# Double trouble in human aneuploidy

Miguel A Brieño-Enríquez & Paula E Cohen

**Crossing over, or reciprocal recombination, is essential for accurate segregation of homologous chromosomes at the first meiotic division, resulting in gametes containing the correct chromosome number. A new study in human oocytes analyzes the genome-wide recombination and segregation patterns in all the products of female meiosis, providing experimental support for existing theories about the origin of human aneuploidies and highlighting a novel reverse segregation mechanism of chromosome segregation during meiosis.**

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Meiosis in human females is notoriously prone to error, with some 20% of oocytes estimated to be aneuploid as a result of errors in meiosis[1]. Many of these errors are thought to arise as a result of defective pairing (or synapsis) and reciprocal recombination (or crossing over) between homologous chromosomes during prophase I. Such events can result in the missegregation (or non-disjunction) of maternal and paternal chromosomes at the first or second meiotic division, leading to aneuploidy, implantation failure, pregnancy loss and congenital disorders[2]. Although the majority of these defects have been attributed to errors in prophase I in females, our understanding of the etiology of such errors is extremely limited. This deficit in our knowledge is attributable to the fact that female meiotic prophase I initiates in fetal life and only culminates after puberty, often decades after meiotic initiation. As such, maternal age is the only verified contributory factor to the high rates of aneuploidy observed in human conceptuses. Previous studies have been limited to assessing recombination initiation maps in men[3] and crossover maps of human oocytes[4]. However, such population-based studies do not show the complete picture of how the recombination landscape may contribute to aneuploidy, both in terms of the establishment of crossovers during fetal development and the maintenance of these intricate structures through adulthood. To investigate whether altered recombination might have a role in the high rates of missegregation observed in human oocytes, Eva Hoffmann, Alan Handyside and colleagues[5] have generated high-resolution MeioMaps through the analysis of all three products of distinct meioses from women.

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## MeioMaps from meiotic products

During female meiosis, unlike the situation in males, only one gamete product arises out of each meiotic event. At the first meiotic division, half of the homologous chromosome content of the diploid oocyte is expelled into a first polar body (PB1; **Fig. 1**), while the other half of the chromosomes remain in the oocyte for progression into meiosis II. At the second meiotic division, half of the sister chromatid content is retained in the gamete, while the other half is deposited in the second polar body (PB2; **Fig. 1**). Whereas most studies of human oocytes have involved only the gamete itself, Ottolini *et al.*[5] analyzed genome-wide recombination and chromosome segregation in all of the products of female meiosis by using PB1 and PB2 together with either the calcium ionophore–activated oocyte (oocyte trios) or the fertilized embryo (embryo trios). Oocyte and embryo trios were analyzed by whole-genome amplification and genotyped at 300,000 SNP loci, with data obtained for more than 4 million SNPs across 23 complete meioses and compared back to the parental genotype. The resulting MeioMaps from euploid embryo trios showed mendelian segregation of SNPs and independent assortment of chromosomes at meiosis I. Crossovers were identified by the transitions between maternal haplotypes along each chromosome, and aneuploidies and rearrangements were evident by the absence or presence of individual SNPs along an entire chromosome or chromosome segment and were confirmed by array comparative genomics hybridization (CGH). All gains or losses were reciprocal, involving gains in oocytes with corresponding losses in one polar body or vice versa. Thus, errors in meiosis are the major source of aneuploidies, rather than defects or mosaicism in germline precursors.

## Recombination and sister chromatid cohesion

The high rate of aneuploidy observed in oocytes from women of advanced age has been postulated to be due to errors in maternal meiosis I. This assumption is based on the fact that the variable recombination rates observed in adult oocytes[4] correlate well with altered processing of recombination events in human fetal oocytes[6]. More recently, however, evidence has pointed to the critical role of cohesins in ensuring appropriate homologous chromosome interaction during prophase I and also in maintaining sister chromatid interactions throughout both meiotic divisions[7–9]. This is further confounded by the fact that the unique and sequential chromosome segregation profiles during meiosis require a specific sequence of cohesion removal. Cohesion is first lost from the chromosome arms at meiosis I to allow for crossover resolution and segregation of homologs and is then lost at the centromeres at meiosis II to allow sister chromatid separation. Such cohesion must remain intact from fetal life until adulthood, and any failure will induce precocious separation of sister chromatids (PSSC) either at meiosis I or meiosis II, as demonstrated in human *in vitro* fertilization

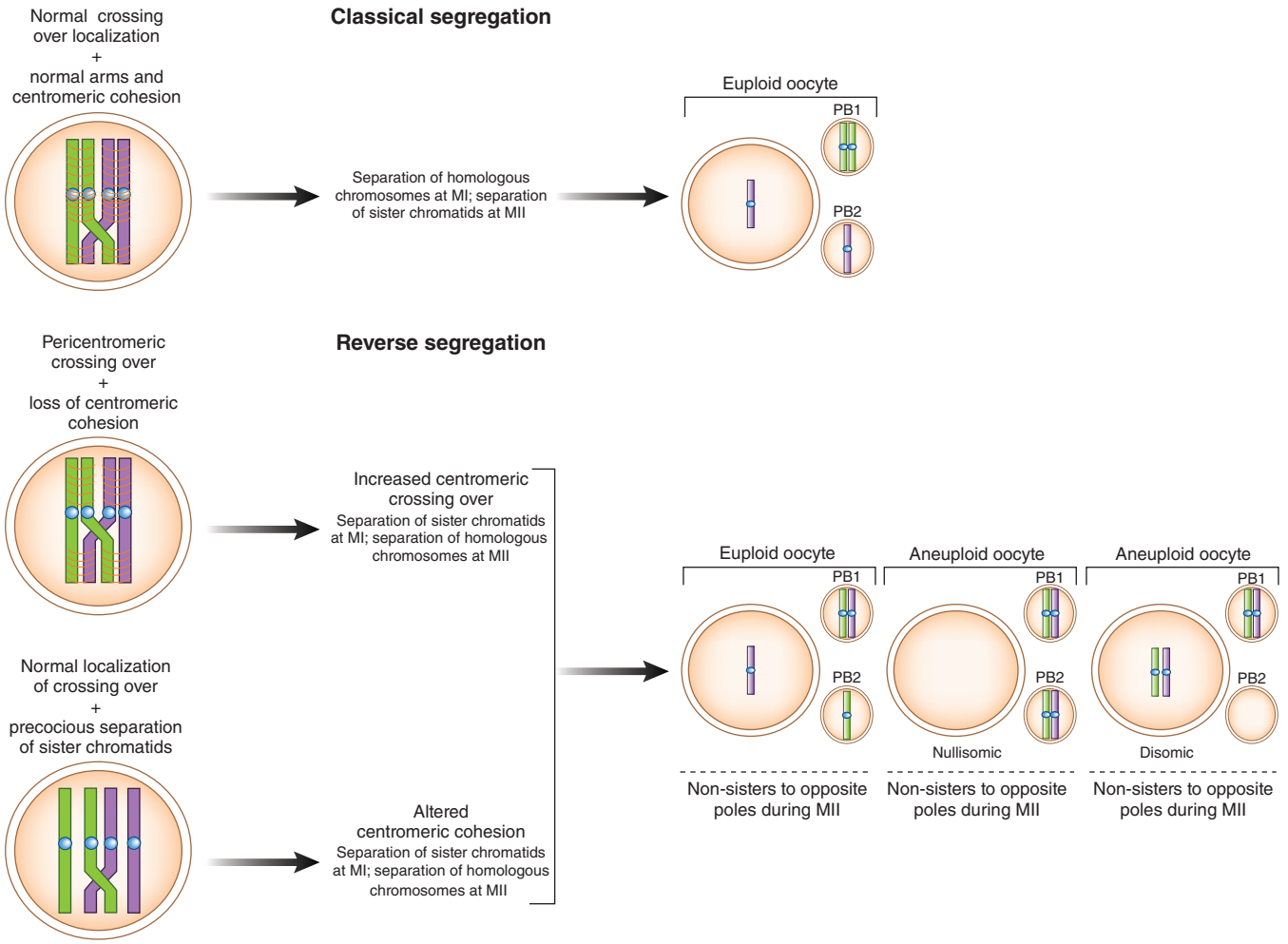


**Figure 1** Altered recombination or cohesin dynamics lead to reverse segregation in human oocytes. Top, classical meiotic segregation resulting from appropriate crossover distribution and frequency together with proper cohesin loading during meiosis I. Bottom, altered centromere recombination (top) and/or cohesin loading (bottom) result in retained homolog interactions and chromatid separation at meiosis I, leading to reverse segregation. MI, meiosis I; MII, meiosis II.

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(IVF) cycles[10]. In confirmation of this model, Ottolini *et al.*[5] assessed chromosome segregation through the analysis of pericentromeric SNPs and determined that the incidence of meiosis I nondisjunction is relatively rare but that PSSC is more prevalent.

Despite an inability to find increased incidence of meiosis I non-disjunction by pericentromeric SNP analysis, Ottolini *et al.* noted highly variable recombination rates in adult oocytes and embryos, similar to those reported for fetal oocytes[6,11]. Moreover, maternal recombination rates were 1.63-fold higher than those in fathers, supporting findings from previous analysis of recombination in prophase I cells from male and female gametes. These values correspond to the total crossover numbers detected by MLH1 foci during prophase I, indicating that presence of MLH1 is a good predictor of recombination events in human oocytes. The analysis of recombination frequency also found a 5.8-fold lower rate of recombination in aneuploid compared to euploid oocytes, supporting the predominant hypothesis that lowered recombination rate leads to higher rates of aneuploidy. Not surprisingly, therefore, the authors demonstrate a natural preference to maintain the highest rates of recombination inside the oocyte, as demonstrated by the 6.6% increase in recombination events in the oocyte in comparison to the PB2. Thus, the oocyte retains the genetic material most favorable for successful pregnancy through a mechanism that remains unknown.

## Reverse segregation

In some distinct plant and insect species, a novel segregation pattern exists for holocentric chromosomes. This segregation pattern was termed 'inverted meiosis' (refs. 12,13) and is characterized by the segregation of sister chromatids during meiosis I followed by crossover resolution and segregation of the homologous chromosomes during meiosis II (**Fig. 1**). Thus, equational division precedes reductional division in this process, which is postulated to arise as a result of the diffuse distribution of the kinetochore on holocentric chromosomes. In such a scenario, it may be more challenging to achieve the sequential release of sister chromatid cohesion that is essential for accurate segregation of homologs and then chromatids at meiosis I and meiosis II, respectively. Instead, inverse meiosis would require that sister chromatids become bioriented at meiosis I, with amphitelic attachment of their kinetochores to opposite spindle poles, resulting in equal segregation. Whether such events are an adaptation to or a consequence of diffuse kinetochores is unclear.

By analysis of segregation patterns in oocyte meiosis using pericentromeric SNPs, Ottolini *et al.*[5] describe a similar process in humans, which they call 'reverse segregation'.

Such reverse segregants make up the major proportion of chromosome segregation errors (8.7%). The majority of these events result in euploid embryos, but with the oocyte and PB2 containing non-sister chromatids and PB1 containing two non-sister chromatids. Such incidences would not result in copy number alterations and so would be unlikely to be identified by array CGH. The authors report different incidences of this phenomenon, but interestingly the chromosomes involved are those that are frequently associated with trisomies or abnormal gametes in IVF clinics (chromosomes 4, 9, 11, 13, 14, 15, 16, 19, 21 and 22). They argue that this novel segregation pattern is not due to two distinct PSSC events at meiosis I and meiosis II. However, it is possible that the altered distribution and frequency of recombination events, particularly at the centromere, may interfere with centromere separation and/or the attachment of sister chromatids to the same spindle at meiosis I, with both resulting in sister chromatid segregation rather than the expected homolog segregation.

Taken together, these studies have elucidated and, in some cases, confirmed many current theories about the origins of human aneuploidy, particularly those that involve cohesion-mediated events. At the same time, although these studies reinforce the importance of regulating the distribution and frequency of crossover events during prophase I, they suggest that altered recombination frequency may not by itself be responsible for meiosis I non-disjunction events leading to aneuploidy. Instead, these data suggest the importance of accurate recombination distribution to facilitate appropriate sequential release of cohesion. Whether reverse segregation is simply one sequela of altered sister chromatid cohesion or whether it relates instead to altered kinetochore behavior, perhaps resulting from increased recombination at the centromere, remains to be seen. Either way, these novel findings force us to reexamine the relationship between recombination and segregation events involving the centromere.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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# Sweet size control in tomato

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**All cells of an adult plant are ultimately derived from divisions that occur in small groups of cells distributed throughout the plant, termed meristems. A new study shows that carbohydrate post-translational modification of a peptide signal influences meristem and, as a consequence, fruit size in tomato.**

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Understanding control of the number, distribution and rate of cell divisions in meristems is key to comprehending plant growth and development. In the shoot apical meristem, from which all above-ground plant mass is derived, a conserved molecular module for the maintenance of cell division has been established[1,2]. At the heart of this module is a homeodomain transcription factor, WUSCHEL (WUS), which acts to promote cell division at the core of the meristem. In response to WUS activity, cells at the periphery of the WUS-expressing region generate a small-peptide signal, CLAVATA3 (CLV3), which feeds back to the inner cells to repress *WUS* gene expression. This loop constitutes a homeostatic mechanism by which any increase in WUS expression (tending to promote cell division in the meristem) leads to increased CLV3 expression, which represses WUS expression, returning cell division to its previous rate. The report by Zachary Lippman and colleagues in this issue[3] demonstrates that a post-translational modification of the CLV3 peptide is required for its signaling activity. This modification involves the addition of a triplet of arabinose sugars to a hydroxyproline residue within the peptide. Bearing in mind the important role that carbohydrate modifications have in cell wall structure and function[4,5], the finding that the activity of a signaling molecule repressing cell division depends on carbohydrate modification identifies a novel potential interface within plant growth and development. Moreover, the link shown in this study between meristem and fruit size highlights the importance of our understanding of fundamental plant biology for advances in agronomy.

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## Arabinosylation and signal activity

Working with tomato, Xu *et al.*[3] identified a series of mutants that had enlarged fruit as a consequence of increased meristem size. Unexpectedly, a number of the underlying mutations (*fin*, *fab2* and *rra3a*) affected genes encoding glycosyltransferases, in particular ones associated with the addition of arabinose to proline- or hydroxyproline-rich proteins, such as the cell wall protein extensin[4]. Although various lines of evidence had already suggested that such post-translational modifications are important in plants[6], the mechanisms underpinning links to phenotype have been obscure. For example, loss-of-function mutants in *Arabidopsis thaliana* that have impaired activity of a series of hydroxyproline O-arabinosyltransferases[6] show pleiotropic phenotypes related to growth, such as altered hypocotyl expansion and decreased cell wall thickness. Potential endogenous substrates for these enzymes (including CLV-related proteins) were identified, but a link to growth control was not shown.

Xu *et al.*[3] observed that the enlarged meristems in the new tomato mutants are reminiscent of those seen in WUS-CLV pathway mutants, so they looked more closely to determine whether arabinosylation might have a role in the CLV signaling pathway, as