Review article

On the parental origin of de novo mutation in man

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Abstract

Studies tracing parental origins of human mutations by means of cytogenetic polymorphisms and RFLPs show that most trisomics arise out of maternal errors of segregation at the first meiotic division in oocytes. Temporal disturbance of meiotic progression seems likely to underly aneuploidy production in the female mouse, and this could equally be true in women, most especially as they approach the menopause when irregular cyclicity sets in. For human monosomy X, a high proportion of cases show loss of the paternal sex chromosome, and from experimental data giving similar findings in the mouse, it seems likely that the error could arise at the pronuclear stage after sperm entry into the egg, rather than at meiosis in the male. For human point mutations and structural rearrangements, a bias exists towards paternal origins. Errors arising during spermatogonial proliferation in men could contribute point mutations, these accumulating over a lifetime to give paternal age effects. For structural rearrangements, the hypersensitive stage is likely to be the post-meiotic differentiating spermatid, a stage not subject to germinal selection, and one which in *Drosophila* has been shown to combine high breakability with enhanced repair. Lack of a comparable cell type to the condensing spermatid of the male might be a reason why balanced structural rearrangements are produced rather rarely in females, at least in the mouse.

Ever since the beginning of human genetic and cytogenetic investigation, studies have been made into tracing the parental origins of chromosome anomalies.

Early studies, based on the inheritance of X linked red-green colour blindness,¹ showed that maternal non-disjunction played a part in some cases of Klinefelter's syndrome (47,XXY), and indicated the maternal origin of the single X chromosome in Turner's syndrome (45,X).² Following the discovery of the X linked gene Xg, Race and Sanger³ showed that for the 47,XXY condition, the extra X was paternal (X^mX^pY) in 33% of cases and maternal (X^mX^mY) in 67%, while for the 45,X condition 77% of cases showed retention of the maternal X chromosome (X^mO).⁴

The extra chromosome in autosomal aneuploid conditions like Down's syndrome (trisomy 21) could not be investigated cytologically for some time, but, nevertheless, it was assumed even in the early days that at least some of the cases originated from malsegregation in oogenesis because of the well known maternal age dependency of the condition. It was after the introduction of banding techniques⁵ that the breakthrough really came, with a rapid realisation that cytogenetic heteromorphisms could be used in tracing the origins of aneuploids and polyploids, and a wealth of data accumulated.^{6,7} Studies into the parental origins of structural rearrangements were also carried out.⁸ Now, in the era of molecular investigation, with RFLPs having been identified on all human chromosomes, the way has been opened up for investigation into the origin of an infinite variety of mutations arising de novo, both in the germline and somatically, and some interesting facts are emerging. It appears that while for most aneuploids, there is a bias towards maternal origins, point mutations and structural rearrangements seem to arise de novo much more commonly in males. Much current debate is focused on this issue and questions regarding whether 'imprinting' might play some role or whether, for example, extra environmental exposure of men could be important, have been raised in various publications. No clear answers, however, have yet emerged.

In this review, I would like to focus on data which have been obtained in other species, notably *Drosophila*

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and the mouse, which I believe can, by extrapolation, give valuable clues to the possible times and mechanisms of origin for some de novo mutations in man. For aneuploid conditions, hard data exist in the mouse which can be extrapolated to the situation of human chromosome loss and non-disjunction, while for point mutation and structural rearrangement, an abundance of data is available relating both to the mouse and *Drosophila*, from mutagenesis studies carried out in the 1950s and 1960s, which may provide clues to times of origin and to the possible reasons for the extra sensitivity of male germ cells over female. It is first necessary, however, to provide a general outline of gametogenesis in the human male and female for readers who may be less familiar with the important stages in germ cell development and to which reference can be made in later sections of this review.

Spermatogenesis

Spermatogenesis is an intricate and involved process requiring, from puberty onwards, a continuous production of spermatozoa by the seminiferous tubules, production which, in quantitative terms, is astronomical over the life span of a person. In man, it has been estimated that 64 days are required for spermatogenesis to be completed in the testis,⁹ a further 10 to 11 days being required for passage of spermatozoa through the epididymis and vas deferens into the ejaculate.

At puberty in the male, gonocytes give rise to spermatogonia which then divide mitotically several times before becoming primary spermatocytes. According to the morphological criteria of Clermont,¹⁰ dark type A (Ad) spermatogonia are stem cells which divide to produce new Ad cells and pale type A (Ap) cells. Ap cells divide to form type B spermatogonia which then differentiate into preleptotene primary spermatocytes (fig 1). As these pass through the

prophase of meiosis, progressive swelling occurs, the nucleus assuming the characteristic configurations of leptotene, zygotene, pachytene, diplotene, and diakinesis. Completion of the first meiotic division produces secondary spermatocytes which divide again to produce haploid spermatids (fig 2). Newly formed spermatids have a small spherical nucleus but, as spermiogenesis proceeds, they pass through an extremely complex series of morphological changes during which the nuclear chromatin condenses into an elongated fusiform body covered by the acrosome (fig 2). Spermatozoa from the testis are not functionally mature; they acquire motility and fertilising capacity during passage through the epididymis. Many details of the process of spermatogonial renewal in man remain still debatable, such as there being only one generation in each class of spermatogonia, spermatogonia of each type dividing once and only once during each cycle of the seminiferous epithelium and not reverting back to the stem cell condition once embarked on their course of differentiation, and the dark type A actually being the stem cell type.¹⁰

Oogenesis

The formation, development, and maturation of the female gamete begins in embryonic life and continues to the time of ovulation. During fetal development there is a period of oogonial division when numbers increase very rapidly; during germ cell migration there are 1700 cells; during the second month of pregnancy 600 000, and at the fifth month 7 million.¹¹ After a finite number of mitoses, the oogonia become transformed into oocytes which then enter the prophase of the first meiotic division, from this time on oocytes being incapable of increasing their numbers and, hence, the population of germ cells only reducing with age. This occurs by the process of atresia and by ovulation. This is in strong contrast to the situation in the male where mitotically active

Figure 1 Model illustrating the development and renewal of spermatogonia in man. Ad, Ap, B, respectively, are dark type A, pale type A, and type B spermatogonia. Pl, preleptotene spermatocytes (from reference 10, with permission).

Figure 2 The steps of spermatogenesis in man. Ad, dark type A spermatogonia; Ap, pale type A spermatogonium; B, type B spermatogonium; PI, preleptotene primary spermatocyte; L, leptotene; Z, zygotene; EP, early pachytene; MP, midpachytene; LP, late pachytene; II, secondary spermatocyte. Spermatids are shown at various stages of spermiogenesis. Spermatozoa are illustrated in lateral (left) and frontal (right) aspects (from reference 10, with permission).

spermatogonia persist basally in the spermatogenic tubules, and spermatocytes pass through the phases of meiosis continuously throughout adult life. By the time of birth, the human ovary contains about 2 million oocytes in the diplotene stage of meiotic prophase when they embark on a prolonged resting phase or 'dictyate' stage. During each reproductive cycle, a crop of growing follicles is stimulated to undergo further growth and maturation, becoming Graafian follicles, which, after an LH surge, pass through a final phase of maturation before ovulation; meiosis is resumed, the egg enters diakinesis (germinal vesicle stage), and metaphase I (MI) ensues rapidly. Ovulation occurs at MII, the whole process from the LH surge to ovulation taking about 36 hours in women.¹¹ The earliest oocyte to resume meiosis does so at the time of puberty and the last may be found in women in their fifties. At the menopause, few oocytes can be detected in histological sections of ovaries.

Types of mutation

Mutations can be conveniently classified into three main types: (1) numerical anomalies, (2) gene or point mutations, and (3) structural chromosome rearrangements.

Point mutations are generally considered not to be visible microscopically, while structural chromosome rearrangements usually are. Nowadays, however, the distinction can become blurred by borderline cases of minor deletion which may only be detectable by molecular analysis and would escape detection by cytological examination. Indeed, it is possible that many point mutations may in reality be small deletions.

Numerical anomalies

AUTOSOMAL ANEUPLOIDS

For trisomy 13,^{12 13} trisomy 18,¹⁴ and trisomy 21^{12 15} a clear bias towards a maternal non-disjunction (ND) event has been found, more than 80% of errors arising in the oocyte, the majority at the first division. Data for trisomies 3, 4, 9, 14, 15, 16, and 22, though more limited, are consistent with this.¹² For most autosomal trisomic conditions, a maternal age effect has been shown, the magnitude varying among individual chromosome pairs with the smallest pairs appearing to show the strongest association.^{12 16}

SEX CHROMOSOME ANEUPLOIDS

For the triple X condition, more than 90% of ND

errors arise maternally, again mostly at the first meiotic division.¹⁷ A maternal age effect is also found. For the XXY condition in men, Jacobs *et al.*,¹⁸ using RFLPs, have shown that the ratio of maternal to paternal errors is about 47% to 53%, slightly but not significantly different from the original estimates of 67% to 33% based on Xg typing.³ Interestingly, but as expected, increased maternal age was found in association only with maternal ND errors (X^mX^mY). RFLP analyses on aborted fetuses and women with Turner's syndrome¹⁹ extend but confirm the original Xg results in showing that some 80% of cases retain a maternal X chromosome (X^mO). The X^pO cases show an association with reduced maternal age, suggesting a meiotic loss or ND mechanism which occurs more frequently in young women.

MECHANISMS OF ORIGIN IN HUMAN MONOSOMY AND TRISOMY

In spite of numerous hypotheses²⁰ to explain the preferential occurrence of non-disjunction during the first meiotic division for autosomal aneuploids as well as the triple X and XXY conditions, and to explain the maternal age effect in women, hard data are difficult to come by. Nevertheless, recent RFLP studies indicate that 'non-conjunction', that is, reduced or absent pairing or recombination or both could be important in the genesis of trisomy 21.^{20a} Studies in *Drosophila* show that asynapsis of pericentromeric heterochromatin, but not complete asynapsis of homologues, is the necessary prerequisite of chromosomal non-disjunction for the X chromosomes and the chromosome 2 pair in females.²¹ Moreover, asynapsis arising in the pericentromeric region can extend distally, causing progressive reductions in chiasma frequency along euchromatic arms. Chromosomes undergoing non-disjunction therefore may show absent or reduced crossing over depending on the extent of asynapsis.²¹ Further probe data will be required to ascertain whether this holds true in man also.

For the female mouse, a shortening in the meiotic prophase appears to be a main predisposing factor in age related aneuploidy, the time available for attachment and alignment of chromosomes on the spindle before anaphase transition being found to be less in old oocytes.²² Changes in hormonal feedback mechanisms at the end of the reproductive lifespan might be causal to these changes in meiotic timing and progression. The first meiotic spindle itself seems not to be abnormal in old mouse oocytes. If meiotic timing is critical, errors in young oocytes leading to aneuploidy might also arise sporadically owing to temporal disturbance of meiotic progression, such errors becoming increasingly common as women reach the end of their reproductive lifespan when menstrual cycle length irregularities set in.

For monosomy X in man, while not ruling out meiotic losses entirely, another time when a paternal sex chromosome might be lost is at the pronuclear stage after sperm entry into the egg. Studies in the mouse²³ have shown that many X^mO mice arise, both spontaneously and by radiation induction, at this sensitive time, paternal sex chromosome losses occurring about 10 times more frequently by irradiation of the male pronucleus than by irradiation of spermatozoa. A significant number of spontaneous paternal sex chromosome losses in man might also come about in this way. A non-meiotic postfertilisation origin for many human X^mO subjects appears not to have been considered previously in published reports as emphasis has usually been placed on the occurrence of a paternal error, by implication meaning loss of a sex chromosome during meiosis in the male.^{18 19}

Point mutation and structural rearrangement

As pointed out in the introduction, a strong bias exists towards a paternal origin for human de novo structural rearrangements⁸ and for germinal mutations in a growing list of conditions such as retinoblastoma (Rb),²⁴⁻²⁶ Prader-Willi syndrome (PWS),²⁷⁻²⁹ NF-1 von Recklinghausen neurofibromatosis (NF-1),³⁰ Wilms' tumour,³¹ and cri-du-chat syndrome.³²

The belief which is widely held is that a paternal bias exists because most mutations arise by 'copy error' at the time of DNA synthesis, males showing lifelong mitotic proliferation from spermatogonial stem cells, while oocytes in adult females are finite in number and arrested at the dictyate stage.^{8 24 33} Penrose³⁴ appears first to have made the 'copy error' suggestion when attempting to explain the strong paternal age effect found in association with achondroplasia and some other dominant disorders, yet paternal age effects have not been shown for diseases like PWS, Rb, NF-1, WT, or for structural rearrangements of paternal origin.

The process of spermatogenesis in man closely resembles that found in *Drosophila* and the mouse, two species for which many publications exist concerning the responsiveness of the male germline to the action of radiation and chemical mutagens. These classical studies showed that while point mutations (sometimes in clusters) were recoverable from all stages including spermatogonia, large lesion mutations and structural chromosome changes were mainly the products of treated meiotic and postmeiotic stages.³⁵⁻⁴⁰ A lower initial sensitivity to induction, combined with the operation of germinal selection, minimised the recovery of chromosome rearrangements from spermatogonia. In *Drosophila*, primary spermatocytes appeared particularly sensitive to the induction of deletions,³⁷ while early spermatids were found to be the hypersensitive stage for the induction

of translocations.³⁸ For the mouse, the early spermatid has recently been found to be particularly prone to deletion formation using the chemical mutagen chlorambucil,⁴¹ investigations at the DNA level showing some of the lesions to be very large.⁴² Earlier investigations in this species using radiation provided further evidence that only point mutations and perhaps minor deletions were recoverable from treated stem cell spermatogonia, larger deletions being recovered solely from post stem cell stages.³⁹

For translocations in the mouse, Ford *et al.*⁴⁰ have shown that only about a half of the rearrangements induced in spermatogonia are recovered in sperm, one particular class of reciprocal rearrangement being notably absent, that is, the male sterile X;autosome translocation. This is because such rearrangements act autonomously during late prophase to kill the germ cells carrying them, recovery thus being possible only from treated postmeiotic stages.⁴³ The same holds true for certain purely autosomal translocations which are male sterile in the mouse.⁴⁴

In man, it is virtually certain that any paternally derived X;autosome reciprocal translocation (and any purely autosomal translocation which is male sterile) will have originated de novo in a spermatid or spermatozoan. In fact, by extrapolation from the mouse and *Drosophila* findings, it would seem likely that most large lesion mutations and structural changes in man would derive from a post-spermatogonial stage of spermatogenesis, only point mutations and other small intragenic changes being recoverable from spermatogonia. As no barriers exist to the recovery of postmeiotically induced gross rearrangements in spermatozoa, their likelihood of survival and transmission to offspring is far greater than for rearrangements which have arisen premeiotically.

FACTORS INFLUENCING MUTATION INDUCTION IN THE MALE GERMLINE

Point mutations can arise by copy error at S phase when spermatogonia are proliferating mitotically, but are there reasons why meiotic and postmeiotic stages in spermatogenesis might be particularly vulnerable to structural chromosome damage? Chromosome aberrations both in somatic cells and in the germline result from an interaction between two (or more) lesions and therefore require that the cell type shows both breakability and repair capacity. Cytogeneticists group chromosome structural changes that can be induced in somatic cells into convenient categories, each one of which might also arise de novo in the germline. The classification includes 'interchanges' leading to reciprocal translocation, 'inter-arm intrachanges' leading to ring chromosomes and pericentric inversions, and 'intra-arm intrachanges', which can produce paracentric inversions and interstitial deletions.⁴⁵ Spatial arrangement of the chromatin in

the nucleus could be one very important factor determining the type of rearrangement produced. For example, Ockey,⁴⁶ using chemically treated root tip chromosomes of *Vicia faba*, showed that while the condensed nuclei of early prophase tended to give a preponderance of *interchanges*, the large late prophase nucleus, when treated, gave mainly *intrachanges*. During spermatogenesis, very extreme size changes and spatial arrangements of chromosomes within nuclei at different stages can be found, the small early prophase nucleus increasing enormously through pachytene, diplotene, and diakinesis, and then diminishing dramatically as early round spermatids condense down into spermatozoa. The large primary spermatocyte nucleus, when bivalents are widely separated from each other, was considered by Chandley and Bateman³⁷ to be a cell stage which favoured *intrachange*, deletions and duplications perhaps arising at this time by unequal crossing over after illegitimate pairing.⁴⁷ On the other hand, the high breakability and enhanced repair capacity in spermatids, combined with large scale chromatin movements which occur during condensation and metamorphosis into the sperm head, were considered to make this a stage when structural rearrangement and particularly *interchange*, that is, translocation, would be favoured.^{36 38} Breaks induced in spermatozoa in *Drosophila* are not repaired until after fertilisation, the sperm being a cell stage showing little, if any, repair capacity.^{36 38}

Mutation in the female

Information on radiation induced mutations in female mice and *Drosophila* is far below the level attained for males. To a large extent, at least in the mouse, this is because the acute doses used to induce mutations in males produce sterility in females owing to killing of oocytes. This can nevertheless be avoided if low intensity doses are administered and, in relation to humans, oocytes are anyway far more resistant to killing by x rays than are those of the mouse.⁴⁸ Since oogonia do not exist in the adult ovary, any exposure received by a female will be delivered to dictyate oocytes, and these have been shown in the mouse to be comparable to post-spermatogonial cells in the male in terms of sensitivity to point mutations and deletions,⁴⁹ deletions induced in oocytes and post-spermatogonial stages tending, moreover, to be larger than those induced in spermatogonia. Studies in females do, however, indicate a much lower overall mutation frequency per treatment dose compared with the male, even when the most sensitive maturing oocyte stages are considered and this is likely also to apply in humans.⁴⁸ These early studies reported a zero or near zero level of mutations for immature oocytes arrested at the dictyate stage, the more sensitive time seeming to be the short period before

ovulation when the oocytes are growing and maturing in the Graafian follicle. More recent studies, however, indicate that significant levels of numerical and structural damage can result from irradiation of immature oocytes of the mouse,^{50 51} although yields of translocation heterozygotes recovered among the progeny of irradiated females appear low, indicating that the production of balanced exchanges is rather rare.⁵²

Conclusions and speculation

From the foregoing, a complex picture emerges from the *Drosophila* and mouse data, when the combined effects of initial sensitivity to mutation for individual germ cell stages and selective recovery are taken together. Great differences exist in the processes of oogenesis and spermatogenesis, and this undoubtedly contributes to the parental biases which are encountered in human mutation. Structural rearrangements, for example, which may arise in males owing to the movements and contractions of the genome during spermatid morphogenesis are unlikely to arise in the female, as the oocyte does not undergo a comparable process of contraction. Added to this, as Penrose³⁴ pointed out, active proliferation of spermatogonia throughout adult life in men could produce many point mutations, these accumulating with age to produce the paternal age effects. A lower overall sensitivity of the female to mutation induction compared with that of the male, combined with the fact that many millions of spermatozoa are required for the successful fertilisation of only a single egg, would all contribute to rendering the male more error prone than the female. However, whether genomic imprinting⁵³ can influence the expression of a disease, depending on its parental origin, remains to be determined.

The human genome is becoming increasingly seen to contain 'sites of instability' or 'hot spots' for crossing over, breakage and rearrangement, fragile site location, etc, and from analysis of the breakpoint regions in many deletions and translocations, clues are beginning to emerge which are helping to elucidate the important features of the genome in these particular regions which might render them error prone. Sequence composition, repetition of DNA, openness of chromatin configuration, ability to bind proteins important perhaps in pairing or recombination, and ability to form secondary DNA structure, could all be important factors. Moreover, those same features which determine exchange or rearrangement in meiotic cells could be crucially important in promoting exchange or deletion at specific sites in somatic cells leading to rearrangements in cancer.⁵⁴ Mitotic crossing over, for example, could arise not simply out of chance collisions within the cell, but as an event predetermined within certain regions of

special chromatin arrangement or sequence composition. Such sites would thus be 'premutational' lesions, capable of increasing susceptibility to error. An additional factor influencing chromosomal interchange, and perhaps gene expression, is chromosome order within the nucleus and studies into this particular aspect of human genome organisation have already begun.⁵⁵

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