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Mutation as a Cause of Genetic Disease [and Discussion]

H. J. Evans and R. Worton

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Mutation as a cause of genetic disease

By H. J. Evans
Medical Research Council, Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh EH4 2XU, U.K.

Mutational changes can be conveniently classified into two sorts: those that appear to involve single genes and are generally referred to as gene mutations, and those that involve chromosomal segments containing many genes, or even whole chromosomes, and are referred to as chromosomal mutations. Both of these kinds of mutation occur in germ-cell lineages and contribute substantially to inherited disease, or predisposition to disease, and both also occur in somatic cells and contribute to acquired disease.

The mutation rates for inherited disease ascribed to mutation in a single gene differ for different genes and are age-dependent. Moreover, a single disease entity, such as haemophilia B, may be the result of any one of a number of different alterations within the gene responsible for the disease. The mutation rate for inherited chromosomal mutation is also age-dependent, particularly so in the case of mutations involving alterations in chromosome number. Studies in experimental animals demonstrate that exposure to physical or chemical mutagens results in increasing the incidence of inherited gene and chromosomal mutations. However, such increases have not been unequivocally demonstrated in human populations exposed to known mutagens.

Studies on mutation in human lymphoid or epithelial somatic cells clearly demonstrate an increased frequency in cells taken from people exposed to ionizing radiations or chemical mutagens or in cells exposed *in vitro*. The consequences of such mutations will depend upon their nature and the origins and functions of the cells in which they occur. Of particular importance are mutations influencing cell growth and proliferation, and both gene and chromosomal mutations are implicated as causal factors in the development of human cancers.

Introduction

My topic is a large one, because, of course, all genetic disease can be considered to be the result of mutation. In many instances, the origin of a particular mutation may date back in time measured by hundreds of generations, in others the mutations are of much more recent occurrence. I shall devote most of my attention to mutations of more recent occurrence and confine my comments to some of the types of mutation involved, to certain observations relevant to the mechanisms involved in their induction, and to some of the environmental agents that may be important in the genesis of these mutations. In considering mutational changes that account for, or predispose to, disease in man, there is a natural tendency to concentrate on those deleterious mutations that are transmitted from parents to offspring. Such emphasis is natural and proper in the context of our attempts to circumvent or ameliorate inherited disease, but we should not overlook some of the consequences that may ensue from mutational changes in somatic cells, and I shall therefore devote some of my time to somatic mutations.

I shall begin by drawing a distinction between 'chromosomal mutations', which can be visualized by examining chromosomes under the microscope, and 'gene mutations', which can not. The distinction is descriptionally useful but is functionally crude, for with our use of the powerful techniques of molecular biology it has become even more evident that many of the so-called single-gene, or point, mutations that we define by altered phenotypes are frequently a consequence of large deletions or large rearrangements of DNA sequences within the gene in question. Single-base substitutions that, for instance, characterize many of the well-known inherited haemoglobin variants, therefore represent but one end of a spectrum of possible mutational changes that ultimately present, in phenotypic terms, as single-gene mutations.

How frequent are these mutational changes in the population, and what do we know about the spontaneous rates of mutation?

1. *Spontaneous mutations transmitted via germ cells*

Spontaneous is usually just another word for 'of unknown cause', and such mutations may arise as a consequence of intrinsic factors within the cell – errors in replication, or DNA damage resulting from the action of 'normal' cellular metabolites, such as hyperactive oxygen radicals – or extrinsic environmental factors.

(*a*) *Gene mutations*

A glance at McKusick's catalogue of Mendelian inheritance in man (McKusick 1986) shows us that at present we know of around 3000 or so inherited conditions in man that have a mutational change as their root cause. These, of course, represent mutations whose effects can be detected at birth or later in life. Germ cell mutations that are expressed as lethals in the pre-embryo, or in the early days after implantation, cannot be detected and one might argue might not be especially relevant in the present context. Taking the genome as a whole, however, there have been various estimates of the overall spontaneous rates of mutational change, usually expressed as rates per nucleotide per year or per generation, or as rates per codon per generation. These calculations are often based on the rates of nucleotide substitution in globin genes and attempt to take into account factors such as silent mutations in the third base of a triplet, mutations in other non-coding sequences, the proportion of the genome that consists of coding DNA, etc. By and large, such calculations end up with figures for mutation rates of somewhere between 10^{-9} and 10^{-8} mutations per nucleotide per year (Vogel & Rathenberg, 1975; Salser 1978; Li *et al.* 1985).

These calculations are all very well if we are discussing evolution and debating selectionist versus neutralist contentions, but for our present discussion I consider it more realistic to ask what are the mutation rates that we observe for defined human diseases – realizing of course that different sorts of mutational change can be responsible for the same disease phenotype? Our best answers to this question come from studies on well-diagnosed conditions with high penetrance, with good family data to ensure unambiguously the sporadic nature of each occurrence considered to be of mutational origin. Data have been published for a whole range of different genetic diseases, with the highest rates being of the order of 10^{-4} per gamete per generation for neurofibromatosis, with almost similar rates for Duchenne muscular dystrophy, in other words 1 mutation per 10000 gametes. At the other extreme there are conditions with mutation rates at least as low as 10^{-7} per gamete per generation, and an average figure for human mutation rates is often quoted as *ca.* 10^{-6} per gamete per generation (Vogel &

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Rathenberg, 1975). What is clear from these studies is that there is a wide difference in mutation rates between different genes, the range spanning at least two or three orders of magnitude.

There are several intrinsic factors that will influence mutation rate and contribute to the different rates that we observe for different genes. These may include: (*a*) gene size and architecture; (*b*) gene location in the genome; (*c*) transcriptional activity; (*d*) base composition and sequence arrangement; and (*e*) parental age. On basic principles one would expect that the probability of mutations in a very large gene, such as that responsible for Duchenne muscular dystrophy which is around 2×10^6 base pairs (b.p.) in length, will be greater than that for a small gene of around 10^3 b.p., such as that coding for a neuropeptide. This follows simply because of the large difference in size of target for the action of intrinsic mutagens as well as the overall increased chance of replication errors, which will be proportional to gene size. The general architecture of the gene will also be important, for two genes with similar contents of coding DNA, but very different amounts of non-coding intronic regions, will present very different targets. One might also expect that genes that are transcriptionally active may be at greater risk because of their conformational state and template activity than quiescent sequences, and indeed there is some evidence for differential rates of DNA repair associated with different DNA states within the genome (Lieberman 1982). There are also reasons for expecting that the mutation rate may be influenced by the location of a gene within the genome and by its proximity to particular types of DNA. There is also much evidence for a significant influence of base composition and sequence arrangement, as well as for parental age on mutation frequencies, and I shall consider these two latter factors briefly.

A number of nearest-neighbour sequencing studies have shown that the proportion of CpG in the human genome is very considerably less than that expected on the basis of a random association of bases for DNA of the same overall base composition. In human DNA the fraction of G + C is 0.4, and so one would expect CpG dinucleotide sequences to occur with a frequency of around 0.04. However, the observed frequency is around 0.008 (Bird 1980; Nussinov 1981). Earlier studies on prokaryotes, and in particular the studies of Coulondre *et al.* (1978) on the *Escherichia coli lac* operon, had shown that the presence of CpG results in an abnormally high rate of mutation at that site. Mutational 'hot spots' of this sort arise because there is a relatively high rate of spontaneous deamination of 5-methylcytosine to thymine (Salser 1978), and such a transition in a coding sequence may result in a mutation, e.g. the deamination of C in the triplet CGA, which codes for arginine, results in TGA, which is a nonsense codon. The DNA of all vertebrate species studied is deficient in CpG, and if this were a consequence of a higher mutation rate at CpG than at other dinucleotides, then the observed deficiency of CpG should be matched by the accumulation of the corresponding dinucleotides TpG and CpA, and Bird (1980) has demonstrated that this is indeed the case.

The suggestion that methylated cytosine in CpG is a mutational hot spot in human DNA has been more recently supported by other kinds of data, not least of which is the general observation that there is a very high frequency of restriction enzyme polymorphisms in human DNA when restriction enzymes are used whose recognition sequences contain a CpG dinucleotide (Barker *et al.* 1984). In a recent analysis using restriction enzymes and complementary oligonucleotide probes on DNAs from 83 unselected patients with haemophilia A, Youssoufian *et al.* (1986) detected and characterized six deletions and ten point mutations. Thus, one third of the mutations were deletions, but rather striking was the fact that nine of

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the ten mutations were detected by the enzyme Taq1, which requires the dinucleotide CpG in its recognition sequence. Of these nine at least four were shown to have involved C–T transitions, so there seems little doubt that CpG sites in man are 'hot spots' for spontaneous mutation.

There is considerable, and long-standing evidence that the mutation frequencies for a variety of different human genes causing disease increase with increasing paternal age. The rates of increase differ between different genes, and a summary of a recent analysis from our laboratory on osteogenesis imperfecta is shown in figure 1 (Carothers *et al.* 1986). Whether these increases are a consequence of a diminishing ability to repair mutational damage with increasing age, or are simply a reflection of a longer exposure to environmental mutagens, is an open question.

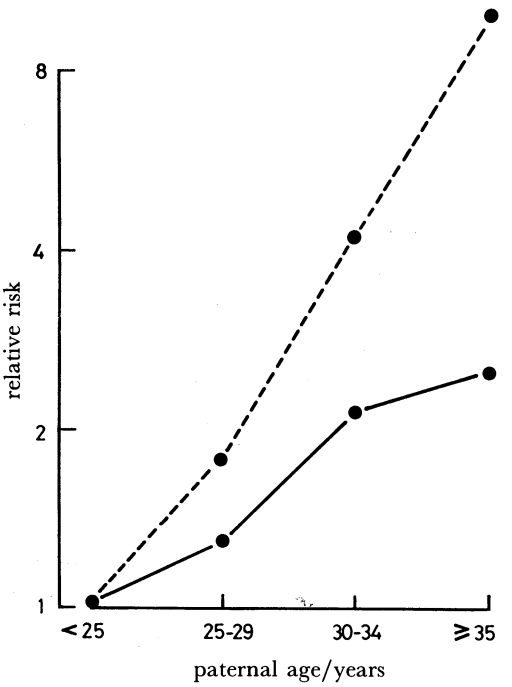

FIGURE 1. Increase of relative risk for mutations for achondroplasia (broken line) and osteogenesis imperfecta (solid line) (from Carothers *et al.* 1986).

(*b*) *Chromosomal mutations*

Chromosomal mutations that can be visualized under the microscope have been intensively studied and are usually considered to fall into two classes: (*a*) those involving alterations in chromosome number, 'numerical changes', and (*b*) those involving changes in chromosome structure, 'structural changes'. In the Edinburgh population a constitutional chromosome anomaly is present in around 1 in every 160 live newborn, i.e. 0.6%, although this figure is decreasing after the introduction of antenatal diagnosis by amniocentesis for mothers 35 or older, with a consequent reduction in the numbers of children born with Down's syndrome (trisomy 21). The vast majority of the inherited chromosome anomalies involve autosomal trisomy or sex chromosome aneuploidy and they are all new mutations. Only a very small proportion are structural anomalies and of these only a fraction are new mutations. Our data on new mutations for balanced and unbalanced rearrangements for example are, respectively 1.94×10^{-4} and 0.46×10^{-4} per gamete per generation, one order of magnitude less than the mutation rate per gamete per generation for numerical anomalies (Evans 1977).

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The chromosome anomalies that we see in the live newborn represent but a small fraction of those that are present in the gametes. In stillborn babies the frequency of chromosome anomalies is around 5%, almost ten times more than the frequency in the live newborn. For spontaneous abortions, the incidence of chromosome anomalies increases dramatically, such that over half of the early abortions are chromosomally abnormal; most of these chromosome anomalies then are lethal mutations (table 1). Estimates of the proportion of recognized pregnancies that abort range from about 15 to 50% (Edmonds *et al.* 1982), so that if approximately 50% of all these abortions were associated with a chromosome anomaly this would imply that the frequencies of chromosomally abnormal gametes in man must be of the order of 3–12%, an extraordinarily high figure.

Table 1. Chromosome anomalies in spontaneous abortions, perinatal death and live newborn (all per 1000 births)

			spontaneous abortions		
anomaly	live births ($n = 43558$)	perinatal deaths ($n = 500$)	< 28 weeks ($n = 941$)	< 18 weeks ($n = 255$)	< 12 weeks ($n = 1498$)
polyploidy	0.13	2.00	53.13	101.96	160.21
autosomal trisomy	1.24	28.00	151.98	250.98	330.44
monosomy X	0.046	2.00	72.26	156.86	93.46
other	4.43	24.00	27.62	39.22	30.70
totals	6.26	56.00	304.99	549.02	614.82

This deduced high rate of chromosomal mutation in human germ cells is underlined by direct studies on human germ-cell chromosomes in sperm, which are made to reveal their chromosomes after penetration of hamster oocytes. Such studies reveal a range of aneuploidy of from 1 to 8% and a frequency of structural rearrangements of from 2 to 13% (table 2). It is possible that some of the structural rearrangements, more than 50% of which are deletions, arise during the 'unnatural' fertilization process, but by far the majority are chromosome-type changes with must have arisen during gametogenesis in the male.

Table 2. Chromosomal mutations detected in human sperm complements visualized after *in vitro* fertilization with hamster oocytes

no. sperm analysed	normal (%)	aneuploid (%)	structural anomaly (%)	reference
60	95.0	5.0	1.7	Rudak *et al.* (1978)
240	90.8	7.5	2.9	Martin *et al.* (1982)
1000	91.5	5.2	4.0	Martin *et al.* (1983)
2468	90.8	1.7	7.7	Brandriff *et al.* (1985)
1091	86.2	0.9	13.0	Kamiguchi & Mikano (1986)
246	96.0	1.0	3.0	R. R. Angell (unpublished)

There are no direct data on the frequencies of chromosome anomalies in human female germ cells, but there are two reports on the chromosome constitutions in fertilized eggs and in early blastomeres from human eggs fertilized *in vitro*. Rudak *et al.* (1984) reported evidence for aneuploidy in 5 out of 24 tripronucleate oocytes and Angell *et al.* (1986), in a series of 20 healthy pre-embryos, showed that 5 were aneuploid, i.e. an aneuploidy rate per gamete of 12.5%, and that 3 contained a structural chromosome change.

It has long been realized that the incidence of certain aneuploid offspring or embryos is

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heavily dependent on maternal age, and this is clear in Down's syndrome and, to a lesser extent, in other chromosome anomalies such as Klinefelter's syndrome (47,XXY) (table 3). The reason for this very marked increased risk with age has been frequently discussed, but the cause(s) remain an enigma. The advent of staining techniques to detect specific chromosome polymorphisms, and more recently the use of DNA probes has, however, enabled a distinction to be made between chromosomes of paternal and maternal origin and so provide information on the origin of additional chromosomes in trisomic aneuploids and indeed, in favourable circumstances, on the meiotic stage at which non-disjunction occurred (Hassold *et al.* 1984). The application of these techniques, for example in a series of cases of trisomy 21, has shown that some 80% of these were a consequence of maternal non-disjunction, and that in the majority of cases (*ca.* 80%) this non-disjunction occurred at the first meiotic division (Evans *et al.* 1986).

TABLE 3. MATERNAL AGE-SPECIFIC RATES FOR FETAL CHROMOSOMAL ABNORMALITIES DETECTED BY AMNIOCENTESIS OF WOMEN NOT YOUNGER THAN 35

(Data from European collaborative study (Ferguson-Smith *et al.* 1984).)

maternal age	Down's syndrome risk per 1000	Down's syndrome risk	all chromosome abnormalities per 1000	all chromosome abnormalities risk
35	3.5	1 in 286	12.9	1 in 78
36	5.7	1 in 175	14.1	1 in 71
37	6.8	1 in 147	15.0	1 in 66
38	8.1	1 in 123	16.5	1 in 60
39	10.9	1 in 92	21.0	1 in 48
40	12.3	1 in 81	23.6	1 in 42
41	14.7	1 in 68	28.3	1 in 35
42	21.9	1 in 45	40.1	1 in 22
43	32.4	1 in 31	50.7	1 in 20
44	29.5	1 in 33	44.3	1 in 22
45	45.3	1 in 22	72.8	1 in 14
46	81.9	1 in 12	103.4	1 in 10

TABLE 4. PROPORTION OF LIVE-BORNS AFFECTED BY HEREDITARY OR PARTIAL HEREDITARY DEFECTS AND DISEASES

(Data from United Nations Scientific Committee on Effects of Atomic Radiations 1977.)

	(%)
autosomal dominants and X-linked	1.0
recessive disease	0.1
congenital malformations	4.3
multifactorial and irregularly inherited	4.7
chromosome abnormalities	0.4
total	10.5

Before considering mutations in somatic cells I summarize the contribution of new mutations and existing mutations transmitted from parent to offspring to disease in man. One such summary is that given by the United Nations Scientific Committee on the Effects of Atomic Radiations (1977) (table 4), and it amounts to a very considerable disease burden.

2. *Spontaneous mutations in somatic cells*

The nature of the molecular and structural changes involved in the production of somatic mutations is similar to those involved in germ-cell mutations. However, it might be expected

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that many mutations that would result in lethality in the early embryo would be tolerated in various somatic cells, so that the spectrum of somatic mutations may be rather wider than that observed in the products of conception. I again consider these mutations under the headings of gene mutations and chromosomal mutations, bearing in mind the artificial distinction between these classes.

(*a*) *Gene mutations*

A major problem in detecting somatic gene mutations *in vivo* is that of identifying single mutant cells, or small colonies, on a background of large numbers of non-mutated cells. This problem is largely overcome in *in vitro* studies where systems for selecting specific mutant cells can be employed. A typical result from such studies is that reported recently by Yandell *et al.* (1986) on somatic mutations at the thymidine kinase locus in a human B-cell lymphoblastoid cell-line heterozygous for a functional gene at the TK locus. TK is a salvage enzyme which is not essential for cell survival unless pyrimidine nucleotide synthesis is blocked, so that TK^- mutants are easily selected on the basis of their resistance to toxic thymidine analogues. Spontaneous mutants were found to arise with a frequency of 1.5×10^{-6} to 5.0×10^{-6} cells. This frequency of mutation is similar to that noted for other loci in human and other mammalian cells *in vitro*, but one particular observation of these authors is worthy of special comment. Using a cloned TK probe, and a range of restriction enzymes, they were able to demonstrate that of 53 spontaneous mutations, 44 involved complete loss of the TK gene, which they conclude was a consequence of a large deletion, resulting in hemizygosity at this locus, or possibly a consequence of mitotic recombination giving homozygosity for the non-functional allele. I wish to emphasize, however, that most of the mutations were deletions and not base substitutions or even small rearrangements within the gene. This finding contrasts with other studies on X-linked genes, for example the HPRT locus, where detectable intragenic changes are responsible for a large proportion of the mutations observed (see, for example, Fenwick *et al.* 1984). Yandell *et al.* (1986) argue that this difference might be accounted for by the hemizygous nature of X-linked genes precluding the occurrence or detection of gene conversion or other recombinational events, and that large deletions may be more often lethal in hemizygous X chromosomes than in autosomes.

Despite the problems inherent in studying *in vivo* somatic gene mutations, several studies have been done, largely utilizing cells of the haematopoietic series. I shall refer briefly to two sorts, one utilizing lymphocytes and the other red blood cells, both of which have also been used in studies on people exposed to mutagens.

Based largely upon the early work by Strauss & Albertini (1979), a number of groups have used selection in thioguanine (TG) to detect lymphocytes lacking functional HPRT. With lymphocyte T-cell cloning techniques these studies give mutation frequencies of $HPRT^-$ cells in peripheral blood of from 2×10^{-6} to 10×10^{-6} (Albertini *et al.* 1982; Morley *et al.* 1983; Vijayalaxmi & Evans 1984), a value similar to that observed in a variety of studies on HPRT mutations in human fibroblasts *in vitro* (see, for example, Cox & Masson 1976). Again, many of these mutations have been shown to be a consequence of major changes involving several kilobases of DNA. Moreover, and in parallel with the findings on certain germ-cell mutations, the incidence of these TG-resistant lymphocytes in blood increases with increasing age of donor (Vijayalaxmi & Evans 1984; Trainor *et al.* 1984) (figure 2).

Very recently, Langlois *et al.* (1986) have described a method utilizing dye-conjugated monoclonal antibodies and flow cytometry to detect rare variant erythrocytes that lack the

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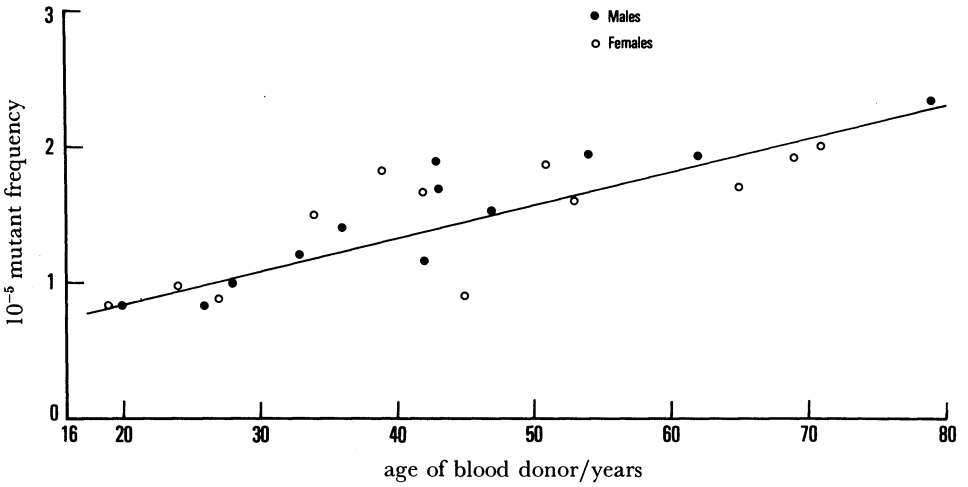

FIGURE 2. Relation between age of donor and frequency of HPRT$^-$ lymphocytes in peripheral blood (Vijayalaxmi & Evans 1984).

expression of one or other of the two allelic forms of glycophorin A, the sialoglycoprotein that is responsible for the M and N blood groups in man. Glycophorin is the product of two codominantly expressed alleles, and the frequencies of variant rbc expressing only one allele in MN heterozygotes has been reported to be 9×10^{-6} to 11×10^{-6} for each of the two hemizygous forms.

Before leaving the topic of gene mutations in somatic cells I should mention the special 'hot spot' for mutations at the Ig loci in B lymphocytes, where the very high mutation rate of 10^{-3} per base pair per cell cycle has been noted for memory cells (Siekevitz *et al.* 1987), and where recent data also suggest a high mutation rate for unrearranged Ig segments (Weiss & Wu 1987). One aberrant consequence of the rearrangements of the V, J and C segments of the Ig gene in B cells and of the T cell receptor segments in T lymphocytes is the production of abnormal chromosome rearrangements associated with certain leukaemias and lymphomas and referred to below.

(*b*) *Chromosomal mutations*

A wealth of data exist on the incidence of chromosomal mutations in human somatic cells, but these are largely concerned with chromosomes in blood lymphocytes stimulated to undergo mitosis in short-term cultures, with some supporting data from short-term cultured bone-marrow cells. In essence, these studies show that the background frequencies of both structural and numerical chromosomal mutations are quite high.

As many as 1% of lymphocytes may show chromatid deletions and some 0.1% chromatid rearrangements. Some of these chromatid-type aberrations reflect events that occurred during culture, as these aberrations are a consequence of misreplication during DNA synthesis, although the lesions that may have given rise to these errors may well have been sustained *in vivo*. Chromosome-type aberrations generally reflect events that had occurred *in vivo* and the frequency of such changes is also high, some 0.5% of cells showing chromosome deletions and around 0.2% chromosomal rearrangements. The frequencies of all these events are, however, quite variable as they are influenced by a variety of factors including age of donor, and in particular exposure to environmental mutagens such as cigarette smoke.

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Numerical chromosomal mutations are even more common than structural mutations; they are observed in direct preparations of bone marrow cells as well as in cultured lymphocytes, are very heavily age-dependent, and losses and gains are not random between chromosomes. In the newborn, children and young adults, up to 1–2% of cells may have lost or gained a chromosome, but this level of aneuploidy increases exponentially with age. In individuals older than 60 a level of 10% aneuploidy is of common occurrence (figure 3), with losses or gains of X chromosomes in females, losses of the Y chromosome in males and losses or gains of chromosomes 21 and 22 being especially prevalent in both sexes (Galloway & Buckton 1978).

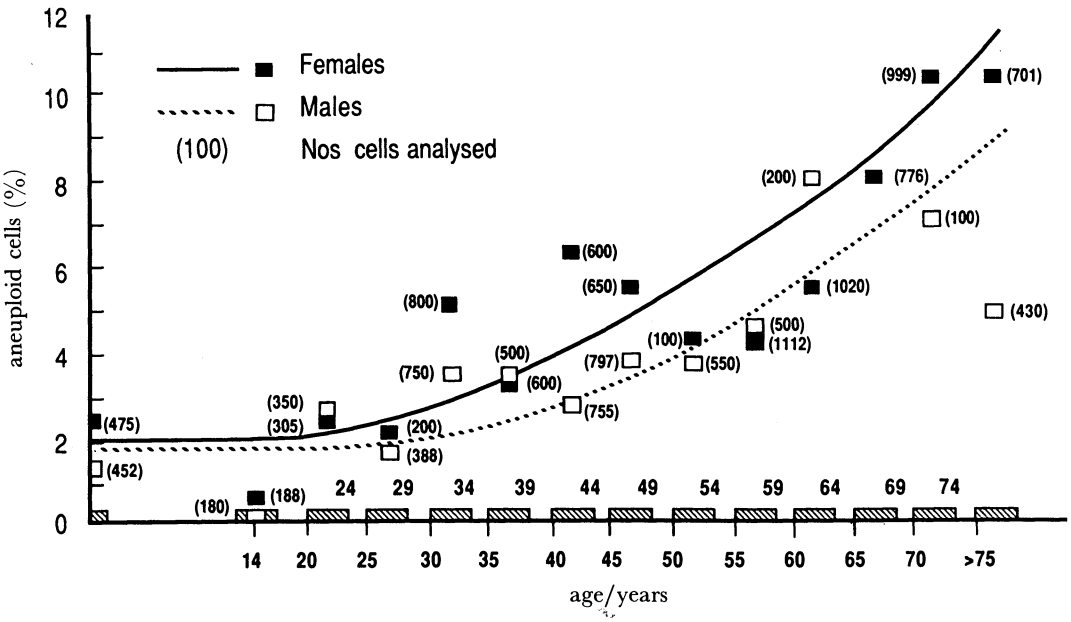

FIGURE 3. Proportion of aneuploid lymphocytes in peripheral blood samples in relation to age and sex of donors.

What then are the consequences of these somatic mutations in terms of disease? Many of the chromosome aberrations that we see may be cell-lethal and may be of no consequence in the mutational sense; however, if their frequency is sufficiently high they may diminish the rate of output of cells in proliferating tissues. Many of the mutations resulting in gene loss or malfunction may be irrelevant, except of course in those tissues in which they are called upon to function. Other mutations may be highly relevant, and in particular those involving genes associated with the control of cellular proliferation or differentiation.

There is, of course, abundant evidence for the importance of both gene mutation and chromosome structural change as early causal events in the initiation of human cancers. The original H-*ras* 1 oncogene of human bladder cancer DNA, which was isolated by gene transfer following transformation, was later shown to owe its transforming activity to a single-point mutation. Since then there have been several examples reported of single-site mutations resulting in transforming activity of a variety of different human oncogenes (see reviews by Varmus 1984; Bishop 1985, 1987). Oncogene activation and indeed amplification is also important in tumour progression, as exemplified in the case of the *n-myc* gene in neuroblastoma (Brodeur *et al.* 1984) and in the recent findings with regard to the c-*erb*B-2 oncogene in human breast cancer (Slamon *et al.* 1987).

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Chromosomal rearrangements and recombinational events are also involved in the genesis of certain solid tumours as exemplified by the 11p13 deletion, in Wilms's tumour (Van Heyningen *et al.* 1985) and the 13p14 deletion in retinoblastoma (Benedict *et al.* 1983); the gene involved in the latter condition has recently been isolated and cloned (Lee *et al.* 1987). The importance of chromosome rearrangements has been especially highlighted in studies of the leukaemias and lymphomas (Evans 1985*a*), where a variety of chromosome translocations have been identified, each one being specific for a given disease type. In the majority of these cases it has been shown that each rearrangement is associated with an activation of, or a mutation within, a c-*onc* gene (table 5), often as a consequence of an abnormality in the normal DNA processing involved in the rearrangement of the Ig or T-cell receptor genes (Haluska *et al.* 1987).

TABLE 5. EXAMPLES OF SPECIFIC TRANSLOCATIONS AND ONC GENE SITES CHARACTERIZING VARIOUS LEUKAEMIAS AND LYMPHOMAS

translocation	malignancy	onc or activated per rearranged gene
t(2;8) (p11–13;q24)	Burkitt's lymphoma	c-*myc* (8q24)
t(8;14) (q24;q32)	ALL-L3	*IgH*, *IgK*, *Igλ*
t(8;22) (q24;q11)		
t(8;21) (q22;q22)	ANLL-M2	*ets-2* (21q22)
t(9;22) (q34;q11)	CML (also ALL)	c-*abl* (9q34)
		bcr (22q11)
t(11;14) (q13;q32)	B cell leuk./lymph.	*bcl-1* (11q13)
t(14;18) (q32;q21)	B cell foll;. lymph.	*bcl-2* (18q21)
t(15;17) (q24;q21)	ANLL-M3	c-*fes* (15q23)
		c-*erb A2* (17q21)
t(14;14) (q11;q32)	T-CLL	*TCRα* (14q11)
		Tcl-1 (14q32)
t(8;14) (q24;q11)	T-ALL	*TCRα* (14q11)
		c-*myc* (8q24)
t(6;9) (p21;q34)	ANLL	c-*pim* (6p21)

There are many examples where chromosomal mutation has occurred in the very early stages of embryogenesis, so that the resultant individuals have a mosaic chromosome constitution. This is well illustrated, for example, in certain patients who are X-chromosome mosaics with a 46,XX:45,X chromosome constitution, and who in consequence suffer from Turner's syndrome. Overall, however, it would appear that the commonest kinds of human disease in which somatic mutation plays a major role are a variety of forms of cancer.

3. *Induced mutations transmitted via germ cells*

There is abundant evidence from animal studies that exposure to well-known environmental mutagens such as ionizing radiations or certain chemical mutagens results in the production of mutations whose effects can be discerned in the offspring of exposed parents. It is also quite evident that human populations are exposed to such mutagens, but so far there has been no unequivocal demonstration of an increased mutation frequency among the offspring of exposed parents. Various studies have been done on the offspring of populations such as those exposed to the atomic radiations from the bombings of Hiroshima and Nagasaki; and on the offspring of patients exposed to diagnostic X-rays. In the case of the studies on the children of X-rayed parents, there have been a number of suggestions of a small increased frequency of Down's syndrome patients, but an equal number of studies have shown no indication of an increase

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(Evans *et al.* 1986). No evidence for an increased mutation frequency has been found in studies on offspring of the bomb survivors in Japan despite a considerable effort to look for a possible increase, and no really systematic attempt has been made to study mutation frequencies in the offspring of cigarette smokers. Few attempts have been made to study systematically offspring of populations occupationally exposed to chemical mutagens, and a major difficulty in these and in other attempts at analysing mutation rates in exposed populations follows from the problems of ascertainment of mutants, defining the background or control frequency and measuring levels of exposure. As a consequence of the high success rate in the treatment of acute childhood leukaemia, a considerable number of children who were treated with mutagenic agents have now reached maturity and are starting families. This population provides a very important source for study and a number of groups worldwide are doing such studies, but no results are available yet.

Despite the paucity of information on induced transmitted gene mutations in man, it is evident that exposure of human germ cells to ionizing radiations does result in chromosomal mutations. Martin *et al.* (1986) recently briefly reported on the use of the human sperm–hamster oocyte technique to reveal chromosomes in sperm of 13 cancer patients before and at regular intervals after testicular radiotherapy. A very large increase in the incidence of structural and numerical chromosomal mutations was noted, with a significant correlation between radiation dose and level of damage, with a maximum frequency of anomalies of 67 % in a sample from one patient.

4. *Induced mutations in somatic cells*

In contrast to the paucity of information on the effects of mutagens on human germ cells, there are very considerable amounts of data on human somatic cells exposed to a range of mutagens *in vitro* or *in vivo*. I shall devote most of my comments to a few of the *in vivo* studies and refer first to a few reports on induced gene mutations.

(*a*) *Gene mutations*

Langlois *et al.* (1986) have utilized their assay for glycophorin variants in a study of red blood cells of cancer patients before and after chemotherapy or radiotherapy or both, and report highly significantly increased variant frequencies after treatment. Earlier, Albertini (1980) using an autoradiographic technique to detect thioguanine-resistant lymphocytes in peripheral blood, described increased frequencies of such variant cells in a range of patients exposed to therapy with various mutagens including X-rays. More recent *in vitro* studies on the induction of $HPRT^-$ mutations in human lymphocytes, as detected by a cloning technique, have shown that increased mutation frequencies can be detected at X-ray doses of less than 100 cGy, that the increase is proportional to dose (figure 4) and that the rate of increase is 1.95×10^{-7} mutations per cell per centigray (Vijayalaxmi & Evans 1984). This rate of increase per centigray is roughly the same as the rate of increase in the spontaneous frequency of $HPRT^-$ cells per year, 2.4×10^{-7} per cell per year, and to X-ray-induced $HPRT^-$ mutations in human fibroblasts in culture of 2.1×10^{-7} per cell per centigray (deRuijter & Simons 1980).

(*b*) *Chromosomal mutations*

The ready availability of peripheral blood lymphocytes for chromosome analysis and the wide ranges of exposure of individuals to mutagens as a result of therapy, occupation, or indeed as a part of their everyday existence, has resulted in the accumulation of a considerable body

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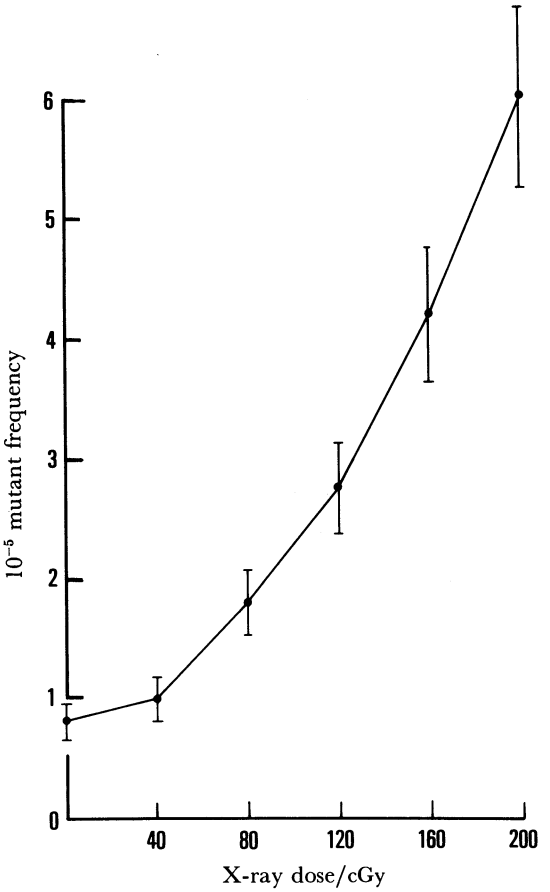

FIGURE 4. Relation between X-ray dose *in vitro* and frequency of HPRT$^-$ cells in irradiated peripheral blood lymphocytes (Vijayalaxmi & Evans 1984).

of data on induced chromosomal mutations in human blood cells. Of particular interest has been the response of human chromosomes to exposure to ionizing radiations. Studies of patients exposed to low dose, 25 cGy, whole-body X-ray therapy reveal an approximately 20-fold increase in the frequency of chromosomal aberrations as a consequence of their exposure. Studies at higher dose levels, given as partial body exposures to patients with ankylosing spondylitis, showed a typical X-ray dose–effect relation and demonstrated the presence of induced chromosomal mutations, particularly those involving translocations and inversions, up to 40 years or more after exposure (figure 5). In many cases cells containing these mutations had undergone proliferation, so that the lymphocyte cell populations frequently contained chromosomally marked cell clones (Evans 1985*b*). The high sensitivity of human chromosomes to radiation damage is also illustrated by the demonstration of significant dose-related increased levels of chromosome damage in blood lymphocytes of workers occupationally exposed to radiation levels within the maximum permitted level of 5 cGy per annum (Evans *et al.* 1979; Lloyd *et al.* 1980). In these studies the rate of aberration induction was shown to be *ca.* 1 per cell per 4×10^{-4} cGy.

Increased chromosome damage is also evident in people exposed to chemical mutagens. At the extreme, high levels of chromosomal aberrations are observed in cancer patients treated with

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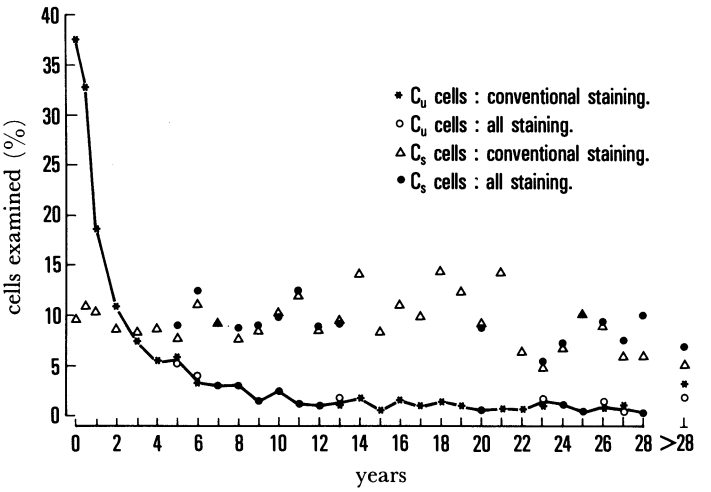

FIGURE 5. Chromosome aberrations in peripheral blood lymphocytes of patients treated with X-rays for ankylosing spondylitis and sampled at various times after treatment. Key: C_u cells, cells with unstable aberrations; C_s cells, cells with stable rearranged aberrant chromosomes. Note the persistent high level of cells with stable rearranged chromosomes and the decline in frequency of cells with unstable rearrangements with time.

high doses of mutagenic cytotoxic drugs. However, increased levels of chromosome damage are also evident in the blood cells of cigarette smokers as compared with non-smokers (Evans 1982*a*), and are frequently observed in individuals exposed to a variety of chemical mutagens in the work place, e.g. various sterilants, pesticides, solvents, etc. (Evans 1982*b*). In this context the recent study of Leonard *et al.* (1984) is worthy of mention. These authors compared aberration frequencies in blood cells of workers at nuclear power stations and conventional fossil-fuel-powered stations, and compared them with controls, taking into account smoking habits and possible medical exposures to mutagens. The results (table 6) showed an increased level of aberrations over controls in nuclear power workers, in line with the earlier findings referred to above, but an even greater increase in fossil-fuel-power workers, a not surprising result in view of the well-known mutagenic properties of the products of combustion of fossil fuels.

TABLE 6. CHROMOSOME ABERRATIONS (%) IN PERIPHERAL BLOOD LYMPHOCYTES OF EMPLOYEES OF FOSSIL-FUELLED AND NUCLEAR POWER PLANTS

(After Leonard *et al.* 1984.)

	controls	nuclear power workers	fossil fuel power workers
no of subjects	23	89	49
no. of cells scored	11500	44500	24500
cells with aberrations	1.23	1.62*	1.98*
chromatid aberrations:			
gaps and break	0.80	1.04*	1.27*
exchanges	0.05	0.04	0.07
chromosome aberrations:			
fragments	0.24	0.38*	0.44*
dicentrics	0.11	0.16	0.22

* $p \leqslant 0.5$.

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What of the consequences of these induced somatic mutations? We have already referred to the fact that gene and chromosomal mutations play a significant role in the initiation and progression of neoplasms in man, so that mutations resulting from exposure to external mutagens might be expected to be associated with an increased risk of cancer. Indeed a majority, but not all, of the agents known to cause cancer in man are known to be mammalian cell mutagens (International Agency for Research in Cancer 1982). As a result of their cytotoxic properties a number of mutagenic agents are used in the treatment of human cancer and, in a number of cases, are clearly responsible for the induction and later development of second primary cancers which arise as a consequence of treatment (see, for example, Day & Boice 1983).

5. *Concluding comments*

Mutations in man are not of uncommon occurrence. Chromosomal mutations are frequent in germ cells and are responsible for a considerable proportion of abortions and of congenital abnormalities in the live-born. Gene mutations vary in frequency between different genes and make a major contribution to disease. The incidence of both kinds of mutation is influenced by a variety of factors, including parental age, and although there has been no unambiguous demonstration of an increased incidence of either type of mutation in the offspring of individuals exposed to mutagens, there is little doubt that such increases must occur. Somatic mutations of both types are also frequent; their incidence is shown to be increased in the somatic cells of people exposed to mutagens, and there is considerable evidence that some of these mutations are involved in the transformation processes that result in human cancers.

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Discussion

R. Worton (*Department of Genetics, Hospital for Sick Children, Toronto, Canada*). In addition to all the types of genetic alteration that Professor Evans has summarized, there is another that is not easy to detect but is of major importance. That is functional and structural homozygosity due to somatic recombination, gene conversion or loss and duplication of homologous chromosomes. Several years ago my laboratory embarked on a programme to generate and map selectable genetic markers on Chinese hamster chromosomes and used the markers to determine if recessive alleles might be expressed after loss of the wild-type gene by somatic recombination or gene conversion or both. Although such events were detected, the major genetic event found in cells expressing the recessive gene was loss of the chromosome carrying the wild-type gene and duplication of the chromosome carrying the recessive (selectable) marker. The selected cells were not only homozygous for the recessive marker but also were homozygous for every testable marker along the length of the chromosome. The importance of this for the expression of recessive cancer genes has become abundantly clear in tumours such as retinoblastoma and Wilms's tumour, where the recessive tumour gene becomes homozygous by a process that involves somatic recombination or chromosome loss and duplication.

I mention this because the mutagenic agents that Professor Evans has discussed result in chromosome breakage leaving recombinogenic ends and also cause non-disjunction at mitosis. Although simple non-disjunction is readily detectable by a chromosome count, the more subtle process of loss and duplication of homologous chromosomes is not so readily detected without resorting to haplotype analysis at several loci. When screening tests are being developed to detect biologically significant alteration in the human genome by mutagenic agents, it might be wise to include at least one test that would detect events that lead to homozygosis of a selectable recessive marker.

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