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Allelic instability in mitosis: A unified model for dominant disorders

(unstable DNA elements/dominant mutations/myotonic dystrophy/fragile X syndrome)

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ABSTRACT **Recent findings indicate that tandemly repeated triplet sequences in certain disease-causing human genes may render these genes highly unstable not only in meiosis but also in mitosis. Typically, a dominant mutation arises upon expansion in the number of these repeated elements. We have considered how mitotic instability of this sort might affect both phenotypic expression and allele transmission. A model based on these considerations leads to the following predictions: (*i*) Phenotypic severity among individuals who inherit an unstable allele should be highly variable due to stochastic variation in the stage of its earliest mutagenic expansion. (*ii*) Strikingly increased severity or decreased age of onset in some offspring should arise because of parental germ-line mosaicism for an expanded or mutant allele. (*iii*) The magnitude of genetic anticipation should be more strongly correlated with paternal than with maternal age at the time of conception. (*iv*) Given a child born with a severe phenotype, the recurrence risk for a second severely affected child should be significantly elevated. (*v*) The severity of phenotype in a child should be positively correlated with that in a parent. Available data on fragile X syndrome, Huntington disease, and myotonic dystrophy are shown to be consistent with the model, and implications for an understanding of achondroplasia and other dominant disorders are discussed.**

Repetitive DNA sequences have been recognized as a source of mutation in human genetic diseases such as fragile X syndrome and myotonic dystrophy (reviewed in refs. 1 and 2; primary references on the fragile X and myotonic dystrophic genes have not been listed due to space limitations) and more recently in Huntington disease (3). These simple periodic sequences are unstable, often undergoing stochastic increases in copy number between one generation and the next. The mechanism causing increased copy number might conceivably entail recombinational events between homologous chromosomes during *meiosis*, but recent data from linkage studies using flanking DNA markers fail to support this possibility (4). On the other hand, variation in copy number has been detected within somatic tissues (5), indicating that stochastic expansion events occurring during mitotic growth of these tissues would result in somatic mosaicism. Given this evidence for substantial mitotic instability, it seems likely that mutational expansion of this sort would arise during mitotic divisions of both the soma and the germ line, thereby leading to somatic and germ-line mosaicism for expanded mutant alleles. In this communication, we propose that somatic mosaicism manifests itself in the variation of phenotypic severity and age of onset that is characteristic of dominant disorders. Germ-line mosaicism, on the other hand, provides a possible basis for understanding the mechanisms responsible (*i*) for the differing probabilities of paternal versus maternal transmission and (*ii*) for "genetic anticipation" (6), wherein one finds increasing severity or earlier age of onset for a disorder in successive generations. We have developed a simple model based on cellular kinetics and have considered its genetic consequences in both types of cells. We show that the available data on myotonic dystrophy, Huntington disease, and fragile X syndrome are consistent with this model, and we discuss other dominant disorders within this framework.

The Model

To simplify derivation of a quantitative model, we assume that just three types of alleles are distinguishable at a given locus of interest: *normal*, unstable *premutant*, and *mutant*. The normal allele, which is the most common in the population, infrequently undergoes conversion into a premutant allele. A premutant allele is, on the other hand, quite unstable in mitosis, frequently becoming converted into a fully mutant allele, which is deleterious at the cellular level and is dominant to either the normal or premutant alleles. When defined according to the length of repetitive DNA sequences (1, 2), the normal allele is specified to contain a short segment of repeats, whereas a premutant allele contains a somewhat greater number of repeats. This moderate increase in the number of repeats renders the gene highly vulnerable to even greater expansion, perhaps then secondarily leading to gene duplication, deletion, and/or chromosomal rearrangement.

Variation in the number of cells that contain the mutant allele in a rapidly dividing cell population is quantifiable by development of a simple model based on cell kinetics. Cell division may be treated as a binary, synchronous process—a single ancestral cell giving rise to 2^n cells after n mitotic generations. The probability that a premutant allele will be converted into the mutant allele during any single mitotic cell division is assumed to be a constant, μ, which would differ between alleles according to their initial sizes (1, 2). Given these assumptions, the number of cells that remain unmutated at generation n (X_n) among descendants of a single ancestral cell that was heterozygous for the premutant allele is a random variable that depends upon the cellular generation in which the first mutational event occurs. If it occurs at an early stage in the exponential expansion of the dividing population, subsequent divisions would lead to a larger number of mutated cells in the final cellular population than if it arises later. Mathematically, X_n is defined by a Bienaymé–Galton–Watson branching process (7). When the number of cell generations is large, the process can reliably be approximated by

$$X_n \sim W' m^n \ (n \text{ large}),$$

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where W' is a random variable with mean $E(W') = 1$ and variance $\sigma^2_{W'} = \sigma^2_{X_1}/(m^2 - m)$ and m [$= E(X_1) = 2 - \mu$] is the mean number of unmutated cells at the first generation (7). Because the sum of unmutated and mutated cells at generation n must equal 2^n, the number of mutated cells at generation n (Y_n) is a random variable definable in terms of X_n; namely,

$$Y_n = 2^n - X_n$$

$$\sim Wm^n \ (n \text{ large}),$$

where $W (= 2^n/m^n - W')$ is a random variable with $E(W) = 2^n/m^n - 1$ and $\sigma^2_W = \sigma^2_{W'}$ (7). The mean fraction of mutated cells in the cell population at generation n can now be computed, yielding $E(Y_n)/2^n = (2^n - m^n)/2^n$ (values of $E(Y_n)/2^n$ increase monotonically with n). We note that occasionally an early mutation will result in a large fraction of cells receiving the mutant allele; this may be considered a "jackpot" phenomenon similar to that described in the fluctuation test of Luria and Delbrück (8).

Variation in Penetrance and Phenotypic Severity. The randomness of W constitutes the principal basis for understanding the random variation both in phenotype and in transmission of an allele that would cause the disorder. Individuals who inherit a premutant allele would by chance possess very different values of W (their tissues contain very different numbers of the mutated cells) and show varying degrees of manifestation. This variation can be expressed in terms of the penetrance of the disorder, which we define as the probability that the random variable W will exceed a threshold T,

$$\text{Penetrance} = P(W > T).$$

Clearly, threshold T will differ from one disorder to another, depending on the nature of the mutant allele and the specific role of the gene product in various target tissues. According to this definition of penetrance, individuals who inherit the mutant allele have no unmutated cells and will invariably manifest the disease (having full penetrance). A low value for threshold T would imply that most people inheriting the premutant allele have more mutated cells in their soma than the minimum number required for clinical manifestation, and the disorder would be judged to have high penetrance. A higher value for threshold T would hold if a much greater proportion of mutated cells were required to cause the mutant phenotype, and the penetrance would be deemed to be lower. This relationship may be presented on a plot (Fig. 1) of the distribution for W, which is assumed for convenience to be a continuous variable. The penetrance, which corresponds to the area of the filled-in region beneath the distribution curve, clearly depends on the location of the threshold level T relative to the peak of the distribution. Intuitively, the threshold level T might be viewed as the stage of embryonic development by which the earliest switch from a premutant allele to a mutant one must have occurred in order to give rise to clinical manifestation (depending on the specific tissue that is affected). A switch occurring after this critical time would generate too few mutant cells to cause a detectable phenotype.

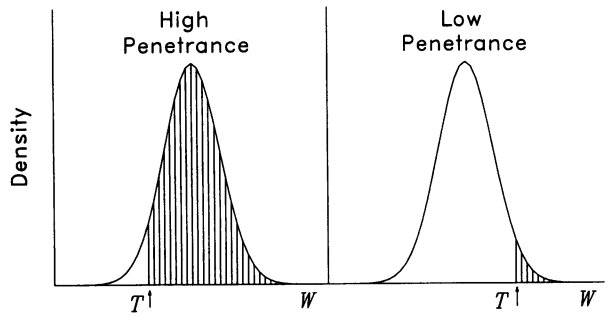

FIG. 1. Hypothetical plot of the distribution of W indicating how penetrance (hatched area) of a dominant disorder would depend on the location of the threshold level T. (*Left*) High penetrance. (*Right*) Low penetrance. Values on the abscissa are arbitrary.

Among individuals who have inherited the premutant allele and have shown at least a minimal manifestation, a great deal of variation in phenotypic severity is expected if the threshold T is low, yielding a probability $P(W > T)$ that is large (see Fig. 1 *Left*). Those individuals who stochastically experience larger values of W (having relatively larger numbers of the mutated cells) would show more severe impairment and earlier onset of the disorder. Those with a small number of mutated cells in their target tissues would have late onset and/or mild symptoms. On the other hand, if the level of threshold T were high and the penetrance low (Fig. 1 *Right*), there will be relatively less variation in phenotypic severity. These latter circumstances would make it difficult to differentiate individuals who inherit the premutant allele from those inheriting the mutant allele.

When the disease locus is X linked, additional variation in phenotype between males and females will be introduced by the random process of X chromosome inactivation, which may confer partial protection from clinical manifestation on women carrying a mutant allele. Correspondingly fewer females than males who carry either the premutant or mutant allele would be expected to show any clinical manifestation of the disease. The average reduction in penetrance in females would vary from disorder to disorder. The expected pattern of X linked dominant inheritance with reduced penetrance might generally be recognizable, but disorders with a low penetrance in females might erroneously be inferred to represent cases of recessive X linked inheritance.

Variation in Genetic Transmission. Whereas somatic mosaicism for the mutant allele has important implications for phenotypic severity (as discussed above), germ-line mosaicism would of course affect transmission to progeny. Consider, for example, those parents who inherit a premutant allele and show only mild symptoms because switches from the premutant to the mutant allele occurred relatively late in their somatic development. Some fraction of their offspring might be expected to inherit a fully mutant allele that arose during mitotic growth of the germ line and would show severe impairment, while other progeny would inherit the nonexpanded premutant allele and show a degree of severity similar to that of the parent. The summation of these two modes of transmission in a family study would contribute to the phenomenon termed genetic anticipation (6).

The magnitude of anticipation should depend on the gender of the transmitting parent. A male parent who receives the premutant allele at conception would be more likely to transmit an expanded allele than his female counterpart, since mitotic expansion of the paternal germ line entails a larger number of mitotic divisions than occur in the maternal germ line, which ceases division during her development *in utero*. In addition, since the number of postembryonic mitotic cell divisions in the paternal germ line should correspond with paternal age, the magnitude of anticipation is further expected to be correlated with paternal age at conception.

Another important prediction is that, once a severely affected child who inherits a mutant allele has been produced, the recurrence risk for a second severely affected child should be significantly elevated. This prediction is readily understood by recognizing that manifestation of the disorder in the first child renders it likely that the carrier parent has a large fraction of mutated cells in the germ line, the mutation having occurred at an early stage to yield a jackpot. If, on the other hand, switches occurred only during meiosis (the

terminal germ-line division), the recurrence risk for a severely affected child would not be elevated.

Correlation Between Somatic and Germ-Line Mosaicisms. In evaluating possible tests for these predictions about the genetic transmission of unstable elements, one should note that the probability of allele expansion will usually depend on the initial number of repeats, which would be subject to a great deal of variation between individuals (1, 2). Within any one individual, however, those very few cells that initially give rise to both soma and germ line would usually bear identical alleles. If expansion of the repeated element were equally probable in all mitotic divisions, then the probability of expansion would necessarily be identical between soma and germ line. Therefore, the proportion of mutant cells in the soma of any individual would be correlated with their proportion in the germ line, and individuals with a higher frequency of mutant cells in their soma would be more likely to transmit a mutant allele to their offspring. Consequently, phenotypic severity in parents should be carefully controlled in statistical analysis of variation in allele transmission.

The population genetics of premutation (9) cannot be dealt with adequately here. Suffice it to point out that the frequency of the premutant allele in a population should exceed that of the fully mutant allele to the extent (*i*) that only a subset of individuals who carry the premutant allele would transmit a fully mutant allele and (*ii*) that individuals who inherit the mutant allele would suffer reduced fitness. We note in addition that the premutant allele would be particularly prevalent among relatives of affected patients who carry the mutant allele. In such pedigrees, the transmission will be most readily observed in the paternal line for the reasons discussed earlier.

Myotonic Dystrophy

In myotonic dystrophy and fragile X syndrome, segments of tandemly repeated triplets within the relevant gene undergo expansion, causing the kind of defects our model addresses (1, 2, 10). The myotonic dystrophy gene on chromosome 19 contains unstable p(CTG)$_n$ repeats. Substantial variation in the age of onset is at least partially attributable to somatic mosaicism, which is more pronounced for alleles of larger initial size. Regression analysis of published pedigree data (11) confirms the prediction of a negative correlation between paternal age at conception and age of onset of the disease in offspring (Table 1). A significant correlation with maternal age is not found in 21 mother/offspring pairs from the same data set.

A paternal effect on anticipation in myotonic dystrophy had already become widely accepted before Harper and Dyken (12) reported in 1972 that congenital cases (onset age younger than 5 years) are almost exclusively born to affected mothers. This finding may appear to refute our model, but we feel that the explanation lies in the fact that the mothers of those congenital cases are generally more severely affected (13, 14). Males with a disorder of comparable severity appear to suffer reduced fertility (6) or tend to be unmarried (15). Because of this confounding variable, we have used multiple regression to control for the father's age of onset in our analysis of paternal-age dependence in myotonic dystrophy (Table 1).

Table 1. Analysis of paternal-age effect on age of onset in myotonic dystrophy

Model	Model coefficient: Intercept *a*	Father's age of onset *b*	Paternal age *c*	Model r^2
1	1.952	0.326	—	0.045
2	4.812	—	−0.447	0.113
3	2.904	0.749	−0.762	0.298*

The logarithm of the age of onset in the offspring was regressed on the logarithms of the age of onset in the father and the father's age at conception. Precisely, $\ln(z) = a + b\ln(x) + c\ln(y)$, where z is the age of onset in offspring, x is the age of onset in father, and y is the paternal age at conception. Models 1 and 2 include either x or y, respectively, as the independent variable, whereas model 3, the full model, contains both x and y as independent variables. Data were collected from ref. 11, including 30 affected father/offspring pairs from 17 families. Age of onset was taken as the age at which cataracts appeared, since this common aspect of myotonic dystrophy is assessed reliably. If an incipient cataract was recorded, the age at onset was assumed to be 4 years after the age of examination, since Bell (11) reported an average interval of about this extent between the onset of incipient and typical cataracts. In this preliminary analysis, multiple contributions from 10 families were included to increase the sample size, but no adjustments were made for correlated observations. Inclusion of such data is in accord with common practice but would be inappropriate for a complete study because it ignores the tendency of recurrence in siblings.
*$P < 0.01$.

Fragile X Syndrome

In fragile X syndrome, the disease gene contains a segment of tandemly repeated p(CGG)$_n$ triplets that is susceptible to amplification. Normal X chromosomes have an allele with about 20 repeated units (range, ≈6 to 50), and the number of repeats in this normal range is stably transmitted. Both male and female fragile X carriers who are of normal mental capacity have an allele that contains between 50 and 200 copies of the repeat. Alleles of this latter type are unstable, stochastically undergoing amplification to a mutant condition in which there are many hundreds, or even thousands, of copies of the triplet. Males who inherit these amplified alleles are more likely than females to be severely affected, possibly because the normal allele borne on the homologous X chromosome in females becomes the only transcriptionally active gene in about half of the cells once random X chromosome inactivation has occurred.

It has been observed that the recurrence risk for fragile X syndrome in brothers and sisters of a transmitting male is less than that in siblings of an affected male (16, 17). A difference in germ-line mosaicism between mothers of transmitting and affected males can readily explain this observation. According to the model, an unaffected transmitting male would have inherited a premutant allele from his mother, whereas an affected male could have inherited a mutant allele. The latter situation would occur more often when the ovary of the mother contained a larger number of mutant oocytes, thereby conferring a greater recurrence risk on other offspring.

Viewed over a longer span of generations, one finds that the recurrence risk for fragile X syndrome in the siblings of a transmitting male is lower than that in children born to his daughters. This observation, termed the Sherman paradox (17, 18), can be resolved in the context of the proposed model quite simply—namely, the fact that the mother of the transmitting male had already given birth to an unaffected son would tend to identify her as an individual whose ovaries contained a relatively small fraction of mutant oocytes. Accordingly, any further children born to her would similarly be unlikely to receive a fully mutant allele. On the other hand, there would be no such ascertainment bias among her granddaughters, some of whom would by chance have undergone a mutational event at an earlier stage of germ-line proliferation. The daughters would therefore be at greater risk of transmitting a mutant allele. This idea resembles and extends a previous explanation for the Sherman paradox based on germ-line mosaicism in mothers (19).

Another puzzling characteristic of fragile X syndrome that must be explained is the fact that almost all affected patients are born to female carriers (17). This phenomenon may appear to contradict the postulated paternal-age effect on allele expansion that is emphasized in our model. The explanation lies, we feel, in the probability that males who inherit a fully expanded allele suffer from macro-orchidism (20). It seems plausible that there is a related defect in any individual germ cell that bears a fully mutant allele as the result of germ-line mosaicism and that the allele therefore cannot be transmitted. On the other hand, an allele expanded to a lesser degree might be transmitted normally, and in this case the paternal-age effect would prevail. This assertion is supported by the report that alleles that are inherited from grandfathers possess a higher penetrance in males than those inherited from grandmothers (21). It should also be noted that many females who carry an expanded allele might be protected from manifestation of the disorder by X chromosome inactivation; many such females (some of whom have learning disabilities) maintain active reproduction, thereby enhancing the bias toward maternal transmission. For example, a recent study found that about 10% of the daughters of normal transmitting males display extensive somatic mosaicism for fully mutant alleles (seen as a smear in Southern blot hybridization), but are relatively unaffected (table I of ref. 22). Among 46 other females known to be obligate carriers on the basis of pedigree analysis (table IV of ref. 23), six of them carried alleles ranging in size from ≈3000 to 13,000 base pairs (≈1000 to 4300 triplet repeats).

A complementary model that has been proposed by Laird (24) invokes an imprinting mechanism to explain the predominance of maternal transmission. According to this interesting hypothesis, the fragile X locus becomes imprinted by a defect in the process of X chromosome reactivation that normally precedes the initiation of meiosis, thereby causing manifestation of the disorder in male offspring who inherit the affected locus on this X chromosome. The correlative proposal that imprinting of this sort represents the abnormal retention of a DNA methylation pattern that normally would be erased during X chromosome reactivation (25) has found support in some, but not all (5), recent data.

Discussion

It has previously been proposed by Sutherland *et al.* (26) that allelic instability in somatic mitosis may be responsible for the genetic variegation, incomplete penetrance, and varied phenotypic expression that are observed in a variety of dominant disorders and that a corresponding instability in meiosis accounts for the patterns of transmission shown for these alleles (1). In the present work, we posit that the same type of mitotic instability that is evident in somatic cells also holds for the germ line. We thus invoke a unified mechanism for dominant disorders based on allele instability in mitosis. Predictions from this model, detailed in the abstract, include (*i*) high variability in phenotypic expression of the disease, (*ii*) genetic anticipation [commonly seen in dominant disorders (Table 2)], which is (*iii*) correlated more with paternal than with maternal age, (*iv*) an increased recurrence risk of a severe phenotype in offspring with a severely affected sibling, and (*v*) correlation in the severity of affliction of parent and child.

A particular benefit from recognizing these various hallmarks of genetic disorders that are already known to result from expansion of DNA repeats is the possibility that one might predict a similar genetic basis for disorders that remain uncharacterized. We had noted during our initial development of this model that many features of Huntington disease were consistent with these hallmarks, leading us to predict that unstable DNA sequences would also be found to underlie Huntington disease. This disorder, which affects the central nervous system, had been attributed to an autosomal-dominant mutation mapping to one of two closely linked candidate positions on the fourth chromosome (28). Evidence for somatic mosaicism was seen in the extremely varied age of onset, which ranges from infancy to more than 70 years (the average is about 40) (29). Germ-line mosaicism for the mutant allele was evident both from the tendency of clustering of early onset offspring in families and from the observation that offspring who inherit the disease allele from their fathers are more likely to develop symptoms at an early age than those who inherit the allele from their mothers (30). This latter effect had been attributed partially to advanced paternal age (31), consistent with the prediction of the current model. Correlation between the probabilities for somatic and germ-line mosaicism is indicated by the correlation in age of onset between parent and offspring (31). The recent discovery (3) that the Huntington disease gene does indeed contain an unstable trinucleotide repeat [p(CAG)$_n$] confirms our prediction and adds yet another example to the set of dominant disorders that are consistent with the model.

The present model may also provide a new perspective on the commonly known paternal-age effect on dominant mutation rates, for it suggests that fathers who are considered asymptomatic may actually carry a premutant allele and show a mild phenotype. In achondroplasia, for example, sporadic cases born to phenotypically normal parents are common (≈80–90%), and the incidence strongly depends on paternal age. Although relatively little variability in achondroplasia *per se* has been suggested, a milder disease (termed hypochondroplasia) has been described (32), and genetic transmission from hypochondroplastic patients to achondroplastic descendants has been observed (33). A traditional explanation for the large number of sporadic cases might be that there is a high mutation rate ($\approx 2 \times 10^{-5}$ per generation), but Opitz (33, 34) has pointed out that this explanation is inconsistent with several reports showing a familial occurrence of achondroplasia in affected individuals related to each other in pedigrees with large numbers of normal individuals. Opitz's proposal of "unstable premutation" in achondroplasia (35), based on an earlier suggestion made by

Table 2. Statistics of anticipation for several genetic disorders

Disorder	No. of parent/child pairs	Mean age of onset, years		
		Parent	Child	Difference
Peroneal atrophy (dominant)	86	24.30	19.36	4.94
Muscular dystrophy (dominant)	90	27.44	21.00	6.44
Hereditary glaucoma	113	42.08	30.66	11.42
Huntington chorea	153	40.80	31.98	8.82
Diabetes mellitus	216	60.29	43.06	17.23
Mental illness (all diagnoses)	1728	50.50	34.20	16.30
Dystrophia myotonia	51	38.48	15.24	23.24

Data are reproduced from table 1 of Penrose (27).

Auerbach (36) in a study of human ectrodactyly, anticipates the idea that is elaborated in the present text.

Finally, it must be recognized that we have chosen to treat the expansion of repeated segments dichotomously, invoking only premutant and mutant alleles in addition to the wild type. The approach must certainly be an oversimplification, because different extents of expansion must differentially affect both the probability of further expansion and the function of the gene. In addition, a biased maternal effect on genetic anticipation would ensue if mutant germ cells were selectively lethal in males or were defective in being chosen for maturation in females. The occurrence of macro-orchidism among fragile X males with reduced fertility (20) suggests a possible case of the former mechanism, whereas enhanced utilization of aneuploid oocytes with increasing maternal age would be expected in the latter [as has been proposed (37)]. Nonetheless, even the simple form of the model that is presented here may lend insight into the genetic mechanisms responsible for certain well-known disorders and may help to develop a representative spectrum of testable predictions about disorders that remain to be characterized at the molecular level.

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