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# Invited Editorial: Anticipation Legitimized: Unstable DNA to the Rescue

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The discovery and recognition of new mechanisms of genome organization and function are not common events. Furthermore, they are usually the result of the gradual accumulation of evidence rather than sudden revelation. Genome properties that are contrary to existing dogma find particular difficulties in being accepted. Transposable elements and introns were beyond most of our imaginations until they had been convincingly demonstrated.

In this issue, Harper et al. (1992) revive and give legitimacy to an old and generally discredited genetic phenomenon, anticipation. Anticipation denotes the increasing severity or earlier age at onset of a genetic disease in successive generations. While clinicians, since early this century, have been impressed by anticipation in myotonic dystrophy (DM), the concept had not found favor with geneticists, for two main reasons. As Harper et al. point out, the concept was associated with "degeneration," and such a concept was not acceptable to the strongly anti-eugenic and highly influential geneticist Lionel Penrose, who ascribed anticipation to no more than ascertainment bias (Penrose 1948). Furthermore, the genome was considered to be stable, and there was no known mechanism by which this virtually Lamarckian entity could operate.

The cloning of the fragile X (Oberlé et al. 1991; Yu et al. 1991) provided a mechanism for anticipation (Fu et al. 1991; Sutherland et al. 1991; Yu et al. 1992). Since the detailed segregation studies by Sherman et al. (1984, 1985), this disorder had been recognized as having unusual genetic properties. These studies confirmed that this X-linked disorder could have asymptomatic male carriers of the mutation, and they also showed that the penetrance of mental impairment in children depended on the phenotype of their mother. The disorder manifested a form of anticipation that became known as the "Sherman Paradox." This paradox was that the mothers and daughters of asymptomatic males who carried the fragile-X mutation had very different risks of having mentally handicapped offspring, yet these two groups of women were supposed to be genetically identical at the fragile-X locus—after all, they both had the same part of the same X chromosome.

The fragile X was rapidly confirmed to be due to a heritable unstable DNA sequence (Nakahori et al. 1991; Verkerk et al. 1991). This instability was due to changes in copy number of a trinucleotide repeat p(CCG)n (Kremer et al. 1991) that appears to be in the 5′-untranslated region of a gene (Yu et al. 1992), *FMR-1* (Verkerk et al. 1991), whose transcription is blocked in fragile-X syndrome (Pieretti et al. 1991).

One fascinating property of the unstable sequence is that the repeat copy number usually increases when it is transmitted by women (decreases have been seen, however), whereas it usually remains the same size or decreases when transmitted by men. This means that no two individuals in a fragile-X family need be genetically identical at this locus. Furthermore, at high copy numbers, the sequence is somatically unstable, such that different cells in a single tissue, as well as different tissues, are also genetically different at this locus. We postulated that such an unstable sequence could account for a number of ill-understood genetic phenomena, including anticipation in DM (Sutherland et al. 1991). Both the isolation of the DM gene (*DM-1*) (Brook et al. 1992; Fu et al. 1992; Mahadevan et al. 1992) and the recognition that it contains an unstable DNA sequence (Aslanidis et al. 1992; Buxton et al. 1992; Harley et al. 1992) provided the mechanism for anticipation that this disorder manifests so strikingly;

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and it gave comfort to the clinicians who had observed the anticipation, only to be told by their geneticist colleagues that it was only a mirage.

The unstable sequence in *DM-1* is also a trinucleotide repeat p(AGC)n of composition identical to that amplified in the androgen-receptor gene of individuals with Kennedy disease (La Spada et al. 1991). In DM it has been observed only to increase in size when transmitted. The copy number of the trinucleotide correlates well with the severity of the disease, although the birth of children with congenital DM only to women with the disease is yet to be accounted for. The repeat is transcribed but appears to be in the 3′-untranslated region of a gene whose product is predicted to be a member of the protein kinase family (Fu et al. 1992).

The recognition that unstable DNA has a different mechanism of mutation requires a reconsideration of what mutation is. Mutations of all types previously recognized were *static* mutations; that is, the product of a mutation was no more likely to undergo further change than was the original DNA sequence. Unstable DNA can be considered to be the result of *dynamic* mutation (Pirsig 1991; Richards and Sutherland 1992), where the initial change to a DNA sequence alters the chance of further changes to it.

The concept that only one type of mutation can account for all disease-gene segregation patterns necessarily colors attempts to make observations fit dogma. When a good fit is not found, the validity of the observations can be doubted, or, traditionally, in genetics, some modifying agent is invoked, and terms such as "incomplete penetrance," "variable expression," "different genetic background," "multifactorial effects," etc., are spawned. While some of these terms undoubtedly describe observations, they all reveal that something else is happening and that we do not know what it is. Now that molecular analysis of DM and fragile-X syndrome has revealed unstable DNA as the basis of their mutational mechanism, it may be time to abandon terminology that indicates ignorance and to refer to these as disorders due to unstable DNA and a dynamic mutation mechanism.

In the immediate future it will be of interest to determine which genetic characteristics—e.g. anticipation, incomplete penetrance, variable expression, etc.—are common to all disorders that have a heritable unstable element as their molecular basis. For this reason there has been some reluctance to include Kennedy disease in the category of disorders caused by dynamic mutation. It should be pointed out, however, that the apparently "normal" genetics in this disorder is still in keeping with the behavior of the unstable element, since, in Kennedy disease, the copy number of the repeat has not yet been observed to amplify to the lengths that accord instability in fragile-X syndrome and DM. It may well be that such amplification either is lethal or causes a substantially different phenotype that as yet is not associated with the androgen-receptor gene. It is worth noting that, in Kennedy disease, the repeat, located in the androgen receptor gene is within the coding region, and that amplification, therefore, has a qualitative effect on the encoded protein—that is, it affects function. The repeats in *DM-1*—and probably those in *FMR-1*—are located in untranslated regions and therefore only have quantitative effects on the gene product. This is exemplified in the case of fragile-X syndrome, where the majority of fragile-X males have no *FMR-1* mRNA (Pieretti et al. 1991).

The discovery of unstable DNA sequences means that genetic concepts that reflect the rules obeyed by static mutations may no longer be appropriate. There are messages in the resurrection of anticipation, for both the laboratory geneticist and the clinical geneticist. Clinical observations are important. The fact that they may be ideologically unpopular or that there is no known mechanism to explain them should not be used as reason to deny their validity.

Many genetic diseases are relatively rare. Because fragile-X syndrome and DM are common, however, they were recognized to have unusual features to their inheritance, and many families were available for study. How many of the less common diseases have similar properties? How many others that do not fit with classical Mendelian inheritance may provide clues to other unstable sequences in the genome or other mechanisms that we have not yet even imagined to exist?

## References

Aslanidis C, Jansen G, Amemiya C, Shutler G, Mahadevan M, Tsilfidis C, Chen C, et al (1992) Cloning of the essential myotonic dystrophy region and mapping of the putative defect. Nature 355:548–551

Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, et al (1992) Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. Cell 68:799–808

Buxton J, Shelbourne P, Davies J, Jones C, Van Tongeren T, Aslanidis C, de Jong P, et al (1992) Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. Nature 355:547–548

Fu Y-H, Kuhl DPA, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJMH, et al (1991) Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 67:1–20

Fu Y-H, Pizzuti A, Fenwick RG Jr, King J, Rajnarayan S, Dunne PW, Dobel J, et al (1992) An unstable triplet repeat in a gene related to myotonic muscular dystrophy. Science 255:1256–1258

Harley HG, Brook DJ, Rundle SA, Crow S, Reardon W, Buckler AJ, Harper PS, et al (1992) Expansion of an unstable DNA region and phenotypic variation in myotonic dystrophy. Nature 355:545–546

Harper PS, Harley HG, Reardon W, Shaw DJ (1992) Anticipation in myotonic dystrophy: new light on an old problem. Am J Hum Genet 50:10–16

Kremer EJ, Pritchard M, Lynch M, Yu S, Holman K, Baker E, Warren ST, et al (1991) Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)n. Science 252:1711–1714

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352:77–79

Mahadevan M, Tsilfidis C, Sabourin L, Shutler G, Amemiya C, Jansen G, Neville C, et al (1992) Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of a candidate gene. Science 255:1253–1255

Nakahori Y, Knight SJL, Holland J, Schwartz C, Roche A, Tarleton J, Wong S, et al (1991) Molecular heterogeneity of the fragile X syndrome. Nucleic Acids Res 19:4355–4359

Oberlé I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boué J, et al (1991) Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. Science 262:1097–1102

Penrose LS (1948) The problem of anticipation in pedigrees of dystrophia myotonica. Ann Eugenics 14:125–132

Pieretti M, Zhang F, Fu Y-H, Warren ST, Caskey CT, Nelson DC (1991) Absence of expression of the FMR-1 gene in fragile X syndrome. Cell 66:817–822

Pirsig RM (1991) Lila: an inquiry into morals. Bantam, London

Richards RI, Sutherland GR (1992) Heritable unstable DNA sequences. Nature Genet 1:7–9

Sherman SL, Jacobs PA, Morten NE, Froster-Iskenius U, Howard-Peebles PN, Nielsen KB, Partington MW, et al (1985) Further segregation analysis of the fragile X syndrome with special reference to transmitting males. Hum Genet 69:289–299

Sherman SL, Morton NE, Jacobs PA, Turner G (1984) The marker (X) syndrome: a cytogenetic and genetic analysis. Ann Hum Genet 48:21–37

Sutherland GR, Haan EA, Kremer E, Lynch M, Pritchard M, Yu S, Richards RI (1991) Hereditary unstable DNA: a new explanation for some old genetic questions. Lancet 358:289–292

Verkerk AJMH, Pieretti M, Sutcliffe JS, Fu Y-H, Kuhl DPA, Pizzuti A, Reiner O, et al (1991) Identification of a gene (*FMR-1*) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905–914

Yu S, Mulley J, Loesch D, Turner G, Donnelly A, Gedeon A, Hillen D, et al (1992) Fragile-X syndrome: unique genetics of the heritable unstable element. Am J Hum Genet 50:968–980

Yu S, Pritchard M, Kremer E, Lynch M, Nancarrow J, Baker E, Holman K, et al (1991) Fragile X genotype characterized by an unstable region of DNA. Science 252:1179–1181