive markers, were collected. Some mutations were re-named or re-interpreted. In mutation nomenclature, 'c.' refers to coding nucleotides. The full selection criteria used in the collation of these mutations and the details of how they were annotated are provided in the [Supplementary Information S1](#) (box) online. See the main text for pathogenic interallelic gene-conversion events and gene-conversion mutations in cancer. [‡]Functional donor genes are indicated by [§]. The solitary case of a 'partially functional' donor gene, *SMN2*, is indicated by ^{|||}. Other donor genes are pseudogenes. [§]The directionality of sequence transfer from the donor gene to the acceptor gene (in the context of the sense strand) is specified whenever the donor and acceptor pairs represent tandem duplications. ^{||}Limits to the length of the converted tract, minimal to maximal (see also FIG. 2d). [¶]De novo mutations. ^{**}Gene-conversion events between gene copies that are not tandem duplications.

Z-DNA

One of several possible double-helical structures of DNA. Z-DNA is a left-handed double-helical structure in which the double helix winds to the left in a zig-zag pattern, rather than to the right, as occurs in the more common B-DNA form.

Concerted evolution

The process by which repetitive DNA sequences are homogenized such that the individual members of a given DNA repeat or multigene family in one species come to show a higher degree of sequence identity with each other than they do with members of the same DNA repeat or multigene family in another species.

not affect the degree to which paralogues are homogenized, it does appear to cause smaller perturbations of orthologous sequence divergence in the preferred donor than in the preferred acceptor gene⁵¹.

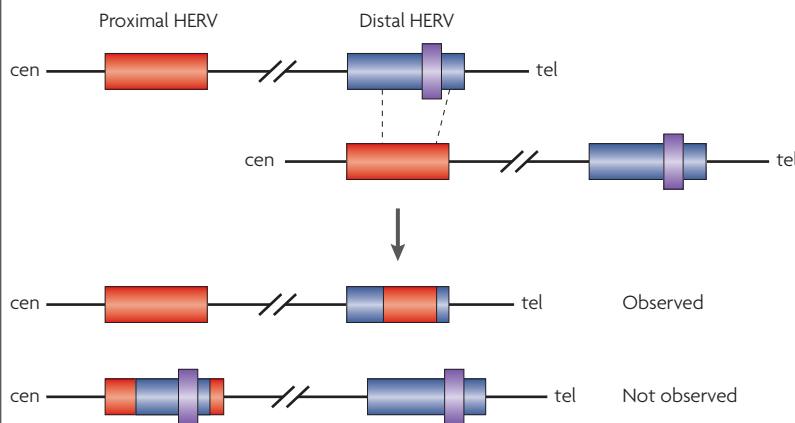
The above notwithstanding, the **impact of inter-locus gene conversion** on the evolution of multigene families has certainly been **affected by other factors**, most notably **selection**. As an obvious consequence of this interaction, interlocus gene conversion has facilitated the **spread of advantageous mutations**. For example, most of the coding polymorphisms in human *OPN1LW* and *OPN1MW* genes, which are tandemly arranged on the X chromosome, lie within exon 3 (7 out of 11 polymorphisms in *OPN1LW* and 6 out of 8 polymorphisms in *OPN1MW*)⁵². That 6 out of 7 of the exon 3 polymorphisms are shared between the two genes is indicative of the magnitude of the imprint of interlocus gene conversion⁵². Human *OPN1LW* replacement SNPs serve to shift λ_{\max} into the 'red–orange' portion of the visual spectrum⁵³. This and other observations⁴³ suggest that **polymorphisms in exon 3 of *OPN1LW* might enhance colour discrimination**.

The spread of an interlocus gene-conversion-derived advantageous mutation through the population might be **accelerated by interallelic gene conversion**. This view is supported by a recent study that traced the evolution of *HLA-E* in eight primate and rodent species⁵⁴.

Interallelic gene conversion generates allelic diversity. Some **regions of the human genome are remarkably polymorphic**. For example, the human *ABO* blood group locus, apart from the heterogeneity that underlies its three major alleles (*A*, *B* and *O*), contains **extensive heterogeneity within the various alleles that constitute the *ABO* subgroups**. Haplotype analysis implies that some of these alleles might be generated by **gene conversion between the parental alleles** (reviewed in REF. 55).

The best known example of interallelic gene conversion is probably the HLA class II region containing *HLA-DR*, *HLA-DQ* and *HLA-DP*; **more than 500 *HLA-DRB1* alleles have been reported** in the human population (see the [International Immunogenetics Project HLA database](#)). Most of the polymorphisms lie in exon 2, which encodes part of the antigen-recognition

Box 2 | Example of a double-crossover event



In humans, gene conversion can never be formally and unambiguously distinguished from double crossover because only one of the products of recombination can be observed. In practice, double crossover is commonly assumed to have occurred when the observed sequence change is considered to be too long (for example, >3 kb) to have been caused by gene conversion. This is exemplified by the sequence exchange between the two ~9-kb human endogenous retroviral (HERV) repeats (shown in red and blue) on human chromosome Yq, in which a ~5-kb stretch, which includes a 1.5-kb insertion (shown as a purple bar) that is present in only the distal repeat copy, was presumed to result from a double crossover between misaligned chromatids (or chromosomes in the case of repeats that are located on autosomes or X chromosomes), rather than from a gene-conversion event⁷⁶. cen, centromere; tel, telomere.

Paralogue

One of a set of homologous genes in the same species that have evolved from a gene duplication, and that can be associated with a subsequent divergence of function.

Segmental duplication

A segment of DNA of larger than 1 kb that occurs in two or more copies per haploid genome, with the different copies sharing > 90% sequence identity.

Linkage disequilibrium

A statistical association between particular alleles at two or more neighbouring loci on the same chromosome that results from a specific ancestral haplotype being common in the population under study.

Tag SNPs

SNPs that are correlated with, and can therefore serve as a proxy for, much of the known remaining common variation in a region.

site of class II proteins. This high degree of polymorphism has been under positive selection in response to the ongoing immunological challenge from diverse pathogens. Direct evidence for interallelic gene conversion has been provided by sperm analysis of the *HLA-DPB1* locus³⁶; furthermore, a recent large-scale population genetic study has suggested that novel alleles in the *HLA* locus are being continually generated by gene conversion⁵⁶.

Gene conversion and human genome variation. On the basis of the realization that interlocus gene conversion can generate allelic diversity, Hurles⁵⁷ challenged the then prevailing assumption that "...there is no reason to expect that polymorphic variation is increased within duplicated regions."⁵⁸ He further speculated that gene conversion could have contributed to the formation of the much greater than expected number (~100,000) of SNPs found within segmental duplications in the human genome⁵⁸. Indeed, many duplcon SNPs have recently been shown to be true SNPs (that is, allelic variants) with only a few of them being paralogous sequence variants⁵⁹. In the meantime, the role of gene conversion in the evolution of segmental duplications has been increasingly recognized, both at specific loci (for example, REFS 35,42,50,60) and at the genomic level⁶¹. In particular, analysis of 24 human duplcon families that together span >8 Mb of DNA (representing some 5% of all known human duplcons⁶²) indicates that most of the segmental duplications contain a significant excess of sites that show signatures of

concerted evolution, as compared with the number that would be expected as a result of nucleotide substitution alone⁶³.

The impact of gene conversion on the human genome has not been limited to duplcons. There is growing evidence that gene conversion has had an important role in shaping fine-scale patterns of linkage disequilibrium (LD) in the human genome^{64,65}. For example, nearly all pairs of loci that are separated by 124 bp on average would be expected to be in complete LD because recombination does not usually occur over such a short interval. The fact that a significant fraction of locus pairs showed only partial LD suggests that gene conversion has increased the apparent rate of recombination between nearby loci⁶⁴.

The public database established by the International HapMap Project⁶⁶, which comprises >3 million SNPs obtained from four different human populations, was originally intended to be a resource of tag SNPs for genome-wide association mapping studies in any human population^{67,68}. However, for technical reasons, duplicated regions in the human genome (which constitute at least 5% of the genome) are generally poorly covered by the HapMap. Given the widespread presence of gene conversion and the short length of the conversion tracts that are usually involved in gene conversion, LD between gene-conversion-generated SNPs and the tagging markers may often be weak, thereby reducing the efficacy of LD-based association studies⁶⁹. Indeed, it is anticipated that the now widely used tagging approach will be replaced by more economical whole-genome sequencing in the near future⁷⁰.

High gene-conversion activity is a common feature of both allelic³⁷ and non-allelic recombination hotspots^{71,72} (at least 25,000 recombination hotspots have been identified across the human genome⁷³). A better understanding of the role of gene conversion in generating or eliminating recombination hotspots in the human genome^{71,72,74} should improve our ability to predict the location of unstable genomic regions⁷⁵. Finally, gene-conversion-mediated sequence homogenization of duplcons increases the likelihood of non-allelic homologous recombination between these sequences, potentially leading to genomic disorders^{76,77}.

The ratio of gene conversion to crossover. Several studies have attempted to estimate the average rate of gene conversion, f , expressed as its ratio to crossover (see REF. 78 for a recent review), using genome-wide population genetic variation data. In a study of 21,840 biallelic SNPs on 20 independent copies of human chromosome 21, f was estimated to be 1.6 assuming a conversion-tract length of 500 bp, and 9.4 assuming a mean conversion-tract length of 50 bp (REF. 79). In a broadly similar study, f was calculated to be 7.3 for a mean tract length of 500 bp (REF. 80). Another study estimated an f value of between 3 and 10, with a ratio of 6 providing the best fit⁶⁴. However, the estimated f is only around 0.3 for Europeans and 1.0 for African Americans⁶⁵. Setting aside the influence of the assumptions about the length of the converted tract, the wide variation in f between

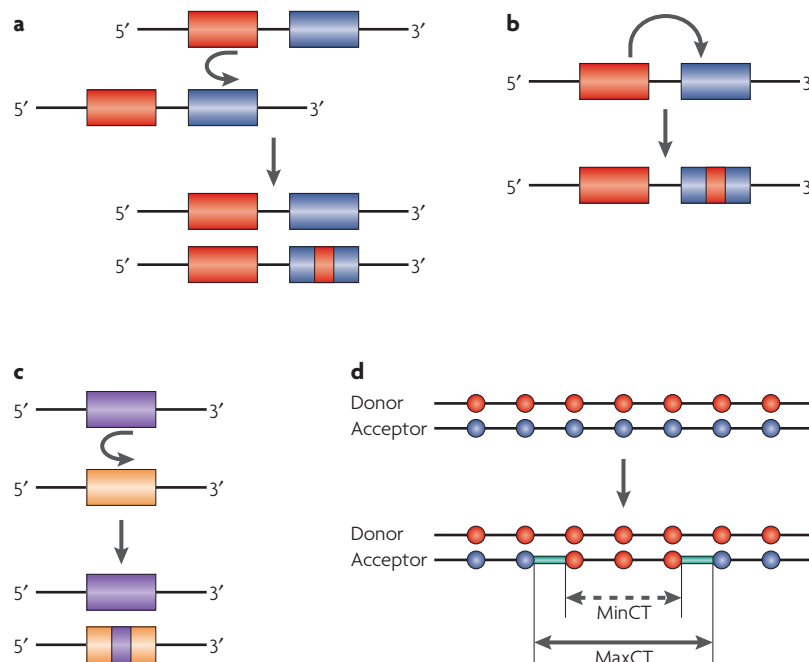


Figure 2 | Types of gene conversion and demarcation of the converted tract.

a | Non-allelic (or interlocus) gene conversion *in trans*, shown as an event occurring between paralogous sequences (represented as red and blue boxes) that reside on sister chromatids or on homologous chromosomes. Gene-conversion events that occur between homologous sequences that reside on different chromosomes are not shown. **b** | Non-allelic gene-conversion events *in cis* (between non-allelic gene copies that reside on the same chromosome). Gene-conversion events, which are depicted in **a** and **b**, are virtually indistinguishable from each other. **c** | Interallelic gene-conversion events occurring between alleles (shown in purple and orange) that reside on homologous chromosomes. **d** | Maximal and minimal converted tracts of a given gene-conversion event. Although the length of the minimal converted tract (MinCT) is usually shorter than the true tract, the length of the maximal converted tract (MaxCT) is usually longer than the true tract. The initiating and terminating points of gene conversion can lie anywhere within the two regions that are marked in green.

the studies might reflect the different populations and genomic regions that are under investigation. Indeed, sperm-typing experiments show that *f*_{is} is between 4 and 15 in the *DNA3* hotspot³⁷, ~0.3 in the *nidogen 1* (*NID1*) hotspot⁸¹ and <0.1 in the β -globin gene (*HBB*) region⁸² (reviewed in REF. 78).

Biased gene conversion. Gene conversion seems to favour some alleles over others, a process known as biased gene conversion (BGC). BGC arises as a consequence of the GC-biased repair of A:C and G:T mismatches that are formed in heteroduplex recombination intermediates during meiosis⁸³. Increasing the probability of the fixation of G and C alleles leads to the GC-enrichment of the sequences involved. BGC has therefore exerted an important influence on GC content during the evolution of mammalian genomes. Indeed, members of multigene families that are closely linked and highly homologous to each other (and hence presumably undergo gene conversion) have a significantly higher GC content than gene singletons (which are presumed to undergo less gene conversion than members of gene families)⁸⁴. Furthermore, chromosomal regions that exhibit a high

rate of recombination become enriched in GC⁸⁵. Thus, G and C allele frequencies tend to be higher at sites of SNPs that lie in the vicinity of recombination hotspots⁸⁶. Nevertheless, using a context-dependent mutation model, Hernandez and colleagues⁸⁷ recently concluded that much of the evidence from human population genetic data that suggests a recent fixation bias favouring GC-content could have been compromised by the misidentification of the ancestral state of each SNP.

Gene conversion and disease

Gene-conversion events, predominantly of the **interlocus variety** (TABLE 1), have been implicated as the molecular cause of an increasing number of human inherited diseases. Although the imprint of gene conversion is not invariably unambiguous, some important features, such as a **high degree of homology** (in the range of 92–99%) between the sequences that are presumed to be involved and the substitution of at least two neighbouring but non-consecutive markers within a short sequence tract, can point to gene conversion rather than single-nucleotide substitution or double crossover as the underlying mutational mechanism.

Nearly 50% of the donor genes are functional or partially functional. Pathogenic gene conversion is often viewed as having resulted almost exclusively from the transfer of genetic information from non-functional pseudogenes to their closely related functional counterparts (see also BOX 4). However, our meta-analysis reveals that, of the 17 acceptor genes known to have been involved in pathogenic interlocus gene-conversion events, 7 had a functional donor counterpart; an additional gene, survival of motor neuron 1 (*SMN1*), had a partially functional counterpart (TABLE 1). Acceptor genes with donors that were functional usually carried only a single mutation, whereas those with donor sequences that were non-functional carried multiple mutations (TABLE 1). This might be at least partially explained by the fact that **non-functional genes generally present a more extensive pathogenic template** than functional genes, simply because non-functional genes tend to carry multiple types of gene-disrupting mutation (for example, nonsense and frameshift).

Functional consequences of interlocus gene-conversion events causing human inherited disease. Our systematic survey of the 44 interlocus gene-conversion events that cause human inherited disease (TABLE 1) reveals that all **events in which pseudogenes have acted as donors resulted in the functional loss of the respective acceptor genes** through the introduction of frameshifting, aberrant splicing, nonsense mutations, deleterious missense mutations and so on. Functional loss of the respective acceptor gene was also associated with nearly all of the gene-conversion events in which a functional gene acted as the donor sequence. The sole exception was *PRSS1*, which encodes cationic trypsinogen, the major isoform of trypsinogen; in this case, gene conversion led to the replacement of at least 289 nucleotides of *PRSS1* by those of *PRSS2*, which encodes anionic trypsinogen,

Box 3 | **Gene conversion involving Alu and LINE-1 sequences**

Amplified to >1.1 million copies and comprising >10% of the genome mass, the Alu repeat constitutes the most abundant short interspersed element family in the human genome. Despite its short length (<300 bp), Alu-mediated gene conversion is not rare: a genome-wide analysis revealed that some 10–20% of the sequence variation in the Ya5 subfamily (one of the youngest Alu subfamilies) was attributable to gene conversion¹¹⁷. A more recent study estimated that some 15,000–85,000 point mutations in the human genome can be attributed to sequence exchanges between neighbouring Alu elements, mainly by gene conversion¹¹⁸.

This high gene-conversion rate is related to the dense distribution of Alu elements in the human genome (on average, one element every 3 kb), their high GC content (~63%) and the remarkable sequence similarity between Alu elements (70–100%). Sequence homogenization between Alu elements would be expected to potentiate Alu-mediated genomic rearrangements in both an evolutionary and a pathological context¹¹⁹.

Long interspersed elements-1 (LINE-1 or L1) constitute ~17% of the human genome sequence. Given the greater length of L1 elements (up to 6–7 kb) and their prevalence in the human genome (>500,000 copies), L1-mediated gene conversion would not be expected to occur infrequently. L1 elements have been experimentally demonstrated to efficiently mediate gene conversion *in vitro* and in transgenic mouse lines^{120,121}. However, only a limited number of gene-conversion events have been documented in humans^{122,123}. Several reasons might account for this. First, although a full-length L1 element is typically of ~6 kb, more than 90% of the L1 copies in the human genome are heterogeneously truncated from their 5' end, and many are either shorter than 2 kb and/or rearranged (for example, REF. 124). Second, the degree of sequence divergence between L1 elements is generally much higher than that between Alu sequences: L1 elements have been amplified in vertebrate genomes for around 170 million years (Myr), beginning before the mammalian radiation, whereas the amplification of Alu sequences has been specific to primate genomes over the last 65 Myr. By comparison, even those L1 elements that have been amplified over the past 70 Myr have diverged by 32% (REF. 125). Third, by comparison with Alu sequences, L1 elements are GC-poor (~43%) and are characterized by a higher average distance between them (one element every 6.3 kb)¹¹⁹. Fourth, L1 elements tend to occur in AT-rich, low-recombination and gene-poor regions of the human genome.

the second major isoform of trypsinogen. *In vitro* functional analysis showed that trypsin levels are increased (through enhanced autocatalytic activation) as a result of a specific missense substitution, N29I, which is brought about by the conversion⁸⁸.

The functional dissection of the only example of a partially functional gene acting as the donor sequence in gene conversion (that is, *SMN2*) has contributed significantly to our current understanding of the determinants of exon recognition. Mutations in *SMN1* cause autosomal recessive spinal muscular atrophy (*SMA*). *SMN2* is located centromeric to *SMN1*, with a sequence similarity of ~99%. *SMN2* is considered partially functional because its expression is impaired by a translationally silent SNP at position 6 of exon 7 (C in *SMN1* versus T in *SMN2*, the only difference between the coding sequences of the two genes), which causes skipping of exon 7 in most *SMN2* transcripts; only a minority of *SMN2* transcripts are correctly spliced and hence capable of encoding a protein identical to *SMN1*. By contrast, most of the wild-type *SMN1* transcripts are full-length. When a minigene was created in which C was artificially replaced by T at position 6 of exon 7 to mimic the *SMA*-causing gene-conversion mutation in *SMN1*, most of the mutant *SMN1* transcripts also lacked

exon 7 (REF. 89). Therefore C→T at position 6 of exon 7 of the *SMN* genes is a key determinant of exon identity and alternative splicing.

Intrachromosomal versus interchromosomal exchange. In nearly all known cases of disease-causing interlocus gene conversion, the acceptor and donor genes are located on the same chromosome. In only one case do the acceptor and donor genes reside on different chromosomes (TABLE 1): the von Willebrand factor gene (*VWF*; 12p13.3) and its pseudogene (*VWFP*; 22q11.22–q11.23). The pseudogene spans exons 23–34 of its functional counterpart and is 97% homologous to it.

This example establishes the precedent for the occurrence, in a pathological context, of gene conversion between unlinked loci in the human genome. However, it is surprising that *VWF* conversions are as common as they are. Of the 17 acceptor genes involved in interlocus gene-conversion events that cause human inherited disease, *VWF* has the highest number (up to 10) of different mutations reported (TABLE 1). In one study, 8 out of 56 (14%) von Willebrand disease (*VWD*) patients were shown to carry mutations that were compatible with the occurrence of pseudogene-mediated gene conversion⁹⁰. This unexpectedly high frequency can be at least partially accounted for by two considerations: first, the *VWF* gene lies at the tip of chromosome 12p, and chromosome ends are known to be hotspots of recombination and DSBs^{91,92}; second, as a bleeding disorder, *VWD* readily comes to clinical attention and is one of the most extensively studied human inherited diseases.

Interlocus versus interallelic events. In stark contrast to the frequent detection of pathogenic interlocus gene conversion (TABLE 1), the occurrence of interallelic gene conversion causing human inherited disease is rare. One putative example involves the T364M missense mutation in the α -L-iduronidase gene (*IDUA*), which was found in the homozygous state in a patient with mucopolysaccharidosis type I (REF. 93); however, only the patient's father carried the mutation, whereas his mother did not. Analysis of additional polymorphic markers suggested that either a *de novo* interallelic gene conversion (with the paternal allele acting as the donor) or a *de novo* genomic deletion (of the maternal allele) had occurred, leading to real or apparent homozygosity for T364M⁹³.

Analogously, apparent homozygosity for a mutation in exon 10 of the follicle-stimulating hormone receptor gene (*FSHR*) in a patient with hypergonadotrophic hypogonadism could in principle have resulted from either a *de novo* interallelic gene conversion (with the paternal allele acting as the donor) or a *de novo* genomic deletion of the maternal allele⁹⁴. In the first case, gene conversion must have occurred postzygotically at an early stage of embryonic development. In the second case, the genomic deletion would probably have occurred in the maternal germ cells. It should be possible to distinguish between these possibilities by quantitative PCR because, in the first case, but not the second case, the patient would be expected to show a degree of mosaicism.

Box 4 | Pseudogene-mediated interlocus gene conversion during evolution

Immunoglobulin (Ig) diversification in mammalian B cells is generated mainly by somatic hypermutation and class switch recombination. By contrast, gene conversion is the primary mechanism used by chicken B cells to generate Ig gene diversity (reviewed in REFS 126, 127). Chickens have a limited number of functional V regions; and the 'reservoir' of potential genetic changes is contained within a series of non-functional V genes. Thus, although chickens have only a single functional light-chain V segment (V_{λ}), after successful recombination with a single J_{λ} segment early on in B-cell development, V_{λ} undergoes diversification by copying sequence from any of its 25 upstream pseudo- V_{λ} genes^{126,127}. Gene conversion is also used, albeit rarely, for Ig diversification by the B cells of rodents (for example, REF. 128) and other mammals including humans (for example, REF. 129).

Introduction of pathogenic mutations into functional genes by pseudogene-mediated gene conversion is a well-known mutational mechanism in human genetic disease (TABLE 1). By analogy, could it be that pseudogenes have also served as templates from which multiple, potentially advantageous changes in their single-copy functional source genes have been derived during the course of human evolution? As long as pseudogene-templated changes in functional genes were not deleterious, they might eventually become fixed. In principle, therefore, pseudogenes could act as a reservoir of sequence variants that could then be transferred to the functional gene in new hitherto untested combinations for selection to act on.

The human sialic acid binding Ig-like lectin 11 (*SIGLEC11*) gene might provide one example of just such an evolutionarily significant gene conversion. Its 5' upstream region and the exons that encode the sialic acid recognition domain (~2 kb) have been converted by the closely flanking *SIGLECP16* pseudogene¹³⁰. This event came to light through a comparison of human *SIGLEC11* and its pseudogene with their homologues in the chimpanzee, bonobo, gorilla and orangutan. The observations that this gene conversion occurred in only the human lineage, that brain cortex acquired prominent *SIGLEC11* expression specifically in the human lineage and that human *SIGLEC11* shows altered substrate binding as compared with its chimpanzee counterpart, are suggestive of an adaptive change that could have been important in the evolution of the genus *Homo*¹³⁰.

How frequently this mechanism of pseudogene-mediated gene conversion has contributed to functional and adaptive changes during human evolution remains unknown, but the *SIGLEC11* example suggests that it can introduce genetic changes that might then become positively selected. The contribution of pseudogene-mediated gene conversion to promoting gene evolution has so far scarcely been explored.

The study of a patient with campomelic dysplasia, bearing a homozygous nonsense mutation (Y440X) in *SRY-box 9* (*SOX9*), led to the identification of an apparent *de novo* interallelic gene-conversion event⁹⁵. Unlike the previous examples, this mutation was absent in both parents. Analysis of intragenic polymorphisms, quantitative real-time PCR of the Y440X-harboured region and the study of the mosaicism that was evident in the patient's leukocytes led the authors to propose an elegant mechanism for how this unique case of spontaneous homozygosity could have been generated. First, a *de novo* Y440X mutation in *SOX9* occurred in the maternal germ line, yielding a zygote that was heterozygous for the mutant maternal *SOX9* allele and that bore a wild-type paternal *SOX9* allele. Following several cell divisions, a somatic interallelic gene conversion resulted in the replacement of at least 440 nucleotides of the wild-type paternal allele by the mutant maternal allele, resulting in somatic mosaicism⁹⁵.

Gene conversion in cancer. Only a few examples of gene-conversion events have been well documented in cancer. The first was noted in a Burkitt lymphoma cell line, in which a ~2kb DNA segment at the *D6S347* locus was

replaced by a partially homologous sequence; this event was presumed to result from a somatic interlocus gene conversion involving an unidentified gene⁹⁶. More recently, multiplex ligation-dependent probe amplification analysis showed that somatic mutL homologue 1 (*MLH1*) and mutS homologue 2 (*MSH2*) deletions are identical to their germline counterparts in up to 55% of hereditary nonpolyposis colorectal cancer (HNPCC) specimens⁹⁷. Given that none of these tumours showed allele loss at markers that flanked the respective gene loci, gene conversion was invoked to account for some of the observed changes.

More recently, a germline gene-conversion event in the gene postmeiotic segregation increased 2 (*PMS2*), involving transfer of 3–23 bp from its centromeric pseudogene, *PMS2CL*, has been reported in a HNPCC patient and his affected sister⁹⁸. *MLH1*, *MSH2* and *PMS2* are all DNA mismatch repair genes, defects of which lead to elevated levels of recombination⁹⁹.

Given that the detection of somatic mutations is technically much more demanding than that of their germline counterparts, it is likely that the occurrence of somatic gene-conversion events in cancer has been seriously underestimated.

De novo pathogenic gene-conversion events. The fairly high prevalence of *de novo* gene-conversion events (6 out of the 44 interlocus events listed in TABLE 1, and all the somatic events in both inherited disease and cancer) is indicative of the dynamic nature of gene conversion. TABLE 2 lists these known *de novo* pathogenic gene-conversion events.

Mutation spreading on different chromosomal backgrounds. Although recurrent mutation can provide one explanation for the occurrence of a specific mutation on different chromosomal backgrounds in different ethnic groups, interallelic gene conversion provides an equally plausible mechanism. Several human *HBB* mutations (reviewed in REF. 34) and a *CBS* mutation, which cause β -thalassaemia and autosomal recessive cystathionine β -synthase (CBS) deficiency (known as homocystinuria), respectively, are two well-known examples.

CBS deficiency is an inborn error of sulphur metabolism. The most common pathogenic *CBS* variant in Western Eurasians is c.833T>C (p.I278T) in exon 8. However, only a small proportion of human chromosomes carry the pathogenic c.833C mutation on its own; a much larger proportion contains a non-pathogenic combination of two mutations *in cis* c.[833C; 844_845ins68] (REF. 100). The non-pathogenic c.[833C; 844_845ins68] chromosomes are common in sub-Saharan Africa (up to 40% of control chromosomes), but less frequent in Europe and America, where the pathogenic c.[833C; -] chromosomes are more prevalent. Haplotype analysis of 69 pathogenic c.[833C; -] chromosomes of predominantly European origin, using 12 intragenic *CBS* polymorphic markers, revealed three unrelated haplotypes, suggesting that the three pathogenic and comparatively prevalent c.[833C; -] (c. stands for coding nucleotides) chromosomes probably originated by recurrent interallelic gene conversion in which

Somatic hypermutation
A process that occurs after immunoglobulin gene rearrangement, whereby the base sequences of part of the immunoglobulin variable regions are mutated more frequently than the rest of the genome. This sequence variation is subject to a selection process in the immune system that favours those cells that express immunoglobulins with the highest affinity for an antigen.

Class switch recombination
The somatic recombination process by which immunoglobulin isotypes are switched to IgG, IgA or IgE, without altering antigen specificity.

Table 2 | **De novo pathogenic gene-conversion events***

Disorder	Donor	Acceptor	Mutation	Type	Origin	Alternative mechanism	Refs
Inherited disease							
Atypical haemolytic uraemic syndrome	<i>CFHR1</i>	<i>CFH</i>	c.[3572C>T;3590T>C]	Interlocus	Germline	–	131
Congenital adrenal hyperplasia	<i>CYP21A1P</i>	<i>CYP21A2</i>	[1688G>T;1767_1768insT]	Interlocus	Germline	–	133
Neural tube defects	<i>FOLR1P</i>	<i>FOLR1</i>	7497_7662 including 13 discriminant nucleotides	Interlocus	Germline	–	138
Neural tube defects	<i>FOLR1P</i>	<i>FOLR1</i>	[7539T>C;7541G>A]	Interlocus	Germline	–	138
Hereditary persistence of fetal haemoglobin	<i>HBG2</i>	<i>HBG1</i>	Conversion involving 9 discriminant nucleotides in the promoter region	Interlocus	Germline	–	145
Shwachman–Diamond syndrome	<i>SBDSP</i>	<i>SBDS</i>	c.[141C>T;183_184TA>CT; 201A>G;258+2T>C]	Interlocus	Germline	–	152
Mucopolysaccharidosis type I (Hurler–Scheie syndrome)	<i>IDUA</i> (Paternal allele)	<i>IDUA</i> (Maternal allele)	Homozygosity of T364M plus five other markers	Interallelic	Somatic	<i>De novo</i> deletion in the maternally inherited allele	93
Hypergonadotrophic hypogonadism	<i>FSHR</i> (Paternal allele)	<i>FSHR</i> (Maternal allele)	Homozygosity of a C>G transversion within exon 10 plus a downstream single nucleotide polymorphism	Interallelic	Somatic	Maternally inherited allele harbours a <i>de novo</i> genomic deletion	94
Campomelic dysplasia	<i>SOX9</i> (Maternal allele)	<i>SOX9</i> (Paternal allele)	Homozygosity of Y440X	Interallelic	Somatic	–	95
Cancer							
Burkitt lymphoma	Unknown	<i>D6S347</i>	Replacement of ~2 kb DNA segment	Interlocus	Somatic	–	96
Hereditary nonpolyposis colorectal cancer	<i>MLH1</i> or <i>MSH2</i>	<i>MLH1</i> or <i>MSH2</i>	Somatic deletions	Interallelic	Somatic	–	97

*Arranged in the same order as in the text. In mutation nomenclature, ‘c.’ refers to coding nucleotides.

the common non-pathogenic c.[833C; 844_845ins68] chromosomes were used as a template¹⁰¹.

Therapeutic gene conversion. Gene conversion of an allele bearing an inherited pathogenic mutation can occasionally lead to the reversion of the disease phenotype, a phenomenon that has been dubbed ‘natural gene therapy’. This is best exemplified by the mitotic gene conversion that underlies the unusual phenotype of an individual with epidermolysis bullosa, who presented with patches of skin that seemed to be unaffected¹⁰². In theory, mitotic natural gene therapy can occur only in either dominantly inherited diseases or recessively inherited diseases that are caused by different mutations in the two alleles, resulting in somatic mosaicism⁴⁹. In the first case, the mutant allele is reverted to normal by the wild-type allele whereas, in the latter case, one mutant allele is converted back to wild type by the other mutant allele. In short, mitotic natural gene therapy seems to invariably involve interallelic gene conversion. An extension to this concept is provided by the exceptional example of the *SMN1* and *SMN2* gene pair. Instances of conversion of *SMN2* by *SMN1* with respect to C→T at nucleotide 6 of exon 7, which would serve to convert the partially functional *SMN2* to a fully functional *SMN2*, have been detected in the general population¹⁰³. A similar process

could also occur in some SMA patients, provided that they carried at least one *SMN1* allele in which C at nucleotide 6 of exon 7 remained intact. The resulting increased production of SMN protein would modulate the clinical manifestation of SMA.

Natural gene therapy raises the exciting prospect of harnessing gene conversion for therapeutic purposes and has, in part, been responsible for fuelling the development of oligonucleotide-based strategies for gene therapy⁴⁹. However, despite considerable progress over the past few years, there is much work to be done before this approach becomes a clinical reality¹⁰⁴.

Conclusions and future directions

A sizeable body of data has accumulated to show that, although gene conversion has been an important driving force in human genome evolution, it can also be the cause of devastating genetic conditions. There is now considerable evidence to support the view that gene conversion derives from distinct pathways, rather than from a random resolution of double HJs. Yet we are only beginning to unravel the basic mechanisms that underlie gene conversion and crossover events in eukaryotic cells. Many important questions remain to be answered. When and how is a recombination event ‘pre-determined’ to a given fate? How does one and the same protein

Multiplex ligation-dependent probe amplification analysis
A semi-quantitative PCR-based method that allows multiple targets to be amplified with only a single primer pair, and that is widely used for detecting copy-number variations.

participate in distinct pathways leading to gene conversion? What determines the selective use of different proteins to promote SDSA? Are the different pathways of gene conversion conserved across the eukaryotic kingdom? What is the nature of the sequences that can promote gene conversion? Which factors influence or determine the frequency, tract length and directionality of gene conversion? Answers to these questions should enhance our understanding of the significance of gene conversion and homologous recombination in ensuring the fidelity of DSB repair and the maintenance of genome integrity.

In the context of evolution, studies of the role of gene conversion in creating specific multigene families have been greatly facilitated by the availability of complete genome sequences from multiple species. The use of population genetic data to make inferences about the impact of gene conversion at the genome level is gaining popularity. These approaches, when combined with new or improved statistical methods, are expected to provide better estimates of the prevalence and spatial distribution of gene-conversion events along human chromosomes. Further integration of data from genome-wide studies, sperm typing and disease analysis should provide a clearer picture of the impact that gene conversion has had on the evolution of human and other mammalian genomes.

Undoubtedly, the spectrum of gene-conversion events causing human disease will continue to expand. In this regard, it should be noted that our meta-analysis deliberately excluded many point mutations that could well have been bona fide gene-conversion events, thereby inadvertently understating the actual contribution of this mutational mechanism to human genetic disease.

Considering the available disease data and the basic mechanism that leads to gene conversion, we speculate that somatic mosaicism resulting from interallelic gene conversion might, until now, have largely escaped our attention. We propose two factors that might account for this possibility. First, leukocyte genomic DNA is usually used for disease-associated mutation screening, and this tissue might be an inappropriate choice if somatic mosaicism is to be considered. Second, even when genomic DNA has been prepared from the tissue that is most relevant to the disease in question, somatic mosaicism is still likely to be overlooked if quantitative PCR is not carried out. Given that a restoration of only 5% of the function of an affected gene can significantly ameliorate the clinical symptoms of a disease (for example, cystic fibrosis), somatic mosaicism caused by interallelic gene conversion could turn out to be a potentially new and important disease modifier.

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