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Review Article: Anticipation in Myotonic Dystrophy: New Light on an Old Problem

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Summary

The concept of anticipation, the occurrence of a genetic disorder at progressively earlier ages in successive generations, has been debated from the early years of this century, with myotonic dystrophy as the most striking example. Throughout most of this period there has been controversy as to whether the phenomenon resulted from observational and ascertainment biases or reflected a more fundamental mechanism. The recent discovery of inherited unstable DNA sequences, first in fragile-X mental retardation and now in myotonic dystrophy, not only confirms that anticipation indeed has a true biological basis but provides a specific molecular mechanism for it; this discovery can explain many of the puzzling anomalies in the inheritance of myotonic dystrophy and may prove relevant to comparable problems in other genetic disorders.

Introduction

"Anticipation" is the term given to the apparent occurrence of an inherited disorder with progressively earlier age at onset in successive generations. It has been discussed in relation to many different disorders, principally those showing autosomal dominant inheritance and variable expression; however, myotonic dystrophy has provided the basis for most of the subsequent data and controversy. Recent developments in molecular genetics, first in relation to fragile-X mental retardation but recently also in myotonic dystrophy, now give for the first time a specific biological explanation for anticipation. In the present article we trace the origin of the concept in relation to myotonic dystrophy, examine the long-running controversy over its validity, and discuss how the new molecular findings can explain how it acts to produce phenotypic variation that varies from generation to generation.

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Historical Background

The concept of anticipation was originally developed in relation to non-Mendelian disorders such as mental illness (Mott 1910, 1911), using institutional patient populations and selected pedigree data, and it was strongly influenced by eugenic concepts of "degeneration" that was considered to be occurring in society. The history of the scientific study of anticipation, though, is, to a remarkable degree, the history of myotonic dystrophy. In 1911, only 2 years after the recognition of myotonic dystrophy as a specific disorder, Greenfield (1911) noted the occurrence of cataract in the earlier generations of a family with myotonic dystrophy. The work of Curschmann (1912) and Fleischer (1916) soon confirmed that cataract was indeed regularly associated with myotonic dystrophy, but it was the later paper of Fleischer (1918), a Swiss ophthalmogist, which showed the presence of cataract in individuals whose descendants had myotonic dystrophy but who themselves had no muscular features of the disease. Fleischer's genealogical studies were able to link different families through these individuals with cataract and through others not known to have any disorder, leading him to suggest that the gene itself might cause no clinical effects in ancestors, that cataract in later life was generally the prinicpal feature in

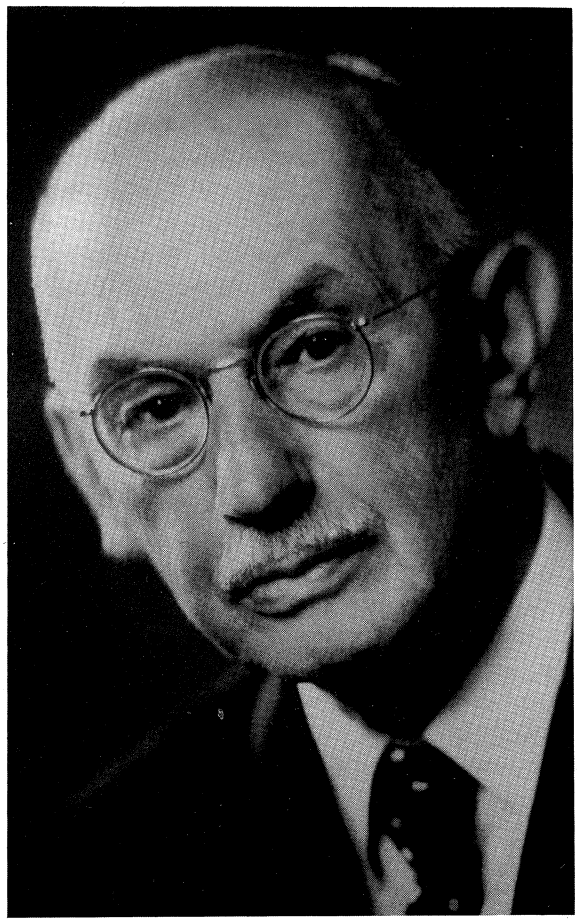

Figure 1 Bruno Fleischer, ophthalmologist (courtesy of Dr. Reinhardt Rudel).

the first generation to be affected, and that muscle disease might occur in subsequent generations. While Fleischer did not use the term "anticipation," his observations accurately established the phenomena in myotonic dystrophy that demand an explanation and that have aroused so much controversy over the subsequent three-quarters of a century.

Fleischer's clinical observations were reinforced by a further report by him in 1922 and by comparable observations by other workers on myotonic dystrophy (Henke and Seeger 1927; Maas 1937). These conclusions did not cause controversy at the time, partly because they were largely descriptive and partly because relatively little was then known about genetic mechanisms that might have made them appear unusual. Henke and Seeger (1927) did discuss possible mechanisms, suggesting a somatic effect that might permanently alter the gene, a possibility that Mott (1911) had earlier invoked with regard to mental illness.

The first attempt at a detailed and quantitative analysis of the genetic aspects of myotonic dystrophy was that of Julia Bell (1947); she assembled pedigree and clinical data on all families reported up to that time, as well as unpublished British data. Her material, while heterogeneous in origin, was critically assessed and formed the foundation not only for her own analyses but for those of Penrose (1948). Bell noted the extreme variability in the clinical features of myotonic dystrophy and of age at onset and death and found, as did her predecessors, evidence of anticipation, which she termed "antedating." Statistical tests (used for the first time) showed a strong correlation (.65) for age at onset between sibs but showed a much weaker value (.32) between parent and child; her data showed a high degree of significance for earlier onset in the second generation than in the first, the χ^2 value of 47.558

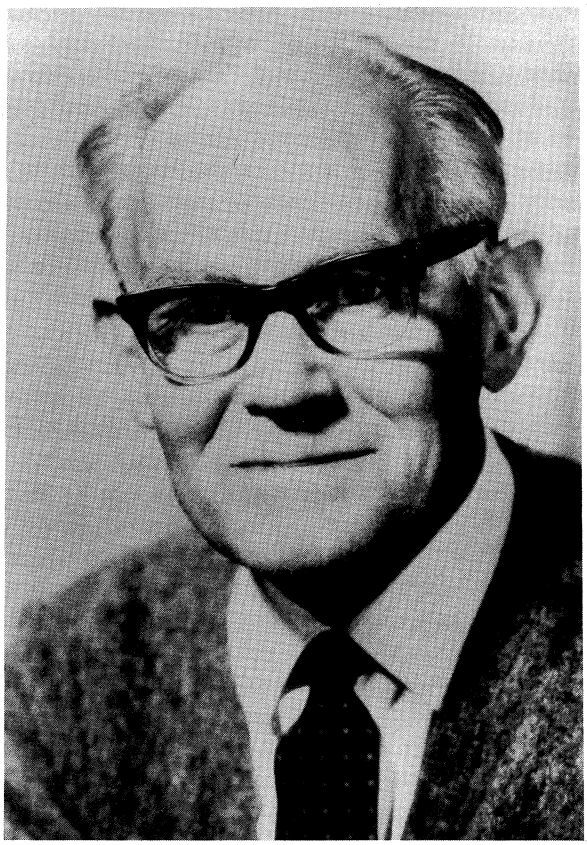

Figure 2 Lionel Penrose, human geneticist (courtesy of Dr. Shirley Hodgson).

"giving an exceedingly remote probability that the observed ages of onset in successive generations provide random samples of the same population" (p. 352). Bell compared the relationship of cataract to muscle disease and concluded that "an examination of the pedigrees must convince the reader that the occurrence of cataract in one or two successive generations is very often found prior to the appearance of dystrophia myotonica in subsequent members of the stock" (p. 360).

In consideration of the general agreement on anticipation in myotonic dystrophy outlined so far, it might seem surprising that it would be debated and largely rejected over the subsequent 40 years, but such was the case. The detailed and thoughtful critique by Höweler (1986) discusses the likely reasons, but two seem predominant. The first was the increasing knowledge of genetic processes by the late 1940s, which appeared to make the modification of a gene from one generation to the next unlikely, whether by somatic or other means. The lack of a plausible mechanism by which to explain anticipation was gradually transmuted into the implausibility of its actual occurrence. The other major factor was the influence of the geneticist Lionel Penrose, who was based at the Galton laboratory in London where Julia Bell's study had been performed and who was working in close conjunction with J.B.S. Haldane and R. A. Fisher. Penrose made a detailed analysis based on Bell's data for myotonic dystrophy and other genetic disorders. He devised an "index of anticipation" based on the parent-child difference and SD and found that myotonic dystrophy gave a much higher value (.9) than did any other disorder analyzed. However, his conclusions were quite different from those of Bell and previous workers; he attributed the anticipation of myotonic dystrophy to a combination of the exceptionally low parent-child age-at-onset correlation (already demonstrated by Bell) and a number of observational biases.

The three main biases identified by Penrose were preferential ascertainment of parents with late-onset disease, since earlier onset would have caused diminished fertility; preferential ascertainment, because of severity, of those in the childhood generation with early onset; and preferential ascertainment of parent-child pairs with simultaneous onset. This last bias would be inevitable in any study performed over a short period, since the opposite situation of early onset in parent and late onset in offspring would require study over decades for detection. Penrose postulated that these "complementary pairs" existed but had not been recorded.

Penrose was not convinced that these biases alone explained anticipation in myotonic dystrophy, but he suggested that the low parent-child correlation exaggerated them, this being produced by a normal allele that affected age at onset and that could not be passed to the offspring along with the allele producing the disease; Goldschmidt (1938) had earlier proposed a somewhat similar mechanism. Penrose did not consider the possibility that the low parent-child correlation might itself be the result of anticipation. His overall conclusion was that anticipation was apparent, rather than real, and that it did not require a novel biological explanation.

It seems puzzling, in retrospect, why Penrose should have reached such conclusions in the face of Bell's and other evidence, but a clue can be obtained if we look at the original papers of Mott (1910, 1911) that had first proposed anticipation. Mott was an ardent eugenicist; his conclusion—that, almost invariably, in the case of insane parents and offspring, the offspring is affected earlier than the parent—was accompanied by inflammatory statements on the degeneration of society and on the threat posed by the mentally ill and "feeble-minded," as well as by other elements. "At the present time in Great Britain restriction of families is occurring in one-half or two-thirds of the people, including nearly all the best, while children are being freely born to the feeble-minded, to the pauper, to the alien Jew, to the Irish Roman Catholic, to the thriftless casual labourers, to the criminals and others" (p. 1251). Mott likened his "law of anticipation in the insane" to "rotten twigs continually dropping off the tree of life" (p. 1255).

It is hardly surprising that Penrose, an ardent anti-eugenicist and supporter of rights for the mentally handicapped, as well as the first worker to apply genetic approaches systematically and critically in the study of mental handicap (Penrose 1933, pp. 66–67, 132–133), should have been more inclined to detect the very real flaws in such material than to accept the possibility that in some situations anticipation might be genuine. The influence of Penrose and his colleagues in all fields of genetics was, and remains, profound and pervasive. Nevertheless, this does not excuse the remarkably uncritical acceptance, by most geneticists over the subsequent decades, of Penrose's dismissal of anticipation. The textbooks by Stern (1960) and by Vogel and Motulsky (1986), in other

respects among the most critical of works, repeat the view of anticipation as purely due to bias, while other books, including those by Bodmer and Cavalli-Sforza (1976) and Gardner and Snustad (1984) and King and Stansfield's (1990) *Dictionary of Genetics,* do not mention it at all. A particular problem arose in the joint work of Caughey and Myrianthopoulos (1963); Myrianthopoulos (a geneticist), insisting that bias was the sole explanation, considered that "some of the proposed explanations concerning cataract and progression in dystrophia myotonica touch on the absurd" (p. 205). Despite this, his clinical coauthor Caughey, like many clinicians, took a different view, observing that "the problem of anticipation is a vexed one. My collaborator, Dr Myrianthopoulos, and I do not see eye to eye in this regard" (p. 70).

Thus from the time of Penrose's (1948) paper until nearly the present time, a remarkable dichotomy of views has existed on anticipation. Geneticists have almost unanimously dismissed anticipation as an artifact resulting from observational biases, whether occurring in myotonic dystrophy or other disorders. Clinicians, by contrast, while grudgingly accepting that geneticists should know best about the behavior of genes, have insisted that in myotonic dystrophy anticipation is a true phenomenon, even though they could provide no obvious mechanism to explain it.

The Emergence of a Biological Basis for Anticipation

The past decade has seen a softening of the traditional concepts of Mendelian inheritance and of the gene as a body with fixed properties that is only to be altered by rare mutation. Genomic imprinting, uniparental disomy, and, most recently, unstable DNA sequences, have all emerged as concepts that are acting to modify the classical patterns of transmission, making phenomena such as anticipation less implausible than to geneticists of previous years. It is relevant to examine this problem as it has influenced anticipation in myotonic dystrophy.

The first important step was the recognition of congenital myotonic dystrophy as a distinctive syndrome (Vanier 1960) and the demonstration that it was invariably transmitted by an affected mother (Dyken and Harper 1973; Harper 1975). The suggestion that the congenital form might result from an intrauterine factor influencing a genetically predisposed fetus (Harper and Dyken 1972) remains unproved to this day, but it provided for the first time a partial explanation for anticipation, since these individuals, who were severely affected and who usually were unable to reproduce, must, on this basis, have had an affected parent. The worsening between parental and childhood generation could thus be explained, though on a presumptively nongenetic basis. At this stage, many considered the anticipation controversy to be resolved, with a true biological factor underlying the congenital form and with observational bias explaining the apparent anticipation in earlier generations. Like many apparently satisfying compromises, this has proved to be wrong.

The next major step in the reemergence and validation of anticipation was the study by Höweler (1986), whose thesis and subsequent paper (Höweler et al. 1989) provided clear genetic evidence that refuted Penrose's explanation of bias as being solely responsible. The thesis is particularly valuable for its detailed family data and full historical critique. Höweler found, as did others before him, that families with myotonic dystrophy showed clear intergenerational differences, with both anticipation and a close correlation between phenotype and age at onset. Analysis of parent-child pairs showed that, in 60 of 61 pairs, age at onset in the child was earlier than that in the parent, while, by segregation analysis, he was also able to show clearly that there was no room for the supposedly "missing" gene carriers, who, according to the Penrose hypothesis, should develop disease at a later date; penetrance of the gene was close to complete, with 46% of offspring affected. He also found that fertility was only slightly reduced for most patients with adult onset, thus removing another of the supposed biases that Penrose had postulated.

Another possible biological explanation emerged in the late 1980s, with the recognition of genomic imprinting as a possible mechanism for intergenerational differences, especially those showing an effect varying with the sex of the transmitting parent (Clarke 1990; Hall 1990). The possibility of imprinting being involved in myotonic dystrophy was strengthened by the finding that the relevant region of chromosome 19 was homologous to a region showing imprinting in the mouse. So far, methylation has not been studied at the myotonic dystrophy locus, nor does imprinting fully explain the progressive and apparently irreversible intergenerational change, when transmitted by either sex, that is characteristic of myotonic dystrophy.

The most fruitful source of a biological basis for

anticipation has come from studies on fragile-X mental retardation. The remarkable similarity between the intergenerational differences seen in this disorder and those of myotonic dystrophy was stressed in the revised edition of Harper (1989), by which point that author had become convinced of the validity of anticipation. However, it remained merely an interesting parallel until the summer of 1991, when the demonstration of an unstable DNA sequence in fragile-X mental retardation (Fu et al. 1991; Oberlé et al. 1991; Verkerk et al. 1991; Yu et al. 1991) not only resolved its molecular basis but provided a clear and satisfying explanation for anticipation. The occurrence of a variable and potentially unstable CCG repeat sequence giving clinical consequences when expanding beyond a certain length can explain the existence of clinically normal transmitting males as well as the occurrence of affected males in the offspring of carrier females, the molecular abnormality showing a close correlation with phenotype. Shortly after this discovery, Sutherland et al. (1991) predicted that unstable DNA sequences might be responsible for other examples of variation in genetic disease, including anticipation in myotonic dystrophy, thus providing a spur to search, in this disorder, for a molecular mechanism comparable to that responsible for fragile X.

By late 1991, therefore, anticipation in myotonic dystrophy had reached the point not only of having clear genetic evidence that it represented reality rather than artifact; it also suggested several biological mechanisms that might produce it, one of which had already been shown to underlie anticipation in another disorder. The discovery, at the end of the year, of an unstable DNA sequence in myotonic dystrophy was thus of general and theoretical importance and represents a major advance in our understanding of the disease itself.

Unstable DNA and Anticipation in Myotonic Dystrophy

The independent finding, by two groups (Buxton et al. 1992; Harley et al. 1992), rapidly confirmed by a third (Aslanidis et al. 1992), of specific molecular abnormalities in myotonic dystrophy, with a variable DNA insert of as much as 5 kb in length, now permits some preliminary conclusions, as well as directed speculation, as to its relationship to anticipation. The conclusions can only be tentative until a larger series of families is studied and until the insert and gene are more fully characterized, but several points are already clear. First, there is at least an approximate correlation between insert size and both severity of disease and age at onset. Second, the insert can be seen to lengthen in successive generations; it has not yet been observed to shorten, although this has been noted for fragile-X mental retardation. Increase in fragment length has been noted in the offspring of both affected males and females and in more than one generation, so that the maternal transmission of congenital myotonic dystrophy is not currently explained by the behavior of the unstable fragment. It is possible that the earlier hypothesis of an intrauterine factor or the operation of imprinting may be involved in this.

Our detailed knowledge of the molecular defect in myotonic dystrophy remains very preliminary in comparison with that in fragile X (Fu et al. 1991), though this is likely to change rapidly, since the gene has now been isolated and since its structure predicts the product to be a member of the protein kinase family (Brook et al. 1992; Fu et al. 1992). However, in one respect, we know more than we know for fragile X, since studies on linkage disequilibrium in myotonic dystrophy (Harley et al. 1991) strongly suggest that a single ancestral mutation has been responsible for most cases of the disorder. If this is indeed the case, it implies that there are a very large number of intervening gene carriers who have few or no clinical abnormalities but who must have had the mutation or premutation. Studies of extensive genealogies, such as those in northern Quebec (Mathieu et al. 1990) or northern Sweden (G. Holmgren, personal communication), have identified kindreds whose common ancestor lived more than 300 years ago, suggesting that the gene may have passed through 15 generations or more without producing obvious effect; it is possible that, as with fragile X, instability and hence anticipation occur only after the unstable sequence passes a certain critical length. Current molecular research should rapidly resolve many of these aspects.

It is worth asking, as did Sutherland et al. (1991), whether anticipation due to unstable DNA is likely to prove to be a widespread phenomenon underlying genetic variation and whether disorders other than myotonic dystrophy and fragile-X mental retardation will prove to be involved. One obvious possibility that deserves careful examination is Huntington disease, for which considerable evidence now exists for anticipation in male-transmitted cases, especially those ending in juvenile disease (Ridley et al. 1988, 1991). As with genomic imprinting, the recognition of unstable DNA as a biological mechanism underlying anticipa-

tion should prompt reanalysis of data separated by generation, to see whether anticipation exists in situations previously unsuspected. Such an approach was attempted previously by Penrose (1948), but data should now be available for a much wider range of disorders. It would be surprising if unstable DNA were not also found to be important in other species, and data from the mouse and *Drosophila* should be sought.

Conclusion

Anticipation can now be considered as a genuine and proven phenomenon in myotonic dystrophy, with unstable DNA as a specific underlying mechanism, although our knowledge of molecular detail is incomplete and additional factors may prove to be involved. The recent developments have vindicated the observations of a long series of clinicians working on myotonic dystrophy over a span of three-quarters of a century and provide a lesson for investigators to be prepared to consider new mechanisms when faced with observations that have no obvious explanation and appear implausible at first sight.

We now recognize that a wide variety of molecular processes can affect the structure and function of genes and that many of these may find practical expression in terms of variability and puzzling inheritance patterns in human genetic disorders. For myotonic dystrophy, the validation and partial explanation of anticipation throw light on a number of long-standing genetic enigmas; for genetic disorders in general, we now have a further factor that must be searched for and taken into account when we try to explain the range of genetic variation that we see in human disease—and which can have important clinical effects for the families in which it occurs.

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