TO ERR (MEIOTICALLY) IS HUMAN: THE GENESIS OF HUMAN ANEUPLOIDY

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Aneuploidy (trisomy or monosomy) is the most commonly identified chromosome abnormality in humans, occurring in at least 5% of all clinically recognized pregnancies. Most aneuploid conceptuses perish *in utero*, which makes this the leading genetic cause of pregnancy loss. However, some aneuploid fetuses survive to term and, as a class, aneuploidy is the most common known cause of mental retardation. Despite the devastating clinical consequences of aneuploidy, relatively little is known of how trisomy and monosomy originate in humans. However, recent molecular and cytogenetic approaches are now beginning to shed light on the non-disjunctional processes that lead to aneuploidy.

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Dosage imbalance of whole chromosomes typically results in inviability. So, it is not surprising that, in most organisms, meiotic non-disjunction is a rare occurrence. In the yeast *Saccharomyces cerevisiae*, for example, the likelihood of an individual chromosome mal-segregating during meiosis is as low as 1 in 10,000 (for example, see REF. 1). Similarly, in *Drosophila melanogaster*, estimates of X-chromosome non-disjunction in the female germ line range from ~1 in 1,700 to ~1 in 6,000 (REF. 2) and autosomal non-disjunction is probably as rare[3]. In mammals, the frequency of meiotic errors seems to be higher; nevertheless, in the organism that has been best studied (the mouse), the overall incidence of aneuploidy (trisomy or monosomy) among fertilized eggs does not exceed 1–2% (REF. 4).

Our species provides a notable exception to this general rule. An estimated 10–30% of fertilized human eggs have the 'wrong' number of chromosomes, with most of these being either trisomic or monosomic. This has profound clinical consequences: approximately one-third of all miscarriages are aneuploid, which makes it the leading known cause of pregnancy loss and, among conceptions that survive to term, aneuploidy is the leading genetic cause of developmental disabilities and mental retardation.

The basis for the difference in incidence between our own and other species remains obscure. However, we now know a lot about the non-disjunctional origin of human aneuploidies, especially those that derive from meiotic errors. In this review, we summarize our current understanding of human aneuploidy by: first, discussing available data on the incidence of aneuploidy in different types of human conception; second, reviewing studies of the mechanism of origin of human monosomies and trisomies; and finally, discussing available information on putative aneuploidy-inducing factors. However, before summarizing these data, it is useful to first review the basics of meiosis and meiotic chromosome segregation in our species.

Meiosis and meiotic abnormalities

The meiotic pathway is extraordinarily conserved and, therefore, it is not surprising that humans follow the same basic programme as do most other organisms. Meiosis generates haploid gametes through a specialized cell division process that consists of one round of DNA replication followed by two cell divisions. The first division, or meiosis I (MI), involves the segregation of homologous chromosomes from each other, whereas meiosis II (MII) involves the seg-

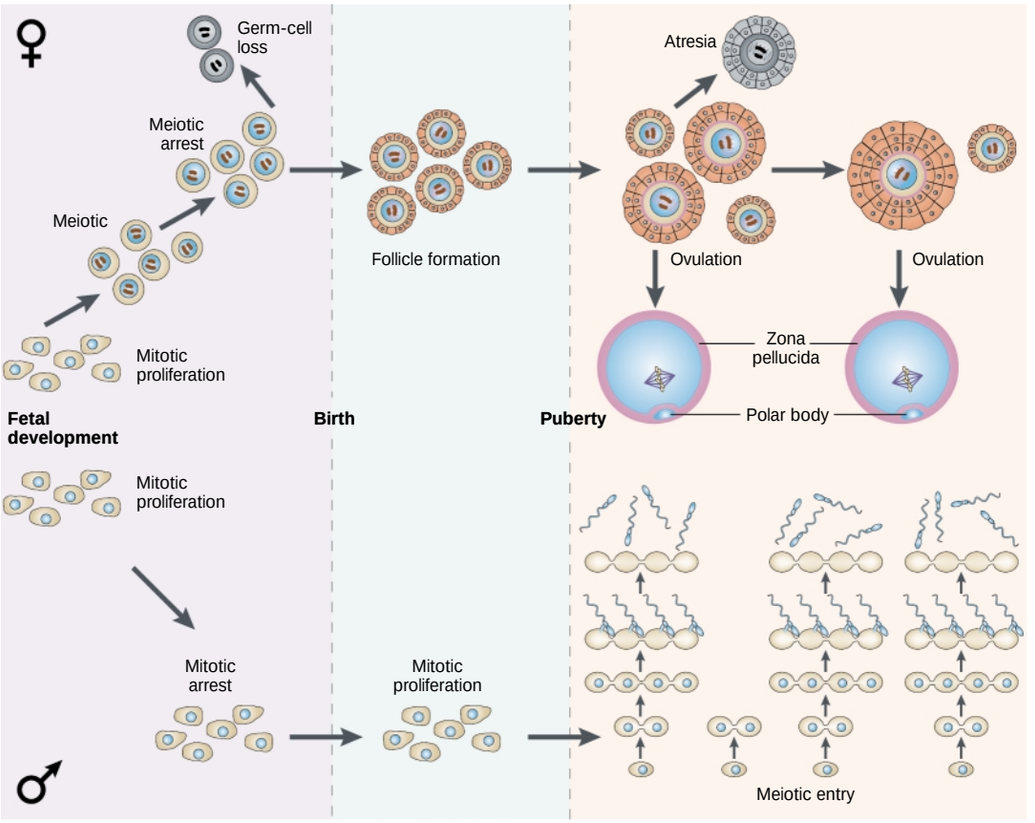

Figure 1 | **Meiotic 'timelines' for humans.** The fate of germ cells is dictated by the somatic environment. In both the developing ovary and the testis, germ cells undergo mitotic proliferation prenatally, but the time of entry into meiosis and the duration of meiosis is strikingly different between the sexes. Females: in the fetal ovary, a brief period of mitotic proliferation is followed by the entry of all cells into meiotic prophase. Several germ cells undergo apoptosis during this time, substantially reducing the pool of developing oocytes. Before birth, all surviving oocytes enter a period of extended meiotic arrest and, by the time of birth, all quiescent oocytes have become surrounded by somatic cells, forming primordial follicles. In a sexually mature woman, individual primordial follicles are stimulated to initiate growth throughout the reproductive lifespan. Typically, one fully grown oocyte is ovulated each month and several growing oocytes become ATRETIC. This process continues until the cohort of oocytes is depleted and the woman enters menopause. Males: in the fetal testis, a brief period of mitotic proliferation is followed by an extended period of mitotic arrest. After birth, the male germ cells, or spermatogonia, resume mitotic proliferation and, with sexual maturity, cells are stimulated to undergo meiotic cell divisions. Because spermatogonia continue to proliferate mitotically and to send daughter cells into meiosis, sperm production is maintained throughout the lifetime of the male. Throughout the meiotic divisions, individual spermatocytes remain connected by cytoplasmic bridges. These connections are lost during the post-meiotic process of spermiogenesis, which involves tight packing of the chromatin, growth of the sperm tail and the sloughing of virtually all the cytoplasm into the residual bodies (depicted as empty cells).

regation of the sister chromatids, and is therefore analogous to a mitotic division. These unique divisions are preceded by an equally unique meiotic prophase, during which homologous chromosomes synapse and undergo recombination.

Although these basic features hold for both human males and females, there are important sex-specific differences in the time of onset, duration and outcome of the meiotic processes (FIG. 1). In the human male, meiosis begins with puberty and the important events are sequential: in the adult testis, cells progress from prophase to metaphase I and on to metaphase II without an intervening delay, and each cell that enters meiosis produces four sperm. By contrast, the meiotic process in the human female is extraordinarily protracted: all oocytes initiate meiosis during fetal development, but after homologous chromosomes undergo synapsis and initiate recombination, the oocyte enters a period of meiotic arrest. Resumption of meiosis and the completion of the first division occur years later in the ovary of the sexually mature woman, just before the oocyte is ovulated. After the completion of MI, the oocyte arrests at the metaphase of MII and, normally, the second division is completed only after the egg is fertilized. Furthermore, the end products differ, as each cell that enters meiosis produces only one egg and two to three POLAR BODIES.

The successful segregation of homologues rather than sister chromatids at the first division requires unique chromosome behaviours that include: first, the maintenance of physical connections between homologues until anaphase I, a role that is fulfilled by the sites of recombination, or chiasmata[5]; and second, some form of physical constraint on the centromeres of sister

ATRESIA
Apoptotic death of follicles.

POLAR BODY
Oogenesis results in only one functional gamete; the remaining products of MI or MII are the polar bodies, which contain chromosomes but virtually no cytoplasm.

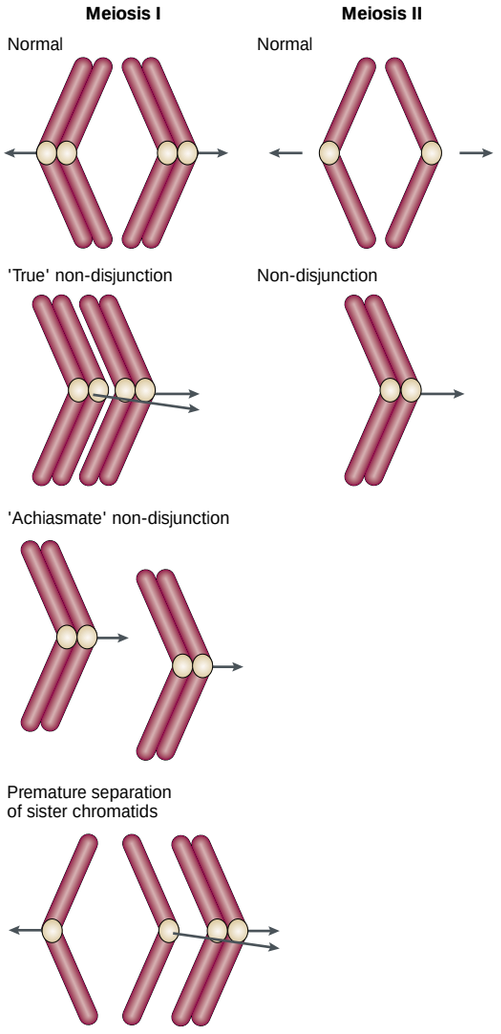

Figure 2 | **Meiotic non-disjunction.** A normal meiosis I (MI) division results in the segregation of homologous chromosomes. There are several possible patterns of abnormal MI segregation: including 'true' non-disjunction, in which homologues travel together to the same pole; 'achiasmate' non-disjunction, in which homologues that have failed to pair and/or recombine travel independently to the same pole; and premature separation of sister chromatids, in which chromatids — rather than homologues — segregate from one another. A normal meiosis II (MII) division involves the segregation of sister chromatids. Non-disjunction at MII is assumed to result from failure of the sisters to separate, although more complicated errors that involve sequential abnormalities at MI and MII have been proposed.

chromatids so that they form attachments to the same, rather than opposing, spindle poles (FIG. 2). In addition, although the second meiotic division is similar to a mitotic cell division, because it involves the segregation of sister chromatids, it follows the first division without an intervening S phase. So, to orchestrate the orderly separation of sister chromatids at MII, cohesion must be released along the chromosome arms at anaphase I (to allow the separation of homologues) but maintained between sister centromeres until anaphase II.

As detailed in the following sections, errors in meiotic chromosome segregation occur frequently in the human female, especially during the first meiotic division. Typically, all such errors are referred to as non-disjunction; however, various mal-segregation mechanisms are possible. As illustrated in FIG. 2, failure to resolve chiasmata between homologous chromosomes at anaphase I results in 'true' non-disjunction, whereby both homologues segregate together. In addition, the premature resolution of chiasmata — or the failure to establish a chiasma between a pair of homologues — can result in the independent segregation of homologues at MI, which leads to an error if both segregate to the same pole of the MI spindle. Finally, an MI error can also involve the segregation of sister chromatids, rather than homologous chromosomes. For example, premature separation of sister chromatids (PSSC) at the first meiotic division can result in the segregation of a whole chromosome, and a single chromatid to each pole (FIG. 2). As detailed below, available evidence indicates that each type of MI error can occur in our species.

Typically, MII errors are thought to result from the failure of sister chromatid separation (FIG. 2). Other, more complicated, models have been proposed to explain the association between aberrant genetic recombination and some MII-derived trisomies; these are discussed in more detail in a later section.

Incidence of aneuploidy

The observed level of aneuploidy in humans varies enormously, depending on the developmental time point being examined (TABLE 1). Among newborns, ~0.3% of liveborns are aneuploid[6] with the most common abnormalities being trisomy 21 and sex-chromosome trisomies (that is, 47,XXX, 47,XXY and 47,XYY chromosome constitutions). The incidence increases by an order of magnitude to ~4% among stillbirths (that is, fetal deaths occurring between ~20 weeks gestation and term), with the types of abnormality being similar to those identified among newborns. Among clinically recognized spontaneous abortions (that is, fetal deaths occurring between ~6–8 weeks and 20 weeks gestation), the incidence again increases tenfold, with ~35% of all such conceptions being trisomic or monosomic. Unlike stillbirths or livebirths, various different aneuploidies are represented among spontaneous abortions, including trisomies for nearly all chromosomes (TABLE 1). The most common specific abnormalities are sex-chromosome monosomy (45,X), accounting for nearly 10% of all spontaneous abortions, and trisomies 16, 21 and 22, which together constitute 50% of all trisomies identified in spontaneous abortions.

Results from these categories of conceptions — representing the three different classes of clinically recognized human pregnancy — allow us to estimate the minimal level of aneuploidy in humans. That is, using the above incidence figures and assuming that ~15% of recognized pregnancies spontaneously abort[7], 1–2% are stillborn[8] and the rest are liveborn, we can estimate that at least 5% of all human conceptions are aneuploid.

Table 1 | Incidence of aneuploidy during development

Gestation (weeks)			*0* — *6–8* — *20* — *40*				
	Sperm	**Oocytes**	**Pre-implantation embryos**	**Pre-clinical abortions**	**Spontaneous abortions**	**Stillbirths**	**Livebirths**
Incidence of aneuploidy	1–2%	~20%	~20%	?	35%	4%	0.3%
Most common aneuploidies	Various	Various	Various	?	45,X; +16; +21; +22	+13; +18; +21	+13; +18; +21 XXX; XXY; XYY

This value, however, clearly underestimates the real incidence of aneuploidy in humans, because it does not include information from 'occult' pregnancies; that is, pregnancies that go undetected because they spontaneously abort during the first few weeks of gestation. Limited data on early pregnancies are available from studies of human pre-implantation embryos that were retrieved in association with human-assisted reproduction procedures, and these indicate that the real incidence of aneuploidy might be much higher than 5%. For example, Jamieson *et al.*[9] karyotyped 178 'spare' diploid embryos obtained from *in vitro* fertilization (IVF) or GAMETE INTRA-FALLOPIAN TRANSFER (GIFT) procedures, and found that nearly 20% were aneuploid. Consistent with this, fluorescence *in situ* hybridization (FISH) studies of IVF-derived pre-implantation embryos indicate possible rates of meiotic- and mitotic-derived aneuploidy of 20% or higher (for example, see REF. 10).

Furthermore, these results are consistent with cytogenetic analyses of human gametes. The FISH studies of human sperm during the past decade indicate chromosome-specific aneuploidy frequencies of ~0.1–0.2% (REF. 11); summing over the entire genome, this indicates that 2% or more of sperm might have missing or additional chromosomes. In oocytes, the value is much higher. Routine cytogenetic studies of over 1,000 oocytes obtained in IVF clinics have now been reported, with the largest studies indicating possible rates of aneuploidy of 20–25% (REF. 12). Furthermore, molecular cytogenetic analyses (for example, SPECTRAL KARYOTYPING) of human oocytes have yielded similarly high values[13]. There has been considerable scepticism about the relevance of these observations to the *in vivo* situation[12] — after all, IVF patients are unlikely to represent the general population of reproducing women, the oocytes come from ovaries that have been stimulated by exogenous hormones and, typically, the oocytes available for study are 'spares' that remained unfertilized after insemination. However, a recent study of 'control' oocytes indicates that the estimates might well be correct. That is, in FISH studies of 90 oocytes obtained from unstimulated ovaries, Volarcik *et al.*[14] analysed MI segregation of four chromosomes — 16, 18, 21 and the X — and identified ten abnormalities; extrapolating these results to the other chromosomes implies an overall rate of aneuploidy in excess of 20%.

Altogether, the combined results from clinically recognized pregnancies, pre-implantation embryos, and gametes indicate an extraordinary level of aneuploidy among human zygotes — at least 5% and possibly as high as 25%. So, for reasons that are as yet unclear, chromosome segregation in meiosis is surprisingly error-prone in our species.

Origin of aneuploidy

Over the past decade, DNA polymorphisms have been used to examine the origin of different aneuploid conditions. For monosomies, information is only available on the 45,X condition, as autosomal monosomies are apparently early embryonic lethals. Several studies of the origin of 45,Xs have now been conducted (for example, REF. 15), with an estimated 70–80% having a single maternally derived X chromosome; that is, it is the paternal X or Y that is lost, either in meiosis or in an early stage in embryogenesis. These results apply to both spontaneously aborted and liveborn 45,X conceptuses, which indicates that the parental source of the X chromosome does not influence the survival of the 45,X conceptus.

Unlike autosomal monosomies, most trisomic conditions are compatible with at least some fetal development and information is therefore available for several different trisomies[16–23]. Results from studies of over 1,000 trisomic fetuses/liveborn individuals are summarized in TABLE 2, with two general principles emerging. First, there is remarkable variation among trisomies with regard to the parent and meiotic stage of origin of the additional chromosome. For example, paternal errors account for nearly 50% of 47,XXYs and trisomy 2, but only 5–10% of most other trisomies, and they are rarely, if ever, the cause of trisomy 16. Similarly, the importance of MI versus MII errors varies among chromosomes. For example, among maternally derived trisomies most, if not all, cases of trisomy 16 seem to be due to MI errors, but for sex-chromosome trisomies one-third of cases are associated with MII errors, and for trisomy 18 most cases involve MII non-disjunction. So, it seems likely that there are *cis* (chromosome-specific) effects that influence the patterns of non-disjunction.

However, overlying this chromosome-specific variation is at least one general theme. That is, maternal MI errors predominate among almost all trisomies. This is perhaps not surprising, because the first division in females is initiated prenatally and is not completed until the time of ovulation (FIG. 1), and involves unique chromosome behaviours to segregate homologous chromosomes rather than sister chromatids. Indeed, the

GAMETE INTRA-FALLOPIAN TRANSFER (GIFT). Assisted reproduction technique in which oocytes and sperm are mixed and placed into the fallopian tubes, where fertilization might occur.

SPECTRAL KARYOTYPING Fluorescence *in situ* hybridization technique in which differentially labelled DNA probes to all chromosomes are used, making it possible to identify every chromosome in the complement in a single hybridization.

GENE CONVERSION
Non-reciprocal recombination event, in which genetic information at one allele is copied into the complementary allele.

BIVALENT
Synapsed pair of homologous chromosomes in meiosis I.

complexity of this division makes it clear that an understanding of the origin of human aneuploidy will require exhaustive analyses of the processes involved in starting, stopping and re-initiating MI in the human female.

Recombination and non-disjunction

Although there are now considerable data on the parent and meiotic stage of origin of different human aneuploidies, we know relatively little about the underlying non-disjunctional mechanisms. However, over the past few years the first molecular correlate of human aneuploidy, namely altered genetic recombination, has been identified and characterized.

Lessons from model organisms. Chiasmata, the physical manifestations of genetic recombination, have a crucial role in tethering homologous chromosomes during the first meiotic division[5]. So, it is not surprising that, in all model organisms studied so far, disturbances in the recombination pathway are associated with abnormalities in chromosome segregation at MI. The most obvious effects involve mutations that reduce, or abolish, recombination: almost invariably, these mutations are associated with meiotic arrest, or with gross abnormalities in chromosome segregation or, at the very least, with increased levels of non-disjunction[24].

In addition to an effect of the number of recombinational events, the location of the exchanges also seems to be important. For example, in meiotic studies that use yeast artificial chromosomes (YACs) or derivatives of budding-yeast natural chromosomes, Dawson and co-workers[25] found that exchanges in different chromosomal intervals had differing abilities to properly segregate chromosomes. Specifically, chromosomes with a single distally located exchange were more likely to non-disjoin than were those with more proximally positioned exchanges. By contrast, Sears *et al.*[26] observed a high frequency of MI segregation errors (either PSSC or non-disjunction) in YACs in which pericentromeric GENE CONVERSION events had occurred. So, these results indicate that exchanges can either be too near the centromere or too far from the centromere, and that both situations impart a risk for non-disjunction.

Table 2 | The origin of human trisomy

		Origin (%)				
		Paternal		Maternal		
Trisomy	No. of cases	MI	MII	MI	MII	Post-zygotic mitosis
2	18	28	–	54	13	6
7	14	–	–	17	26	57
15	34	–	15	76	9	–
16	104	–	–	100	–	–
18	143	–	–	33	56	11
21	642	3	5	65	23	3
22	38	3	–	94	3	–
XXY	142	46	–	38	14	3
XXX	50	–	6	60	16	18

(MI, meiosis I; MII, meiosis II.)
(Adapted from REF. 6.)

In flies, also, there seems to be a link between the location of meiotic exchanges and the likelihood of non-disjunction. For example, in an analysis of spontaneous X-chromosome non-disjunction in *Drosophila* females, Koehler *et al.*[27] observed an increase in BIVALENTS with a single distally located exchange in MI errors, and an increase in extremely proximal exchanges in MII errors. So, as in yeast, exchanges too close to or too far from the centromere seem to increase the risk of non-disjunction. The link between distal cross-overs and mal-segregation has also been supported by mutational analyses. That is, several mutations that cause non-disjunction of non-exchange bivalents in *Drosophila* females (for example, *nod* (*no distributive disjunction*), *Axs* (*Abnormal X segregation*), *Dub* (*Double or nothing*) and *ncd* (*non-claret disjunctional*)) also increase non-disjunction of exchange chromosomes; in virtually all these cases, single cross-overs are distally positioned (for example, REFS 28,29, and R. S. Hawley, personal communication), which indicates that such bivalents might be more susceptible to non-disjunction than are those with more proximally located chiasmata.

Altogether, the data from these and other model organisms (for example, REFS 30,31) indicate that absent or reduced levels of recombination, or suboptimally positioned recombinational events, increase the likelihood of non-disjunction. So, an obvious question is whether or not these effects also apply to humans.

Human non-disjunction. By using genetic mapping techniques to study the inheritance of DNA polymorphisms in trisomic conceptuses, it is possible to recapitulate the recombinational events that occurred in the trisomy-generating meioses[32]. During the past decade, several laboratories have used this approach to study the relationship of recombination and human non-disjunction, by comparing the frequency and distribution of meiotic exchanges in trisomy-generating meioses with those from chromosomally normal meioses (for example, REFS 17,18). Several general principles have emerged from these analyses and are discussed in the following paragrahs.

Significant reductions in recombination are a feature of all MI-derived trisomies so far studied. This includes paternally derived cases of trisomy 21 and Klinefelter syndrome (47,XXY), and maternally derived cases of trisomies 15, 16, 18, 21, and sex-chromosome trisomies[16–19,33–37](FIG. 3). The magnitude of the effect is variable: the most pronounced reduction is observed for paternally derived XXYs, in which the genetic map of the XY pairing region is decreased four- to fivefold, from ~50 cM in normals to ~10–15 cM in trisomy-generating meioses[34,35]. For others (for example, trisomy 15), the effect is subtler[36]; nevertheless, it seems likely that diminished recombination is a correlate of all human trisomic conditions.

Conceptually, either of two processes might be responsible for the reduced map lengths of the different trisomic conditions. First, a proportion of cases might involve chromosomes that failed to recombine;

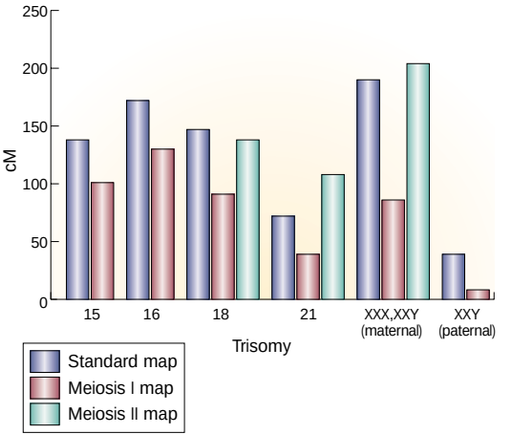

Figure 3 | **Genetic maps of normal and trisomic meioses.** Chromosome-specific genetic maps based on analyses of normal or trisomy-generating meioses. Standard maps were based on conventional genetic linkage analyses of CEPH families (CEPH — Centre d'Étude du Polymorphisme Humain; a repository containing cell lines from large, well-characterized families that have formed the basis for most human genetic maps). Trisomic maps were constructed using centromere mapping analyses of trisomic conceptions and their parents[32]. Except for paternally derived XXYs, all trisomic maps are based on maternal meiotic errors, and have been divided into cases of meiosis I (MI) and meiosis II (MII) origin. These maps make possible comparisons of the amount of recombination between homologues that segregated normally and those that non-disjoined. Recombination is reduced for all MI-derived trisomies so far studied, including maternal MI trisomies 15, 16, 21 and XXX/XXYs, and paternal MI-derived XXYs. Increased recombination seems to be a feature of maternal MII-derived cases of trisomy 21, but not trisomy 18 or XXX/XXYs. (cM, centimorgan.)

that is, the non-disjoining bivalent was 'achiasmate'. Alternatively, subtler reductions in recombination might be involved; for example, on a chromosome normally joined by two to three chiasmata, only a single, suboptimally positioned chiasma was present. In fact, both effects have been observed, although their relative importance varies widely among the different trisomic conditions. For example, for paternally derived trisomy 21 and the 47,XXY condition, and for maternally derived trisomies 15, 18 and sex-chromosome trisomies, there is no evidence that cross-overs — when detected — are unusually positioned on the chromosomes[16,33,35–37]; so, for these trisomies the reductions in recombination seem to involve achiasmate homologues.

Similarly, an estimated 40% of maternal MI-derived cases of trisomy 21 involve an achiasmate bivalent[18]. However, in this instance there is a secondary recombination effect, which involves the location of exchanges. Specifically, in those cases in which a single exchange is present, the cross-over typically is placed distally[18]; so, similar to the situation in yeast and flies, the presence of a single, distally placed chiasma seems to be a risk factor for trisomy 21.

Unlike other trisomies so far studied, failure to recombine seems unimportant to the genesis of trisomy 16 (REF. 17). Instead, the 'typical' non-disjoining chromosome 16 bivalent seems to be joined by one to two chiasmata, but with the exchange(s) much more distally located than expected. Indeed, Hassold *et al.*[17] reported a 20-fold reduction in recombination in proximal regions of chromosome 16 in trisomy-generating meioses by comparison with normal meioses. So, for trisomy 16 it seems that a shift in exchange position, rather than reduced recombination *per se*, is the important determinant of non-disjunction.

All the above observations pertain to MI-derived trisomies; indeed, because recombination occurs at MI, there was little reason to suspect that altered recombination would be associated with MII-derived trisomies. So, it was surprising when Lamb *et al.*[19] reported just such an effect. Specifically, they observed an increase in recombination in maternal MII-derived cases of trisomy 21 by comparison with controls, with the effect being especially noticeable in the region closest to the centromere. So, as reported for yeast and flies[26,27], exchanges that occur too close to the centromere seem to be a risk factor for human non-disjunction as well.

The observations of an effect of an MI process (recombination) on MII non-disjunction indicate an obvious question: Did these trisomies really originate at MII? Lamb *et al.*[19] concluded that the answer is no. They suggested that the presence of a pericentromeric exchange might increase the likelihood of chromosome 'entanglement' or PSSC at MI. Subsequent segregation at MII would result in a disomic gamete having identical centromeres — so the case would be scored as originating at MII even though the precipitating event occurred at MI (FIG. 4).

A clear implication of this interpretation is that most, if not all, cases of human female non-disjunction have their origin at the first meiotic division. Although this might be the case for trisomy 21, subsequent studies indicate that other chromosomes behave differently. Specifically, there are no obvious changes in the amount or location of recombinational events in maternal MII-derived cases of trisomy 18 or sex-chromosome trisomy[16,37]. So, at least for these conditions, it seems that non-disjunctional events can, indeed, originate at the second meiotic division.

Premature separation of sister chromatids

Although several laboratories have used a molecular approach to study the origin of human trisomies, other groups have applied cytogenetic methodology to analyse directly meiotic chromosome segregation in humans. One of the questions that has received considerable attention relates to the way in which meiotic chromosomes 'misbehave' in humans; that is, via classical non-disjunction or because of PSSC at MI.

So far, all such studies have focused on the human oocyte. These analyses have been hampered by the fact that the desired object of study — the fully mature, recently ovulated egg — is virtually impossible to

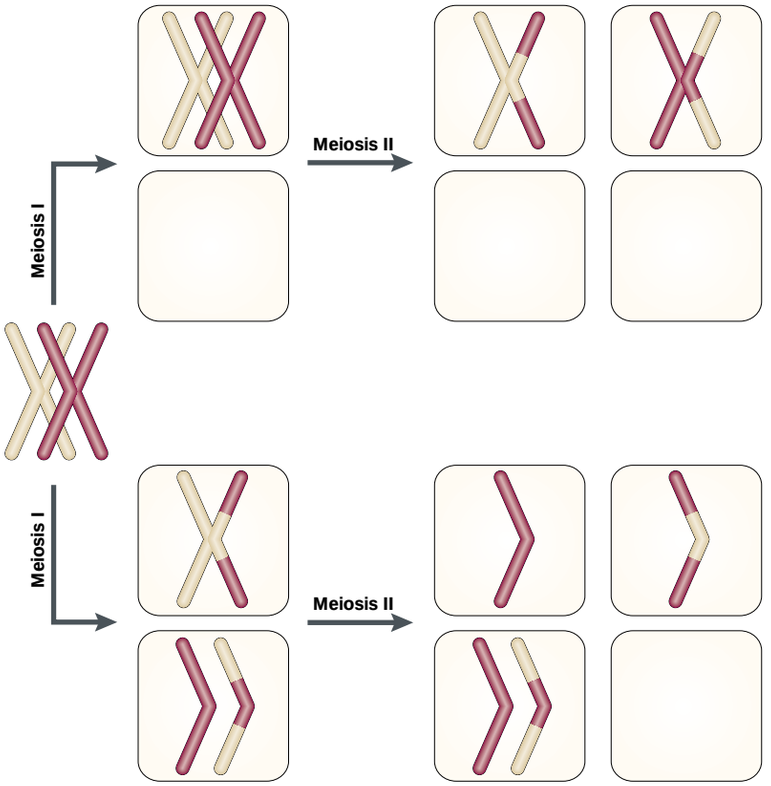

Figure 4 | **Recombination and meiosis-II-derived trisomies.** For trisomy 21, increases in recombination (especially in the pericentromeric region) have been linked to cases scored as arising at meiosis II (MII). Two possible explanations for this surprising correlation have been suggested[18]. Top: It is possible that extremely proximal exchanges lead to chromosome 'entanglement', so that the bivalent remains intact until it is positioned on the MII plate, at which time the two homologues finally separate from one another. The result will be two 'disomic' products, each containing two chromatids with identical centromeres. Scoring of the meiotic stage of origin of trisomies is based on the centromere, with meiosis I (MI)-derived cases having genetically distinguishable centromeres, and MII-derived cases having identical centromeres. So, in this scenario the case would be scored as originating at MII, even though the precipitating event occurred at MI. Bottom: Alternatively, pericentromeric exchanges might disrupt sister chromatid cohesion, resulting in the premature separation of sisters at MI. If the two sisters travel to the same pole in anaphase of MI and MII, the result will be the same as that for chromosome entanglement — that is, a disomic gamete, with the two chromatids having identical centromeres.

obtain. As a result, only limited information is as yet available, and most of it is based on studies of those 'spare' oocytes that remain unfertilized after attempted *in vitro* fertilization. The largest data set comes from conventional cytogenetic analyses conducted by Angell at the University of Edinburgh[38–40]. In her initial report, Angell[39] identified abnormalities that resulted from PSSC but found none that derived from true non-disjunction events[39] (see FIGS 2,5). These results were confirmed on additional analyses[38,40], with Angell hypothesizing PSSC to be the main source of human aneuploidy[41] (see also REF. 42). In subsequent molecular cytogenetic studies of spare oocytes, this claim has been challenged: true non-disjunction as well as PSSC errors have been observed[43] and some investigators have suggested that PSSC is largely an artefact of cell culture[44].

More recent studies that use another source of oocytes, and new methodology, indicate that PSSC might be important but that it is not the only source of human aneuploidy. Specifically, Hunt and colleagues[14] obtained oocytes from the unstimulated ovaries of fertile donors that had undergone elective abdominal surgery; this circumvents at least some of the concerns associated with the analysis of IVF-derived material. Furthermore, they combined immunofluorescence and FISH technology to study intact MII-arrested oocytes; this makes it possible to visualize the chromosomes and the spindle apparatus, and to assess the chromosome content of both the polar body and the MII oocyte (FIG. 5). Using this approach to analyse segregation of chromosomes 16, 18, 21 and the X chromosome in ~400 oocytes, they have identified non-disjunctional and PSSC errors, with the former accounting for approximately two-thirds of all events (P.H., unpublished observations). However, the distribution seems to vary with age and among the different chromosomes and, given the limited number of oocytes so far examined, the relative contributions of non-disjunction and PSSC to human aneuploidy are not yet certain.

Maternal-age effect on trisomy

Despite the high frequency and clinical importance of human aneuploidy, we know surprisingly little about factors that modulate the risk of meiotic non-disjunction. In this section, we discuss the one factor incontrovertably linked to human aneuploidy — increasing maternal age.

The association between increasing maternal age and Down syndrome was recognized as early as 1933 (REF. 45), more than 25 years before it was determined that Down syndrome was caused by trisomy 21. Subsequently, studies of other human trisomies have shown that most, if not all, are affected by increasing maternal age, although the exact relationship varies among trisomies[46,47]. The magnitude of the effect is extraordinary: among women under the age of 25 years ~2% of all clinically recognized pregnancies are trisomic, but among women over 40 years this value approaches 35% (FIG. 6). Furthermore, the effect seems to be 'hard-wired' into our species; that is, there is no known influence of race, geography, or socio-economic status on maternal-age-specific rates of trisomy.

Despite its importance, we know very little about the basis of the maternal-age effect. Indeed, until relatively recently one popular model attributed the effect to the uterine environment, indicating that there might be an age-related decline in the ability to recognize and then abort trisomic fetuses[48]. Studies of the parental origin of trisomies, described above, have shown that the effect of maternal age is restricted to cases of maternal origin; so, it is clear that it is the egg, and not the uterine environment, that is the source of the age effect.

It also seems reasonable to conclude that the effect involves biological, and not chronological, ageing. For example, Kline *et al.*[49] recently analysed the age at menopause of women previously identified as having a

SYNAPTONEMAL COMPLEX
A tripartite, meiosis-specific structure that binds the homologous chromosomes together during meiosis I.

trisomic spontaneous abortion, and compared the results with those of women with a chromosomally normal index pregnancy. On average, women with a known trisomic pregnancy entered menopause about one year earlier than did those in the control group. These results are consistent with the "limited oocyte pool" hypothesis[54], a model indicating that the age effect might be due to the relative scarcity of oocytes at optimal stages of maturation. Furthermore, they are consistent with a recent epidemiological study of Down syndrome, in which mothers of Down syndrome individuals were significantly more likely than controls to have a 'reduced ovarian complement', either as a result of ovarian surgery or because of congenital absence of one ovary[50].

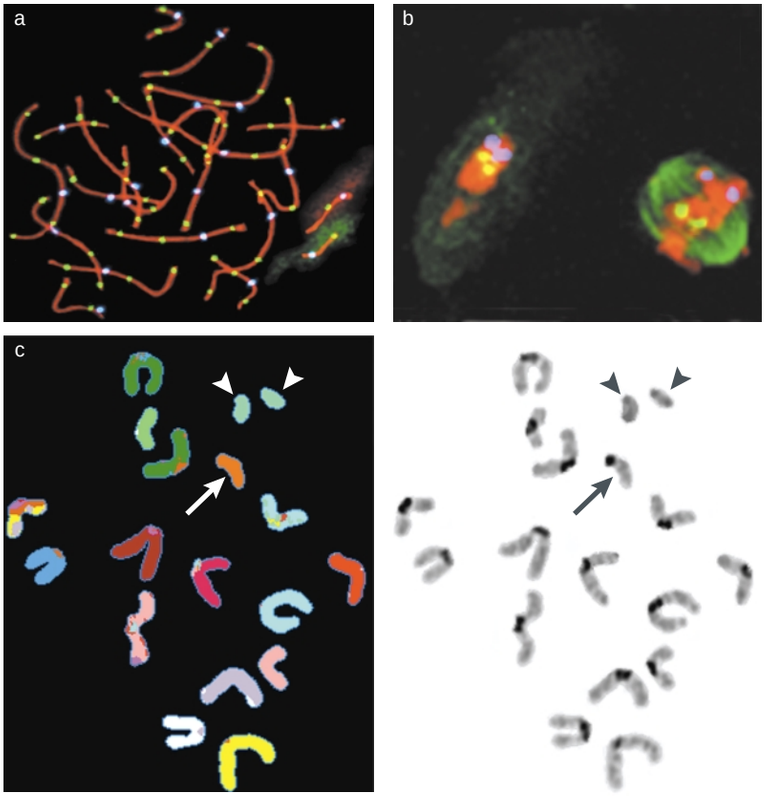

Figure 5 | **Molecular cytogenetic approaches to studying gametes.** Over the past few years, several new cytological approaches to the analysis of mammalian meiosis have been introduced, including the following. **a** | Immunofluorescence/fluorescence *in situ* hybridization (FISH) analysis of meiotic recombination: a human male pachytene preparation, using antibodies against SCP3 (synaptonemal complex protein 3) to identify the SYNAPTONEMAL COMPLEX (SC; shown in red) and antibodies against the DNA mismatch-repair protein MLH1 (mutL homologue 1; thought to identify the sites of meiotic exchanges; shown in green), and using CREST antiserum to detect the centromeric regions (shown in blue). Subsequent FISH analysis of these preparations allows identification of individual chromosomes or chromosome regions, making it possible to determine the number and location of exchanges on individual chromosomes. For example, in this figure, paint probes have been used to identify chromosomes 21 (shown as green cloud) and 22 (shown as red cloud); because DNA loops out from the SC, the chromosomal material appears as 'clouds' surrounding the SC. Note that a single MLH1 focus is observed for each of the two chromosomes, consistent with a single meiotic exchange for each. **b** | Immunofluorescence/FISH analysis of human oocytes. By fixing intact oocytes, it is possible to maintain the three-dimensional structure of the oocyte, thus allowing examination of both spindle morphology and chromosome behaviour. Here is shown a meiosis II (MII)-arrested egg (right) and the first polar body (left), probed with X-chromosome and chromosome-18 FISH probes (the spindle is shown in green, the metaphase chromosomes in red, the X-chromosome centromere in yellow and the chromosome-18 centromere in blue). In both the oocyte and the polar body, two signals (representing the two sister chromatids) are observed for each of the two chromosomes. So, both chromosome 18 and the X chromsome must have segregated normally at meiosis I (MI). **c** | Spectral karyotyping of gametes — this can be used to analyse segregation of individual chromosomes. A partial spectral karyotype of an MII-arrested egg from an X,Y sex-reversal female mouse (with the conventionally stained image of the cell for comparison, right) is shown here. The cell has two obvious abnormalities — first, the sister chromatids of the Y chromosome are prematurely separated (arrowheads); and second, chromosome 10 is represented by only a single sister chromatid (arrow), indicating premature separation of the sisters of at least one of the two chromosome 10 homologues at the previous MI.

These observations aside, little else is certain about the maternal-age effect. Common sense dictates that it involves MI — the stage of oogenesis that requires at least 10–15 years and as many as 40–45 years to complete (FIG. 1) — and this is consistent with most studies that have correlated the meiotic stage of origin of trisomy with maternal age (for example, REF. 37). However, the timing of the precipating event is unclear. Does the effect arise: in the fetal pre-meiotic stage of germ-cell development, during which time rapid mitotic divisions occur; in fetal MI, during which time pairing and recombination occur; in the prolonged diplotene stage, during which time the oocyte is meiotically 'arrested'; or in the peri-ovulatory stage, at which time MI is resumed and completed? Each of these time points has been suggested to be the source of the maternal-age effect (for example, REFS 51–54), but there is little hard evidence to discriminate between the various models. Nevertheless, it seems unlikely that the age effect occurs simply because of something that happened prenatally, so several recent models have proposed multi-step abnormalities that involve different stages of MI.

One of the more provocative of these models indicates a link between altered recombination and maternal-age-related non-disjunction[19]. Specifically, Lamb *et al.*[19] proposed that at least two 'hits' are required for age-dependent trisomy. The first involves the establishment in the fetal ovary of a susceptible bivalent (for example, a bivalent with a single, distally placed exchange); this component would be age independent. The second hit involves abnormal processing of the susceptible bivalent at metaphase I, in the adult ovary; this would be the age-dependent component of the process. If this model is correct, it implies that non-disjunctional mechanisms are similar in older and younger women, and that the age effect occurs simply because the older ovary is less efficient at segregating susceptible bivalents. Furthermore, the model makes two predictions: first, recombination should be similarly altered in non-disjunctional meioses that involve younger and older women; and second, processes associated with follicular growth or with re-initiation of MI in the adult ovary degenerate with age, and do so in such a way that susceptible chiasmate configurations are more likely to non-disjoin than are bivalents with 'normal' exchange patterns. Evidence that supports the first of these two predictions has been presented for trisomies 16 and 21, but contradictory evidence has been reported for trisomies 15 and maternal sex-chromosome trisomy[55]; so, if the model is correct, it probably pertains to a subset of human chromosomes. The second prediction is harder to examine, owing to the difficulties in obtaining and analysing

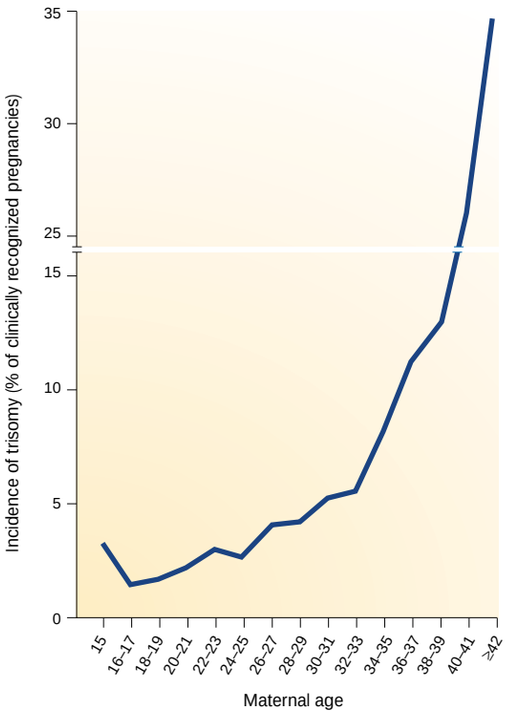

Figure 6 | **Maternal age and trisomy.** This shows maternal-age-specific estimates of trisomy among all clinically recognized human pregnancies, generated by combining data from individual trisomies and assuming a spontaneous abortion rate of ~15% (REF. 85). Not all individual trisomies manifest the same slope as seen here; for example, for trisomy 16, the commonest of all human trisomies, the increase is essentially linear. So, non-disjunctional mechanisms associated with maternal age must vary among different human chromosomes. There is also an apparent 'bump' in trisomy among teenage girls. This slight increase has also been observed in several studies of Down syndrome, and might reflect a tendency to non-disjoin in the earliest ovarian cycles of the human female.

suitable material — that is, oocytes from volunteer donors. However, two such analyses of MII oocytes, one studying the ANTRAL FOLLICLES of unstimulated ovaries[14] and the other PERIOVULATORY FOLLICLES exposed to exogenous hormone[56], found virtually identical age-related abnormalities in spindle formation and chromosome alignment. Furthermore, Hunt and co-workers[14] (and P.H, unpublished observations) have also noted striking age-related abnormalities in the congression of chromosomes to the MI plate. Possibly, abnormalities in spindle formation or in spindle-checkpoint control[57] make it more likely that susceptible bivalents will become mal-aligned than will bivalents with normal exchange patterns.

Regardless of the correctness of the two-hit model, it seems unlikely that it will apply to all trisomic conditions. Various approaches — including the recently described cytological technology to study recombination in MI cells (FIG. 5), spectral karyotyping of oocytes from younger and older women (FIG. 5) and development of appropriate mammalian models — will probably be needed to unravel the mechanisms that are responsible for generating the maternal-age effect. For example, in our laboratories we have been interested in asking whether female mice that are heterozygous for structural chromosome abnormalities that are known to disturb recombination (for example, inversions) are at an increased risk of non-disjunction and, if so, whether the effect is heightened in older animals. Other possible approaches that make use of animal models include analysis of recombination and non-disjunction in appropriate knockout mice: several such meiotic mutants have now been generated and, in those in which the female is fertile (for example, REF. 58) it will be important to ask whether recombination is altered, and if meiotic non-disjunction (and age-dependent non-disjunction) is a feature of the phenotype. As the maternal-age effect on trisomy is arguably the most important aetiological factor in any human genetic disease, the pay-off associated with development of an appropriate animal model will be considerable.

Other predisposing factors

Despite years of intensive study, increasing maternal age remains the only factor indisputably linked to human aneuploidy. A large number of other environmental or genetic risk factors have been suggested, including parental irradiation[59], oral contraceptives and fertility drugs[60], thyroid antibodies[61], alcohol consumption[62], seasonality[63], parity[64], maternal diabetes[65], consanguinity[66], allelic combinations at specific loci (for example, REF. 67) and the presence of certain types of chromosome polymorphisms[68]. However, none of these or any other associations have been proven (for example, REFS 69–76). Possibly there are no such factors or, if they do exist, their impact is so trivial that they escape detection. However, it might also be that they exist, but that we have failed to identify the correct ones to study: for example, as discussed below, the putative association between folate metabolism and Down syndrome represents an initial attempt to link maternal genotype and nutrition with non-disjunction. Alternatively, it might be that the study designs that we have used are inadequate. For example, previous epidemiological analyses of trisomies have pooled all cases, making the assumption that non-disjunction is homogeneous. This is almost certainly incorrect: factors that affect MI undoubtedly are different from those affecting MII or mitosis, and factors that affect spermatogenesis are different from those affecting oogenesis. So, analyses of putative aneuploidy-inducing agents would profit from knowledge of the parent and meiotic/mitotic stage of the origin of trisomy. As described below, recent studies analysing the possible association of Down syndrome with maternal smoking have used just this approach.

Maternal folate polymorphisms and human trisomies. In 1999, considerable excitement was generated[77] by a report that linked Down syndrome to a maternal

ANTRAL FOLLICLE
Final stage in the growth of the oocyte, when the follicle develops a fluid-filled cavity — the antrum.

PERIOVULATORY FOLLICLE
The follicle around the time of ovulation; at this stage, the oocyte, which has been suspended in prophase, will resume and complete meiosis I in response to the preovulatory surge of gonadotrophins ('LH surge').

polymorphism for an enzyme involved in FOLIC ACID metabolism. Specifically, James *et al.*[78] studied the frequency of a commonly occurring point mutation in methylenetetrahydrofolate reductase (MTHFR) in mothers of Down syndrome individuals and age-matched controls. The mutation leads to reduced enzyme activity in heterozygotes and mutant homozygotes; it affects both folate metabolism and cellular methylation reactions, and is a known risk factor for neural tube defects[79]. James *et al.*[78] proposed that aberrant methylation as a result of the mutation might increase the likelihood of meiotic non-disjunction, thus making the mutation a risk factor for Down syndrome as well as for neural tube defects. Their analyses fit this idea, as they identified a highly significant increase in the proportion of heterozygotes and mutant homozygotes among mothers of Down syndrome individuals.

In a subsequent report[80], James and co-workers expanded their study population and analysed maternal polymorphisms at a second gene in the folate pathway, methionine synthase reductase (MTRR); a commonly occurring point mutation in MTRR has been linked to an increase in spina bifida[81]. They observed a highly significant increase in mutant homozygotes at MTRR among Down syndrome mothers and confirmed the initial observations of a link between MTHFR and Down syndrome. Furthermore, the combined presence of both mutations seemed to increase the risk, as the highest odds ratios were observed among women carrying 'susceptible' genotypes at both MTHFR and MTRR.

These observations are provocative, for two reasons. First, the magnitude of the effect is remarkable, given the relatively small number of cases and controls examined, and implies an important role of MTHFR and MTRR variants in the genesis of Down syndrome. Second, the results indicate the possibility of relatively straightforward preventative measures, because dietary folate supplementation might be expected to overcome the risk of non-disjunction associated with the susceptible genotypes. Indeed, some suppliers of vitamins are making this inference in their advertisements.

So, the confirmation of a link between folate metabolism variants and Down syndrome would represent an important milestone in human aneuploidy research. Unfortunately, however, recent studies indicate that this link might be less important than originally thought, or missing altogether. That is, Petersen *et al.*[82] were unable to demonstrate an increase in MTHFR mutations in mothers of Down syndrome individuals by comparison with controls. Furthermore, in studies of over 200 trisomies that involve other chromosomes (that is, cases of trisomies 2, 7, 10, 13, 14, 15, 16, 18, 22, and sex-chromosome trisomies), there were no obvious increases in MTHFR or MTRR mutations in case-mothers by comparison with controls (T. Hassold, P. Jacobs and N. Thomas, unpublished data). So, there is no evidence that maternal folate polymorphisms alter the risk of non-disjunction for chromosomes other than 21, and the evidence for trisomy 21 is now equivocal. Nevertheless, the importance of the effect, if confirmed, and the media attention that the observations have drawn, make it essential that additional analyses be conducted to clarify the situation.

Maternal smoking and Down syndrome. There has been persistent conjecture that maternal smoking might be a risk factor for Down syndrome, but the results have been contradictory[83]. Recently, Sherman and co-workers re-investigated this possibility, using a new approach: they combined a questionnaire-based case–control study of putative risk factors with a molecular analysis of the origin of the extra chromosome 21. The initial results on 244 trisomy-21 liveborns and 297 control liveborns are intriguing[84]. When all maternally derived trisomies were combined, there was no significant association between maternal smoking at the time of conception and the risk of non-disjunction of chromosome 21. However, when cases were divided by meiotic stage of origin (MI or MII) and by maternal age (<35 or ≥35 years), a significant association emerged, with the effect being confined to MII cases that involve younger women. Furthermore, in an examination of the possible interaction of smoking and oral contraceptive use around the time of conception, there was a significantly increased odds ratio compared with that for smoking alone. These results are clearly preliminary and need to be confirmed on a more extensive series of cases. Nevertheless, they provide optimism that, by recognizing the heterogeneous nature of non-disjunction, it finally might be possible to identify agents that contribute to human trisomies.

Conclusion

The past decade has witnessed important advances in our understanding of human aneuploidy. We have characterized the parental and meiotic origins of the most important aneuploid conditions and have identified the first molecular correlate of human non-disjunction, that is, alterations in meiotic recombination. However, an understanding of the molecular mechanisms responsible for meiotic non-disjunction remains elusive, and we are still ignorant of the basis of the maternal-age effect on trisomy. New approaches to the study of meiotic chromosome segregation — including the development of appropriate mammalian model systems, and the application of new molecular and cytogenetic methodology (FIG. 5) to the study of human gametes — will be essential if we are to gain an understanding of the genesis of this most common class of human genetic disorder.

FOLIC ACID
One of the B-vitamins; folic acid is essential for cellular methylation reactions and for *de novo* synthesis of nucleotide precursors in DNA synthesis.

Links

DATABASE LINKS trisomy 21 | *nod* | *Axs* | *Dub* | *ncd* | Klinefelter syndrome | MTHFR | MTRR | SCP3 | MLH1
FURTHER INFORMATION Terry Hassold's lab | Patricia Hunt's lab

1. Sears, D. D., Hegemann, J. H. & Hieter, P. Meiotic recombination and segregation of human-derived artificial chromosomes in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA* **89**, 5296–5300 (1992).
2. Koehler, K. E., Hawley, R. S., Sherman, S. & Hassold, T. Recombination and nondisjunction in humans and flies. *Hum. Mol. Genet.* **5**, 1495–1504 (1996).
3. Sandler, L. in *Trisomy 21* (*Down Syndrome*): *Research Perspectives* (eds de la Cruz, F. & Gerald, P.) 181–197 (Academic, New York, 1981).
4. Bond, D. & Chandley, A. in *Aneuploidy* 86–91 (Oxford Univ. Press, Oxford, 1983).
5. Smith, K. N. & Nicolas, A. Recombination at work for meiosis. *Curr. Opin. Genet. Dev.* **8**, 200–211 (1998).
6. Hassold, T. *et al.* Human aneuploidy: incidence, origin, and etiology. *Environ. Mol. Mutagen.* **28**, 167–175 (1996).
7. Warburton, D. & Fraser, C. Spontaneous abortion risks in man: data from reproductive histories collected in a medical genetics unit. *Am. J. Hum. Genet.* **16**, 1–27 (1964).
8. Angell, R. R., Sandison, A. & Bain, A. D. Chromosome variation in perinatal mortality: a survey of 500 cases. *J. Med. Genet.* **21**, 39–44 (1984).
9. Jamieson, M. E., Coutts, J. R. & Connor, J. M. The chromosome constitution of human preimplantation embryos fertilized *in vitro*. *Hum. Reprod.* **9**, 709–715 (1994).
10. Delhanty, J. D. Chromosome analysis by FISH in human preimplantation genetics. *Hum. Reprod.* **12**, 153–155 (1997).
11. Hassold, T. J. Nondisjunction in the human male. *Curr. Top. Dev. Biol.* **37**, 383–406 (1998).
12. Jacobs, P. A. The chromosome complement of human gametes. *Oxf. Rev. Reprod. Biol.* **14**, 47–72 (1992).
13. Marquez, C., Cohen, J. & Munne, S. Chromosome identification in human oocytes and polar bodies by spectral karyotyping. *Cytogenet. Cell Genet.* **81**, 254–258 (1998).
14. Volarcik, K. *et al.* The meiotic competence of *in-vitro* matured human oocytes is influenced by donor age: evidence that folliculogenesis is compromised in the reproductively aged ovary. *Hum. Reprod.* **13**, 154–160 (1998).
15. Jacobs, P. *et al.* Turner syndrome: a cytogenetic and molecular study. *Ann. Hum. Genet.* **61**, 471–483 (1997).
16. Bugge, M. *et al.* Non-disjunction of chromosome 18. *Hum. Mol. Genet.* **7**, 661–669 (1998).
17. Hassold, T., Merrill, M., Adkins, K., Freeman, S. & Sherman, S. Recombination and maternal age-dependent nondisjunction: molecular studies of trisomy 16. *Am. J. Hum. Genet.* **57**, 867–874 (1995).
18. Lamb, N. E. *et al.* Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. *Hum. Mol. Genet.* **6**, 1391–1399 (1997).
19. Lamb, N. E. *et al.* Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. *Nature Genet.* **14**, 400–405 (1996).
Study that links errors scored as arising at meiosis II, as well as those originating at meiosis I, to the genesis of trisomy 21.
20. Robinson, W. P. *et al.* Nondisjunction of chromosome 15: origin and recombination. *Am. J. Hum. Genet.* **53**, 740–751 (1993).
21. MacDonald, M. *et al.* The origin of 47,XXY and 47,XXX aneuploidy: heterogeneous mechanisms and role of aberrant recombination. *Hum. Mol. Genet.* **3**, 1365–1371 (1994).
22. Zaragoza, M. V. *et al.* Nondisjunction of human acrocentric chromosomes: studies of 432 trisomic fetuses and liveborns. *Hum. Genet.* **94**, 411–417 (1994).
23. Zaragoza, M. V., Millie, E., Redline, R. W. & Hassold, T. J. Studies of non-disjunction in trisomies 2, 7, 15, and 22: does the parental origin of trisomy influence placental morphology? *J. Med. Genet.* **35**, 924–931 (1998).
24. Roeder, G. S. Meiotic chromosomes: it takes two to tango. *Genes Dev.* **11**, 2600–2621 (1997).
25. Ross, L. O., Maxfield, R. & Dawson, D. Exchanges are not equally able to enhance meiotic chromosome segregation in yeast. *Proc. Natl Acad. Sci. USA* **93**, 4979–4983 (1996).
26. Sears, D. D., Hegemann, J. H., Shero, J. H. & Hieter, P. *Cis*-acting determinants affecting centromere function, sister-chromatid cohesion and reciprocal recombination during meiosis in *Saccharomyces cerevisiae*. *Genetics* **139**, 1159–1173 (1995).
27. Koehler, K. E. *et al.* Spontaneous X chromosome MI and MII nondisjunction events in *Drosophila melanogaster* oocytes have different recombinational histories. *Nature Genet.* **14**, 406–414 (1996).
Comprehensive analysis of X-chromosome non-disjunction in female flies, demonstrating an association between recombination and non-disjunction that is remarkably similar to that observed in humans.
28. Rasooly, R. S., New, C. M., Zhang, P., Hawley, R. S. & Baker, B. S. The lethal(1)TW-6cs mutation of *Drosophila melanogaster* is a dominant antimorphic allele of *nod* and is associated with a single base change in the putative ATP-binding domain. *Genetics* **129**, 409–422 (1991).
29. Moore, D. P., Miyazaki, W. Y., Tomkiel, J. E. & Orr-Weaver, T. L. Double or nothing: a *Drosophila* mutation affecting meiotic chromosome segregation in both females and males. *Genetics* **136**, 953–964 (1994).
30. Krawchuk, M. D. & Wahls, W. P. Centromere mapping functions for aneuploid meiotic products: analysis of rec8, rec10 and rec11 mutants of the fission yeast *Schizosaccharomyces pombe*. *Genetics* **153**, 49–55 (1999).
31. Zetka, M. C. & Rose, A. M. Mutant *rec-1* eliminates the meiotic pattern of crossing over in *Caenorhabditis elegans*. *Genetics* **141**, 1339–1349 (1995).
32. Chakravarti, A. *et al. Gene-Centromere Mapping and the Study of Non-Disjunction in Autosomal Trisomies and Ovarian Teratomas* (eds Hassold, T. & Epstein, C.) (Alan R. Liss, New York City, 1989).
33. Savage, A. R. *et al.* Elucidating the mechanisms of paternal non-disjunction of chromosome 21 in humans. *Hum. Mol. Genet.* **7**, 1221–1227 (1998).
34. Hassold, T. J., Sherman, S. L., Pettay, D., Page, D. C. & Jacobs, P. A. XY chromosome nondisjunction in man is associated with diminished recombination in the pseudoautosomal region. *Am. J. Hum. Genet.* **49**, 253–260 (1991).
35. Thomas, N. S., Collins, A. R., Hassold, T. J. & Jacobs, P. A. A reinvestigation of non-disjunction resulting in 47,XXY males of paternal origin. *Eur. J. Hum. Genet.* **8**, 805–808 (2000).
36. Robinson, W. P. *et al.* Maternal meiosis I non-disjunction of chromosome 15: dependence of the maternal age effect on level of recombination. *Hum. Mol. Genet.* **7**, 1011–1019 (1998).
37. Thomas, N. *et al.* The origin of maternal sex chromosome non-disjunction. *Hum. Mol. Genet.* **10**, 243–250 (2001).
38. Angell, R. R. Aneuploidy in older women. Higher rates of aneuploidy in oocytes from older women. *Hum. Reprod.* **9**, 199–200 (1994).
39. Angell, R. R. Predivision in human oocytes at meiosis I: a mechanism for trisomy formation in man. *Hum. Genet.* **86**, 383–387 (1991).
40. Angell, R. First-meiotic-division nondisjunction in human oocytes. *Am. J. Hum. Genet.* **61**, 23–32 (1997).
41. Angell, R. R., Xian, J. & Keith, J. Chromosome anomalies in human oocytes in relation to age. *Hum. Reprod.* **8**, 1047–1054 (1993).
42. Wolstenholme, J. & Angell, R. R. Maternal age and trisomy — a unifying mechanism of formation. *Chromosoma* **109**, 435–438 (2000).
This paper describes a model linking maternal-age-dependent trisomy and premature separation of sister chromatids at meiosis I.
43. Mahmood, R., Brierley, C. H., Faed, M. J., Mills, J. A. & Delhanty, J. D. Mechanisms of maternal aneuploidy: FISH analysis of oocytes and polar bodies in patients undergoing assisted conception. *Hum. Genet.* **106**, 620–626 (2000).
44. Dailey, T., Dale, B., Cohen, J. & Munne, S. Association between nondisjunction and maternal age in meiosis-II human oocytes. *Am. J. Hum. Genet.* **59**, 176–184 (1996).
45. Penrose, L. The relative effects of paternal and maternal age in mongolism. *J. Genet.* **27**, 219–224 (1933).
46. Morton, N. E., Jacobs, P. A., Hassold, T. & Wu, D. Maternal age in trisomy. *Ann. Hum. Genet.* **52**, 227–235 (1988).
47. Risch, N., Stein, Z., Kline, J. & Warburton, D. The relationship between maternal age and chromosome size in autosomal trisomy. *Am. J. Hum. Genet.* **39**, 68–78 (1986).
48. Ayme, S. & Lippman-Hand, A. Maternal-age effect in aneuploidy: does altered embryonic selection play a role? *Am. J. Hum. Genet.* **34**, 558–565 (1982).
49. Kline, J., Kinney, A., Levin, B. & Warburton, D. Trisomic pregnancy and earlier age at menopause. *Am. J. Hum. Genet.* **67**, 395–404 (2000).
50. Freeman, S. B., Yang, Q., Allran, K., Taft, L. F. & Sherman, S. L. Women with a reduced ovarian complement may have an increased risk for a child with Down syndrome. *Am. J. Hum. Genet.* **66**, 1680–1683 (2000).
51. Zheng, C. J. & Byers, B. Oocyte selection: a new model for the maternal-age dependence of Down syndrome. *Hum. Genet.* **90**, 1–6 (1992).
52. Henderson, S. A. & Edwards, R. G. Chiasma frequency and maternal age in mammals. *Nature* **217**, 22–28 (1968).
53. Hawley, R. S., Frazier, J. A. & Rasooly, R. Separation anxiety: the etiology of nondisjunction in flies and people. *Hum. Mol. Genet.* **3**, 1521–1528 (1994).
54. Warburton, D. The effect of maternal age on the frequency of trisomy: change in meiosis or *in utero* selection? *Prog. Clin. Biol. Res.* **311**, 165–181 (1989).
Thoughtful review of possible mechanisms leading to the generation of the maternal-age effect, with a description of a 'limited oocyte pool' hypothesis to explain it.
55. Hassold, T., Sherman, S. & Hunt, P. Counting cross-overs: characterizing meiotic recombination in mammals. *Hum. Mol. Genet.* **9**, 2409–2419 (2000).
56. Battaglia, D., Goodwin, P. & Klein, N. Influence of maternal age on meiotic spindle assembly in oocytes from naturally cycling women. *Hum. Reprod.* **11**, 2217–2222 (1996).
Study that demonstrates an effect of increasing maternal age on meiotic spindle morphology.
57. Shonn, M. A., McCarroll, R. & Murray, A. W. Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. *Science* **289**, 300–303 (2000).
Study demonstrating that in yeast, spindle-checkpoint mutations preferentially affect meiosis I chromosome segregation, and indicating that checkpoint defects might contribute to Down syndrome.
58. Yuan, L. *et al.* The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. *Mol. Cell* **5**, 73–83 (2000).
Generation and phenotypic analysis of mice in which a component of the lateral element of the synaptonemal complex has been knocked out.
59. Uchida, I. & Curtis, E. A possible association between maternal radiation and mongolism. *Lancet* **2**, 848–850 (1961).
60. Harlap, S. *et al.* Chromosome abnormalities in oral contraceptive breakthrough pregnancies. *Lancet* **1**, 1342–1343 (1979).
61. Torfs, C. P., van den Berg, B. J., Oechsli, F. W. & Christianson, R. E. Thyroid antibodies as a risk factor for Down syndrome and other trisomies. *Am. J. Hum. Genet.* **47**, 727–734 (1990).
62. Kaufman, M. H. The teratogenic effects of alcohol following exposure during pregnancy, and its influence on the chromosome constitution of the pre-ovulatory egg. *Alcohol Alcohol.* **32**, 113–128 (1997).
63. Jongbloet, P. H., Mulder, A. & Hamers, A. J. Seasonality of pre-ovulatory non-disjunction and the aetiology of Down syndrome. A European collaborative study. *Hum. Genet.* **62**, 134–138 (1982).
64. Schimmel, M. S., Eidelman, A. I., Zadka, P., Kornbluth, E. & Hammerman, C. Increased parity and risk of trisomy 21: review of 37,110 live births. *Br. Med. J.* **314**, 720–721 (1997).
65. Narchi, H. & Kulaylat, N. High incidence of Down's syndrome in infants of diabetic mothers. *Arch. Dis. Child.* **77**, 242–244 (1997).
66. Alfi, O. S., Chang, R. & Azen, S. P. Evidence for genetic control of nondisjunction in man. *Am. J. Hum. Genet.* **32**, 477–483 (1980).
67. Avramopoulos, D., Mikkelsen, M., Vassilopoulos, D., Grigoriadou, M. & Petersen, M. B. Apolipoprotein E allele distribution in parents of Down's syndrome children. *Lancet* **347**, 862–865 (1996).
68. Jackson-Cook, C. K., Flannery, D. B., Corey, L. A., Nance, W. E. & Brown, J. A. Nucleolar organizer region variants as a risk factor for Down syndrome. *Am. J. Hum. Genet.* **37**, 1049–1061 (1985).
69. Verger, P. Down syndrome and ionizing radiation. *Hlth Phys.* **73**, 882–893 (1997).
70. Hassold, T. J. & Jacobs, P. A. Trisomy in man. *Annu. Rev. Genet.* **18**, 69–97 (1984).
71. Sayee, R. & Thomas, I. M. Consanguinity, non-disjunction, parental age and Down's syndrome. *J. Indian Med. Assoc.* **96**, 335–337 (1998).

72. Morris, J. K., Alberman, E. & Mutton, D. Is there evidence of clustering in Down syndrome? *Int. J. Epidemiol.* **27**, 495–498 (1998).
73. Pelz, J. & Kunze, J. Down's syndrome in infants of diabetic mothers. *Arch. Dis. Child.* **79**, 199–200 (1998).
74. Stolwijk, A. M., Jongbloet, P. H., Zielhuis, G. A. & Gabreels, F. J. Seasonal variation in the prevalence of Down syndrome at birth: a review. *J. Epidemiol. Commun. Hlth* **51**, 350–353 (1997).
75. Ezquerra, M. *et al.* Apolipoprotein E ε4 alleles and meiotic origin of non- disjunction in Down syndrome children and in their corresponding fathers and mothers. *Neurosci. Lett.* **248**, 1–4 (1998).
76. Hassold, T., Jacobs, P. A. & Pettay, D. Analysis of nucleolar organizing regions in parents of trisomic spontaneous abortions. *Hum. Genet.* **76**, 381–384 (1987).
77. Rubin, R. Folic acid might reduce risk of Down syndrome. *USA Today* **Sept 29**, D1 (1999).
78. James, S. J. *et al.* Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. *Am. J. Clin. Nutr.* **70**, 495–501 (1999).
79. van der Put, N. M. *et al.* A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am. J. Hum. Genet.* **62**, 1044–1051 (1998).
80. Hobbs, C. A. *et al.* Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. *Am. J. Hum. Genet.* **67**, 623–630 (2000).
81. Wilson, A. *et al.* A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk for spina bifida. *Mol. Genet. Metab.* **67**, 317–323 (1999).
82. Petersen, M., Grigoriadou, M. & Mikkelsen, M. A common mutation in the methylenetetrahydrofolate reductase gene is not a risk factor for Down syndrome. *Am. J. Hum. Genet.* **67**, S141 (2000).
83. Chen, C. L., Gilbert, T. J. & Daling, J. R. Maternal smoking and Down syndrome: the confounding effect of maternal age. *Am. J. Epidemiol.* **149**, 442–446 (1999).
84. Yang, Q. *et al.* Risk factors for trisomy: maternal cigarette smoking and oral contraceptive use in a population-based case-control study. *Genet. Med.* **1**, 80–88 (1999).
Combined epidemiological and molecular analysis of trisomy 21, in which potential aetiological agents can be linked to the parent and meiotic stage of origin of the extra chromosome.
85. Hassold, T. & Chiu, D. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. *Hum. Genet.* **70**, 11–17 (1985).

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