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THE DEVELOPMENTAL NEUROPSYCHOLOGY OF FEMALE CARRIERS OF THE FRAGILE X GENE

by

VERONICA J. HINTON

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

THE DEVELOPMENTAL NEUROPSYCHOLOGY OF

FEMALE CARRIERS OF THE FRAGILE X GENE

by

Veronica J. Hinton

Adviser: Professor Jeffery M. Halperin

This dissertation examined the developmental neuropsychology of female carriers of fra(X). The relationship among inheritance, gene structure and cognitive skills was investigated in female carriers of the fragile X gene. Subjects were studied molecularly by examining gene state and cognitively by examining performance on a neuropsychological test battery.

Results from 16 women with a maternally inherited fra(X) gene, 16 women with a paternally inherited fra(X) gene, and 18 controls (mothers and sisters of persons with Down Syndrome) indicated: 1) Mode of inheritance influences both behavioral and molecular expression of fra(X). Only a subgroup of the women who inherited the fra(X) gene from their mothers (56%) were affected; 2) Changes in the genomic structure of the fra(X) gene is present in all fra(X) females with mild cognitive deficits. Even in nonretarded subjects, lower IQ scores (e.g. WAIS-R Full Scale IQ $\geq$ 1 standard deviation below the mean) were associated with the full fragile X mutation. Women with the premutation were no different from controls on measures of IQ; and 3) The cognitive

deficits observed are specific. After controlling for differences in overall intellectual functioning, the subgroup of maternal inheritance women with the full mutation had impaired visual attention, while their verbal and general memory skills remained intact. The maternal and paternal inheritance groups with the premutation did not differ from controls or each other on any of the measures.

This work took a genetic approach to the study of brain-behavior relationships. The differential effects observed on cognitive profiles were inferred to be due to varying amounts of a specific gene product. Variation from controls on cognitive measures may be either a direct result of the lack of the product or due to developmental adaptive responses of the CNS to that selective loss. By effectively manipulating one variable in physiological development, the study of a single gene disorder such as fra(X) provides an optimal model for the study of developmental neuropsychology.

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Table of Contents

1. Approaches to the study of developmental neuropsychology 1

2. Fragile X Syndrome

A. Background/ Significance 13

B. Inheritance characteristics 15

C. The fragile X gene 20

D. Cognitive phenotype of females 30

3. Questions to be addressed 50

4. Paper I: Mode of inheritance influences behavioral expression and molecular control of cognitive deficits in female carriers of the Fragile X Syndrome 51

5. Paper II: Molecular and cognitive aspects of Fragile X 66

Table of Contents

6. Discussion

A. Review 85

B. Limitations of the Work 87

C. Specificity of Test Measures 90

D. Functional Specificity 91

E. Performance Specificity 94

F. Etiological Specificity 94

G. Neurological Specificity 96

H. Specificity of the Cognitive Profile 98

I. From Gene to Cognition 100

J. The Fra(X) Female: More Subtle Effects 105

7. Conclusion 107

8. References 108

List of Figures

1. Familial position influences penetrance 16

2. Hypothetical pedigree 19

3. Fragile X chromosome 21

4. Three forms of FMR-1 22

5 Relationship between CGG insert size and risk on subsequent generation 25

6. WAIS-R FSIQ scores in three groups, # 1 58

7. WAIS-R factor scores in three groups 60

8. BVRT performance 61

9 Autoradiograph # 1 63

10. WAIS-R FSIQ scores in three groups, # 2 75

11. Autoradiograph # 2 76

12. Association between genomic structural changes and mild cognitive deficits 78

13. Index by group interaction on WMS-R 81

14. Fragile X as a model for developmental neuroscience 102

Approaches to the Study of Developmental Neuropsychology

Developmental neuropsychology is the study of the maturation of brain-behavior relationships. It focuses on the progressive differentiation of the central nervous system and the concurrent manifestation of increasingly complex behaviors. Thus, it explores the contributions, physiological and environmental, to the development of human cognition. The field tries to determine why we are the way we are, and what caused us to develop that way.

Traditionally, neuropsychology has explored the role of insult or injury to an otherwise normal CNS and then tried to infer functions on the basis of what has observably changed. In this way, measuring behavioral differences between persons with and without the brain injury allows for generalizations about the role of the injured area in the mediation of the behavior. By adding emphasis to the role of development in neuropsychology, the focus shifts from a static system to one that is continually changing and responding to a variety of influences. A myriad of factors contribute to the development of human cognition. Study of developmental disorders, rather than brain lesions, can offer insight into the complex workings of normal cognitive development. Thus, the study of developmental neuropsychology includes studies of developmental disorders of unknown etiology, disorders due to teratogenic agents, and disorders of known genetic etiology (chromosomal, polygenic or single-gene disorders).

Standard psychometric methods used in neuropsychology have been found to be

useful for assessing skills in developmental disorders, but interpretation of performance may be very different from that of normal development. A mature CNS that has developed normally and then is injured may not offer an adequate explanation for the workings of a system that has developed abnormally. A small change in development may have far ranging effects. One thing gone awry early in life may have broader consequences, as developments that occur later are dependent on those that occurred before. Further, since the CNS is known to be somewhat "plastic," behaviors normally assigned to one area or system may be taken over by different substrates as an adaptive consequence to insult in development[1]. A nervous system may "hook up" differently in a developmental disorder. In addition, different areas of the CNS may mediate what appears to be the same function at different stages of development[2]. As such, study of a developmental disorder may be very different from an acquired one. With developmental aberrations, there is no a priori reason to assume either that there will be specific behavioral effects, or that those functions observed to be affected are mediated by the same areas of the brain known to be involved in a healthy, adult nervous system.

Nevertheless, much of the work in developmental neuropsychology is focused on

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[1] Just as the right hemisphere apparently maintains the capacity to take over much of the role of the left hemisphere when it is removed (as demonstrated in cases of children with hemispherectomy (Vargha-Khadem, O'Gorman, & Watters, 1985), so perhaps can other physiological variables compensate - to a degree - for loss or imbalance of a system.

[2] Work by Goldman-Rakic (1974) has demonstrated in monkeys that dorsolateral frontal lobe lesions interfere with expression of certain behaviors at some ages but not at others, suggesting that the same function is subserved by different brain areas at different times during normal development.

investigations of behavioral attributes and tries to define a physiological basis for them, relying on what is known about the normal nervous system. This is necessary because for many developmental disorders, such as dyslexia and attention deficit disorder, the etiology is unknown. This approach is pragmatically useful for guiding treatment. First, in order to determine if something is a definable disorder, symptoms are grouped together and individual variation on a number of measures is assessed. Symptoms that consistently cluster together are reasoned to have a specific etiology. However, behaviors that clinically present similarly actually may stem from very different causes. Just as a stomach ache may be caused by a variety of factors, so may poor attention skills[3]. The etiology of the symptom clusters is then inferred by understanding of the function in a healthy system, and surmising what may be different. The shortcomings of this approach were described earlier. Because of the dynamic and "plastic" nature of the developing nervous system, the same function could be served by different substrates. Studies of developmental disorders with unknown etiology may be clinically relevant, yet they offer only a murky understanding of normal cognitive development.

Another approach to the study of developmental neuropsychology is to manipulate variables in development and then observe subsequent differences in behavioral outcome. This is standard procedure (although ethically suspect) in animal experimentation. Work

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[3] Physiological substrates believed involved in attention include cortical areas such as the temporoparietal-occipital junction, limbic areas such as the cingulate gyrus, and subcortical areas such as the thalamus and mesencephalic reticular formation. Neurotransmitters implicated include norepinephrine, dopamine and acetylcholine. Disturbance in any of these areas/systems may result in the behavioral manifestation of inattention (e.g. Heilman, Watson & Valenstein, 1985).

examining the role of teratogenic agents, such as drugs, radiation or certain viruses, examines the effects of introduction of a toxic agent on development. Yet, the ethical implications of tinkering with human development in order to see what will happen are immense. If an agent is a known teratogen, every preventative effort should be made to avoid exposure. As such, human clinical work in this area tends to be done "after the fact." This leads to numerous methodological problems. Determination of the type and amount of agent, and the stage in development when exposure occurred, is dependent on historical report. In most cases, the mother will be asked to relate details of exposure on the developing fetus or child. The accuracy of the report depends on the woman's knowledge of exposure, her memory and her willingness to comply. Since many teratogens are contraband there may be good reason for her not to divulge accurate information. Confounding the situation further, in cases of drug abuse, many persons tend to be poly-substance abusers, so it is difficult to determine the specific effects of any one substance. Poor general health, malnutrition and lack of adequate health care are often other confounding factors. Further, because of the dynamic nature of the nervous system, exposure at different periods in development may have different effects; there may be "sensitive periods" in development when the nervous system is more susceptible to the effects of specific agents (e.g., Bornstein, 1989). In addition, all of the reservations described above about inferring function from a model based on normal development also apply. Thus, this approach to the study of human cognitive development is also problematic.

Still another approach to studying developmental neuropsychology is to examine the behavioral consequences of genetic disorders. This has the distinct advantage of studying symptoms with a known etiology; the "manipulation" in development is definable. Thus, the

underlying causal factors are known, not presumed, and they are due to too much of or a lack of substances that normally are involved in development. The influence of these physiological variables on behavior can be measured across individuals. In addition, how that influence is mediated can be studied. These disorders can be subdivided into chromosomal, polygenic and single gene disorders. Human chromosomal abnormalities are quite common and generally result in spontaneous abortion. Those fetuses that do survive usually have multiple defects, including mental retardation. This is because chromosomal abnormalities involve gross genetic imbalance. Cells have either too much or too little chromosomal material, so vast numbers of gene products are likely affected. The best known example of this is trisomy-21, or Down syndrome. Down syndrome generally presents clinically as mental retardation, with verbal and motor skills being particularly poor. Usually, persons with this disorder have an extra copy of one chromosome (the smallest autosome[5]) in every cell[6]. The effect of the extra chromosome copy probably results in greater doses of the gene products normally coded for by the genes on the chromosome, which cause multiple developmental anomalies. Even more severe effects are seen in cases where chromosomal material is missing. Monosomy, or only

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[5] The triplicate chromosome is the smallest (actually number 22 by size), yet the name trisomy-21 is maintained for historical reasons.

[6] This is due to nondisjunction during meiosis; the chromatids failed to separate, so one gamete ended up with both copies (instead of one) of the chromosome. After fertilization, three copies of the chromosome are maintained. Less severe cases, where some cells maintain the normal complement of chromosomes while some have the triplicate copies, are known as mosaics. In translocations, where segments of one chromosome attach to another, the resultant effects may be similar to having multiple copies of the chromosome because the extra piece can be found in conjunction with the normal chromosomal complement.

one copy of a chromosome, is lethal (except for the X chromosome). Cases of deletions of portions of a chromosome likely result in too small amounts of crucial gene products necessary for normal development[7]. Thus, by studying chromosomal abnormalities, the relative contribution to neuropsychological development of the multiple gene products (most of which are unidentified) can be inferred. However, because of both the severity of the behavioral effects and the multiple variables involved, understanding of specific mechanisms leading to selective effects is difficult at best.

Disorders of the sex chromosomes have more specific, less severe effects. Normally, females have two X chromosomes (although in each cell only one is active)[8] and males have one X and one Y (which is very small). The X chromosome has the greatest contributing effect. Cells with only a Y chromosome are not viable. In cases where there is only one X, or Turner's Syndrome, the X remains active in every cell, but selective deficits are observed. Females with the X0 genotype have prototypical physical characteristics (e.g. sterility, short stature, webbed neck), and overall normal IQ scores with selective deficits in spatial ability and directional sense (Money, 1968). Thus, even though in every cell there is one active X

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[7] An example of this is "cri du chat," where a portion of the short arm of chromosome 4 or 5 is missing. The syndrome is named for the unusual cry (cry of the cat) during the first months of life. It results in severe retardation.

[8] In the third week of gestation, one X chromosome in each cell is inactivated, and subsequent cell proliferation in that line maintains the same inactive X. Thus, females are mosaics for that X chromosome; in some of their cells it comes from their mother, while in other cells it comes from their father. The inactive X chromosome is called a chromatin, or Barr, body (Lyon, 1961). Further, during segregation of the germ cells, only one X chromosome enters each cell; the inactivated X chromosome must be reactivated at this point.

chromosome, the lack of variation in origin of the X may interfere with normal cognitive development. Another possibility is that the inactivated X may contribute something to the system. Again, because of the amount of genetic material on the X chromosome, the variables are not easily specified. Yet the idea that origin of gene products, rather than dose, may be necessary for proper development is intriguing.

Other sex chromosome disorders, involving multiple copies of either X or Y, also result in cognitive impairment and in some cases mental retardation. Extra X chromosomes tend to be inactivated, but as the number increases, so does the severity of symptoms. Thus, inactivation is not sufficient to prevent the chromosomes from having effects. Multiple Ys also affect cognitive skills. The finding of an elevated crime rate for XYY men was once believed due to increased aggressiveness, yet it is now accepted more as a reflection of lower intelligence; they are more likely to get caught (Witkin et al., 1976). What is of interest is that multiple copies of the sex chromosomes generally do not have as severe an effect on cognitive development as do multiple copies of the autosomes. Thus, they may have fewer gene products related to the expression of cognitive functions. Still, the amount of genetic material and the number of physiological variables involved is great, so few, if any, inferences about specific contributions to cognitive development can be made.

Disorders involving multiple genes are probably most common and the least well understood. Many of the developmental disorders described earlier as having unknown etiology likely fall into this category, and with time their basis will likely be determined. Many also appear to develop only when a complex combination of environmental and genetic influences occur together. Advances in molecular genetics, particularly the use of linkage

analysis, allow for study of the relationship between linkage of observed traits and chromosomal markers by examining populations of persons grouped by behavior and probed for similarities in their DNA[9]. In polygenic disorders, genetic and environmental variability can be studied using this approach. For example, dyslexia (which is likely a heterogeneous group of disorders), can be linked to chromosome 15 for 30% of the persons studied (Lubs, et al.,1991). This explains a great deal about the genetic contribution to the manifestation of the disorder, but offers little in the way of understanding the underlying mechanisms of its expression.

A more controlled approach to the study of developmental neuropsychology comes from the examination of single gene disorders. There are over 100 known single gene disorders that clinically present as mental retardation (McKusick, 1986). A mutation in a single gene can have devastating consequences on cognitive development, and the contribution of that gene product to normal development can be assessed. This method is akin to the classic CNS lesion method of study, but on a molecular level. Instead of removing a particular area of the CNS and studying the behavioral effects, single gene disorders demonstrate the consequences of removing a particular protein. The protein may play many roles in development and have far ranging effects, and the detrimental effects on cognition may be secondary. This is true of many of the inborn errors of metabolism. The best known

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Linkage analysis involves the use of restriction fragment-length polymorphisms (RFLPs), pieces of DNA of variable size that reflect the presence of sites known to be sensitive to enzyme cuts, or particular DNA sequences. This involves the presence of normal varaition in the DNA that makes it possible to distinguish homologous chromosomes, (e.g., to ditinguish one X chromosome from another).

example of this is phenylketonuria (PKU), where a mutation in a single gene results in the lack of an enzyme necessary for the conversion of phenylalanine to tyrosine. If the conversion is blocked, levels of blood phenylalanine increase, with the resulting effect of depressing blood levels of other amino acids necessary for development of the nervous system. If untreated, the behavioral outcome is progressive worsening of cognitive skills, leading to mental retardation.[10] Thus, single gene disorders, like CNS lesions, may have nonspecific consequences. Ablation of a CNS area may disrupt function of all the areas normally influenced by the lesioned site, so the behavioral consequences cannot be inferred to be due directly to the function of the removed area. Similarly, behavioral effects of a missing gene product may not be due directly to that product. Yet, in single gene disorders the first step in the causal chain can be established.

Most of the known single gene disorders have severe effects. This is partially because those disorders are more obvious to characterize, so it is easier (using molecular linkage techniques) to find the gene. Distinct behavioral features increase the likelihood of locating a single gene, not of having it as a cause. Thus, subtle cognitive traits are likely linked to single genes, but they are more elusive to identification.

Some single gene disorders that have mild cognitive effects are known. These provide a model paradigm for the study of developmental neuropsychology. The specific cognitive consequences of removing (or decreasing the amount of) a single gene product crucial to a

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10 This disorder can be treated, and the cognitive decline prevented, by restricting the amount of phenylalanine in the diet.

developing system can be studied. The nature of neuropsychological assessment can be much more detailed than in disorders presenting with mental retardation. Therefore, natural selective manipulation of a single physiological variable can be assessed more thoroughly. Nevertheless, there is still a great deal of phenotypic variability in single gene disorders, reflecting the dynamic nature of development.

Neurofibromatosis-1 (NF-1) is an example of such a disorder. A defect in a single gene on chromosome 17 results in neurocutaneous problems. Persons with NF-1 generally have normal IQs (although scores are lower than those of control siblings), and specific visual-spatial deficits (Eldridge et al.,1989). Similarly, persons with PKU, in whom levels of blood phenylalanine are not high enough to result in mental retardation, often show cognitive deficits in problem solving skills (Welsh, Pennington, Ozonoff, Rouse & McCabe, 1990). And women with Fragile X syndrome (fra X) who have one functional and one mutated copy of a single gene (which is linked to mental retardation when it occurs by itself) may show selective cognitive deficits (Baumgardener, et al., 1992; Miezejeski & Hinton, 1992; Pennington, et al., 1991).

Thus, variability in cognitive traits can be reduced to the level of a single gene. This is not to say that a link between one gene - one behavior is established. Nor is it meant to lessen the obvious importance that environmental influences have in shaping the occurrence and degree of behavioral expression. Understanding what genes do and how they contribute to the development of the nervous system and behavior is essential to the study of developmental disorders. "Genes are not magical elements that somehow blossom into behavior patterns, as when the puppeteer pulls a puppet's strings. Genes are segments of

DNA that code for protein production. In that sense, all aspects of ourselves - our bones as well as our behavior - are by-products of this process," (Plomin, DeFries & McClearn, 1990, p. 84). Understanding of a single gene disorder offers insight into the foundations of human cognitive development.

This dissertation describes one such disorder, Fragile X syndrome. In fra(X), understanding of a single gene defect can be studied both by examining its behavioral consequences and by understanding its molecular basis. Inheritance-related changes in the structure of the gene are observed. Thus, "fra(X) may be one of the first disorders for which the primary diagnosis will be based on the direct analysis of a mutation at the DNA level," (Shapiro, 1991, p. 1737). Definitive molecular characterization of the disorder can be made. Nonetheless, there is a very wide range of phenotypic presentation. Innumerable physiological and environmental factors contribute to development, so that each person's phenotypic rendition of the effects of the mutation is distinct. Still, some definite within-group similarities in behavior are observable. The full mutation results in mental retardation with characteristic behavioral attributes in males. For females, the effects are more subtle.

In fra (X), the effects of inheritance, changes in the DNA structure and cognitive impairment are known to be linked in mentally retarded persons. Whether they are also linked in mildly affected, nonretarded women with fra(X), and whether there are specific cognitive domains that are more likely affected, was investigated in the present study. Although the nature and function of the fra(X) gene product is unknown, associations between the molecular mechanisms that appear to control its transcription and cognitive performance can be made. Speculations regarding involvement of specific neural substrates or systems,

although intriguing, are unsubstantiated.

Study of the most basic genomic start point and the most complex behavioral endpoint in a developmental disorder potentially allows the myriad of intervening steps involved in cognitive development to be worked out. Fra(X) is an ideal, naturally occurring system for the study of developmental neuropsychology.

Fragile X Syndrome

Background/Significance

The study of fra(X) is well suited for the study of genetic influences on neuropsychological development. Fra(X) is the most common form of inherited mental retardation and is associated with a known mutated gene on the X chromosome (Verkerk et al., 1991). The name fra(X) derives from the observation that cells of an affected individual cultured in a folate deficient medium show a characteristic fragile site at position Xq27.3 on the distal arm of the X chromosome . Fra (X) accounts, in part, for the increased incidence of mental retardation in males over females. As an X-linked disorder, fra(X) mainly affects males, causing mental retardation and some autistic-like symptoms in 1/1,200 males. Other behavioral characteristics associated with fra (X) include gaze aversion (Cohen, et al., 1988), speech and language deficits (Sudhalter, Scarborough & Cohen, 1991; Sudhalter, Maranion & Brooks, 1992), and hyperactivity (Bregman, Leckman & Ort, 1988). Females may also be affected, but the effects are generally less severe. The possibility of females with fra(X) having a specific cognitive profile has been suggested (Baumgardner, Green & Reiss, 1992; Miezejeski & Hinton, 1992; Pennington, O'Connor & Sudhalter, 1991), and makes this group of particular interest for the study of developmental neuropsychology. For both males and females, the physical phenotype is considerably more subtle than that of many other syndromes associated with mental retardation. Persons with fra(X) generally do not look obviously dysmorphic. Yet there are some physical characteristics commonly associated with fra(X). These include macro-orchidism in males, hyperextensible joints, long face and large

ears (Hagerman, 1991). In addition, persons with fra(X) are at an increased risk for seizure disorder, mitral-valve prolapse, joint dislocations and lazy eye.

The genetic aspects of fra(X) are particularly intriguing. Fra(X) has an uncommon, non-Mendelian, inheritance pattern. A person's position in a family (relative to that of the affected proband) appears to determine his/her risk of mental retardation (Sherman et al., 1984, 1985). In addition, the fra(X) gene has a propensity to mutate, and appears to change structure in passage from one generation to the next (Kremer et al., 1992; Oberle et al., 1991; Yu et al, 1991). Both of these characteristics may be related to "genomic imprinting," a differential gender effect on passage of the gene. Three apparently different states of the gene are known to exist: 1) that of persons not at risk, 2) that of carriers, and 3) that of persons affected with fra(X). In general, however, the behavioral phenotype of "affected" has been grossly associated with mental retardation. Where mildly impaired persons fall in the molecular spectrum of gene structure is as yet unclear. Thus, in fra (X), a single gene defect can be studied both by examining its behavioral consequences and its molecular basis. Specifically, study of nonretarded females with the fra(X) gene enables investigation of the roles of inheritance, gene structure, and the cognitive domains that are more susceptible to the fra(X) gene's mediating deficits. This offers insight into a single gene's effects on cognition. As fra(X) can be studied from either a top-down (reducing behavioral characteristics to their possible physiological substrates) or bottom-up (speculating on the role of the gene product on nervous system function) approach, the myriad of complex contributions to cognitive development can potentially be determined.

Inheritance of fra(X)

The inheritance characteristics of fra(X) are unusual. Like the X-linked disorders Duchenne muscular dystrophy and Lesch-Nyhan Syndrome, fra(X) mainly affects males. Unlike those disorders, however, some fra(X) males (about 20%) exhibit incomplete penetrance. That is, 20% of the males who have been determined to be carriers for fra(X) are cognitively unimpaired and display no distinguishing phenotype. Females who inherit their fra(X) gene from these nonpenetrant transmitting males, also appear to be phenotypically normal. Their offspring who inherit the gene, however, are frequently impaired. Thus, grandsons of the non-penetrant transmitting male are often mentally retarded and granddaughters may show some cognitive impairment (see Figure 1). Further, the likelihood of expressing the disorder appears to increase with successive generations, a phenomenon known as "genetic anticipation" (Sherman, et al, 1984; 1985).

All males inherit their fra(X) chromosome from their mothers. Females can inherit their fra(X) chromosome from either their mothers or their fathers. Yet, assuming that males with mental retardation do not reproduce, evidence suggests that only inheritance from a female results in cognitive impairment (Sherman et al., 1984; 1985). These data, based on retrospective analyses of pedigrees, suggest that sex of parental origin of the fra(X) gene can be a determinant of effects in offspring. This phenomenon is known as "genomic imprinting"[11]. Another novel aspect of the inheritance characteristics of fra(X) is the

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11 Genomic imprinting has been demonstrated in other human disorders including Prader-Willi Syndrome and Huntington's chorea, as well as in animal models (Hall, 1989).

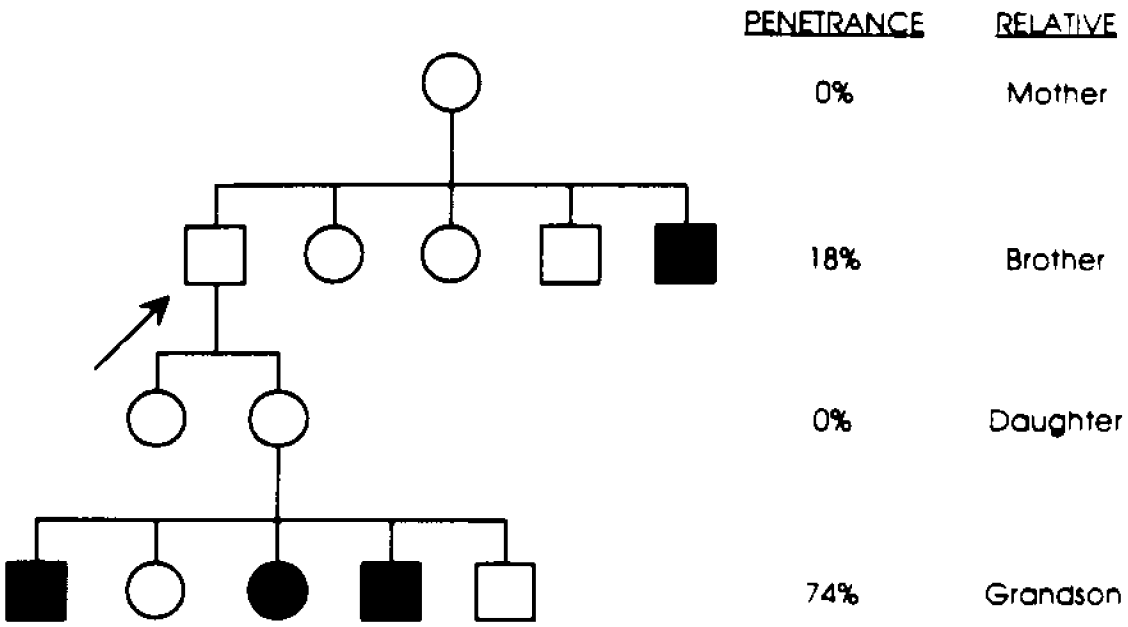

Figure 1.
Pedigree showing that penetrance, or risk of being affected with Fragile X, varies depending on position in a family (from Sherman, 1991).

observation that mothers as well as daughters of transmitting males are rarely, if ever, mentally retarded, and brothers of affected males are more likely to be retarded than are brothers of nonpenetrant males. Analysis of information from over 200 families affected with fra(X) followed the presumed inter-generational route of the gene (Sherman, 1984, 1985). Findings demonstrated that position within a family (e.g. relative to the affected proband) determines risk of mental retardation. This is known as the "Sherman paradox." The paradox is that females who appear to be phenotypically similar, and cognitively normal, have a different likelihood of having affected offspring. If this is so, then even if genomic imprinting is playing a role in the expression of the gene, pedigree analyses suggest not all fra(X) genes mutate when carried by women. Passage by a woman may be necessary, but not sufficient, for the offspring to be affected. It is also possible that women in different positions within a family are not phenotypically similar. That is, even though women may appear to be cognitively normal, subtle cognitive deficits may go unnoticed without psychometric testing. In Sherman's (1984, 1985) classic collaborative study, subjects were not rigorously tested for intellectual skills. Probands were identified cytogenetically; others in the pedigrees were designated "affected" if they had signs of mental impairment. "This limited phenotypic measurement of the fra(X) syndrome was sufficient for males because most have overt mental retardation. However, it was not accurate for females because they have milder mental retardation and their level of intellectual functioning was usually judged subjectively by a family member or the investigator," (Sherman, 1991, p. 84). Only 3 of the 46 daughters of transmitting males, (all of whom must be obligate carriers), studied had intellectual handicaps. It was concluded that inheritance from a nonpenetrant male does not result in fra(X) related

mental retardation. What remained unclear was whether milder cognitive deficits, those not easily subjectively judged, might be present, and if so, whether their occurrence was related to inheritance.

The role of inheritance in fra(X) has led to a number of different models of its genetic basis. One model posits that X inactivation and resultant "imprinting" are necessary for fra(X) expression (Laird, 1987). Another model suggests that a premutation involved in a recombination event is the basis for the heritable differences (Pembrey et al., 1985). Yet another model suggests that unequal crossing over, leading to amplification of a pyrimidine-rich sequence, could contribute to fra(X) expression (Nussbaum et al., 1986). Each of these models, although differing in their molecular mechanisms, predict the same three possible outcomes for female fra(X) carriers: 1) Females who inherit their fra(X) chromosome from an unaffected nonpenetrant male should not be affected because the X has been neither inactivated nor recombined. 2) Females who inherit their fra(X) from their mothers should fall into two groups: a) Those who will not be affected, (where presumably the X has not been imprinted or recombined) and b) those who will have cognitive deficits (where the X may have been either inactivated or recombined). The models, as depicted in figure 2, were based on the Sherman's retrospective data of subjectively determined cognitive skills. The derived predictions can be tested prospectively.

A number of questions regarding inheritance remain unanswered. Do carriers who appear to be cognitively normal differ from matched controls when tested psychometrically? Are there differences in cognitive skills related to mode of inheritance in persons who are not obviously retarded? Does inheritance from a mother result in two different distinct

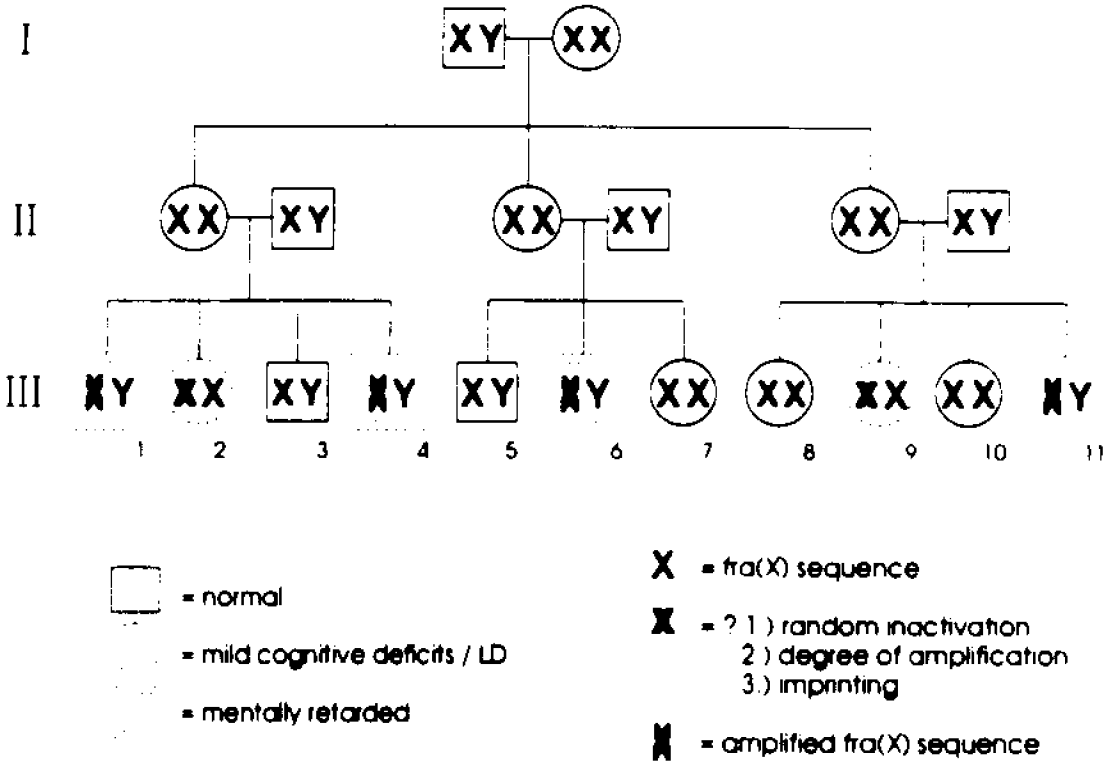

Figure 2.
Hypothetical pedigree demonstrating what may occur in Fragile X as the gene is passed from generation to generation. Females who inherit from males are predicted to be unaffected Females who inherit from females are predicted to fall into two subgroups: some will be affected and some will be unaffected.

phenotypes while inheritance from a father result in only one? Grouping of female subjects according to gender of inheritance, followed by rigorous intellectual testing can potentially address these questions.

The fragile X gene

Recent findings have offered insight into the mechanisms underlying the Sherman paradox. The fra(X) gene has been found to have a simple repeat expansion mutation (Kremer et al., 1992; Oberle et al., 1991; Yu et al, 1991)[12]. Structural changes in an unstable region of the fra(X) gene containing a CGG repeat are related to both transmission and expression of fra(X). Figures 3 and 4 show a representation of this region. Three structurally different states of the fra(X) gene have been identified. The method of identification involves Southern analysis of genomic DNA digested with two restriction endonucleases (one of which is methylation sensitive) and probed for the gene sequence. Normal individuals have a CGG repeat of 90 to 150 base pairs (bp), which corresponds to a 2.8 kilobase (kb) band on Southern analysis. "Transmitting" male and female carriers both have a CGG repeat of 150 to 500 bp, detected as a >3 kb band. This has been termed the "premutation." Affected individuals, however, have a much greater amplification (ranging from 500 to over 3000 bp,

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12

Prior to the discovery of this last year, no other example of this was known. Since then, three other human neurological disorders have been found to have simple repeat expansion mutations. These include myotonic dystrophy, where the triplet CTG is amplified, and spinal-bulbar muscular atrophy (Kennedy's disease) and Huntington's disease, where the triplet CAG is amplified (Davies, 1992; MacDonald, et al., 1993).

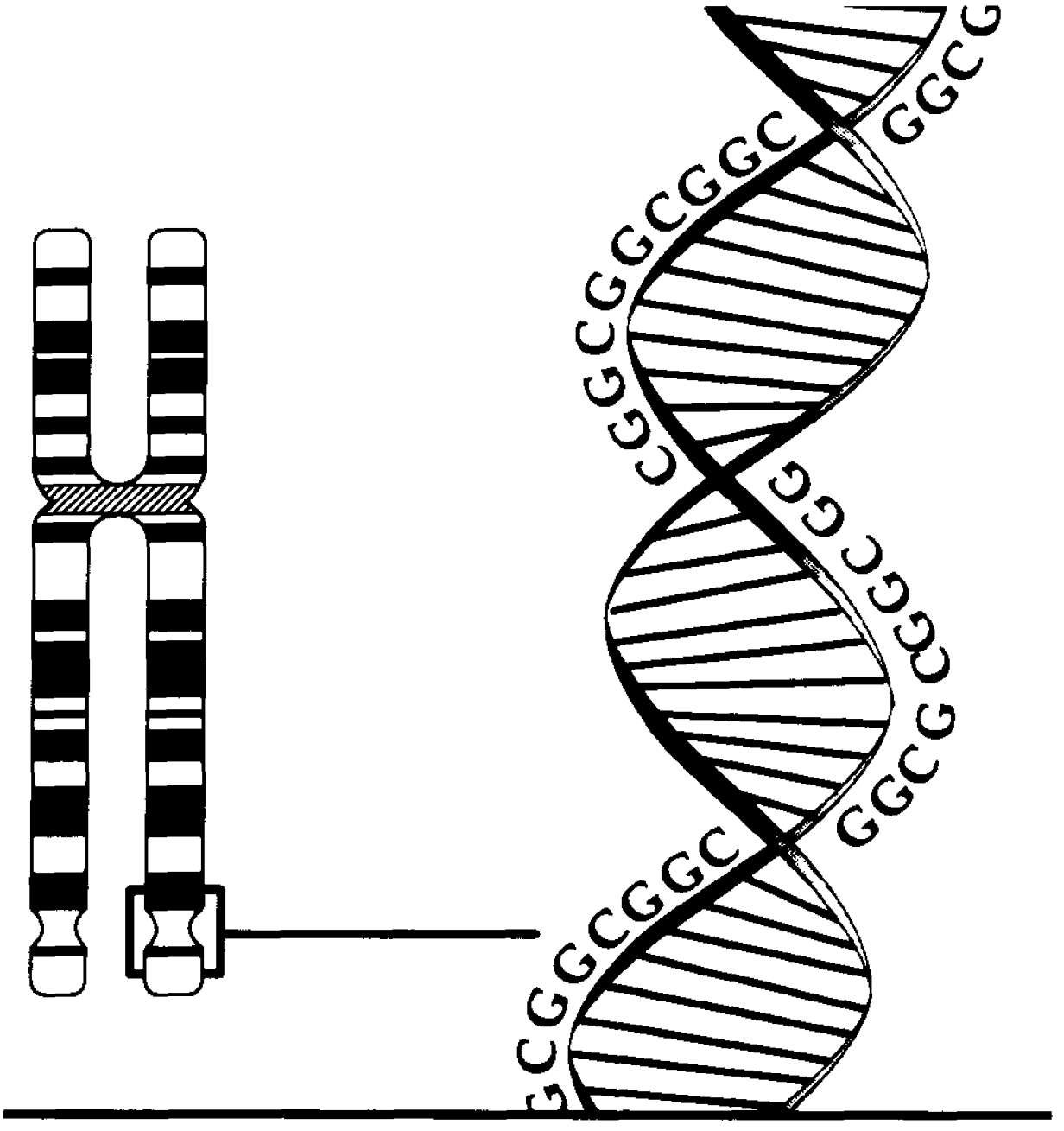

Figure 3.
Figure shows a representation of the X chromosome with a fragile site on the distal arm. Theoretically, if the "fragile" region were greatly magnified, it would show the DNA double helix made up of repeating CGG trinucleotide sequences.

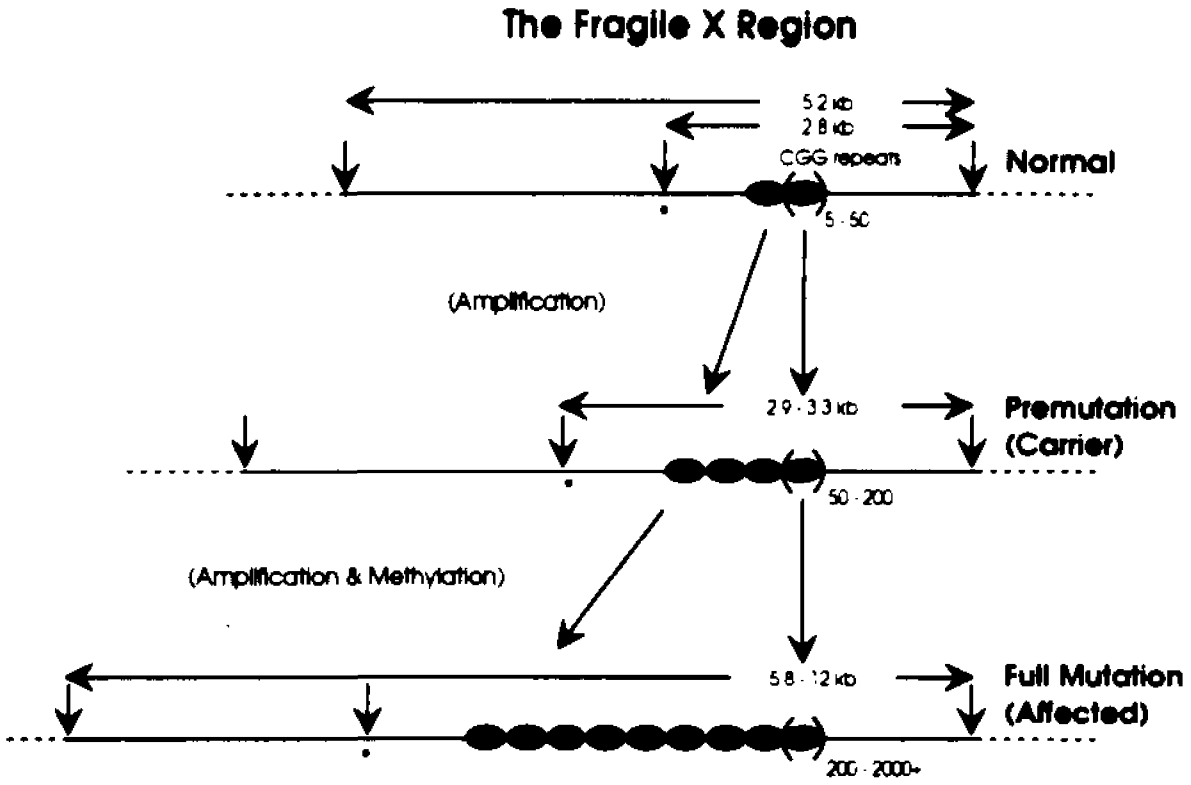

Figure 4.

Figure representing part of the fragile X region containing the CGG repeat that is amplified in carriers and affected individuals. Fragments of the DNA are cut by restriction enzymes (EcoR I, Eag I) at the vertical arrows. DNA methylation prevents cleavage at the position marked by an asterisk (*). Only the fragments containing the CGG repeat are detected by probe StB12.3. A 5.2 kb fragment is detected in normal methylated inactive X chromosomes and a 2.8 kb fragment is detected in normal unmethylated active X chromosomes. Amplification of the CGG repeat leads to an increase in the size of these fragments which is most readily detected in the smaller one (e.g. the $\geq$ 3 kb band). In affected individuals the CGG repeat is both amplified and the DNA is methylated preventing cleavage at the (*) sensitive restriction site, so the fragment detected is larger than 6 kb

corresponding to a >6kb band), as well as hypermethylation of an adjacent "CpG island" region (Bell et al., 1991; Vincent et al., 1991). This has been termed the "full mutation." Thus, two structural elements are involved in the mutation - expansion of the CGG triplet and hypermethylation.

Investigation of the expansion of the CGG repeat clarifies, in part, the Sherman paradox. Marked amplification has been noted only when transmitted by carrier females, not when transmitted by males (Oberle et al., 1991; Yu et al., 1991). Further, Yu et al. (1991) noted that the risk of an affected female carrier having an affected son increased as the length of her amplified sequence increased. Women with inserts less than 200 bp had either transmitting or affected sons, whereas those with inserts greater than 200 bp had only affected sons. The gene has a propensity to mutate, and as the amplification increases, it becomes more unstable. This accounts for the observed increased risk in successive generations, (genetic anticipation)[13]. Since significant amplification has only been noted to occur with female transmission, the sequence is believed to be meiotically unstable[14]. Yet the gene is also

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13

Genetic anticipation is also observed in myotonic dystrophy. Increased copies of the CTG repeat are associated with a worsening of symptoms (not risk as in fra(X)) with successive generations. Spinal bulbar muscular atrophy does not show genetic anticipation (Davies, 1992).

14

In myotonic dystrophy, CTG amplification occurs whether the mutation is passed through either a male or female, yet severe congenital cases are almost exclusively maternally transmitted. In Kennedy's disease, CAG repeats increase with either male or female transmission (Davies, 1992). Whereas in Huntington's disease, paternal transmission is related to the largest trinucleotide repeat expansions, seen

mitotically unstable[15]; within somatic cell lines a variation of length of insert is seen (once the repeat is markedly amplified), which presents as a smear rather than a distinct line on a Southern blot.

Fu et al. (1991) examined in more detail the range of allele sizes and the risk of expansion to the full mutation and found the data fit remarkably well to Sherman's (1984, 1985) risk assessment determined from pedigree data (Figure 5). Again, the risk of a premutation allele expanding to a full mutation (with >500 bp insert and hypermethylation) increases as the size of the premutation allele increases. The variability of insert length is not believed to be associated with degree of impairment (Yu et al., 1991). The size changes are believed to have a threshold effect on the function of the gene. The premutation, although structurally variable and at risk for expanding to the full mutation is still believed to function, while the full mutation is believed to effectively shut down expression (Pieretti et al., 1991).

Interpretation of phenotypic effects of the molecular changes is, at best, imprecise. The problems associated with determination of cognitive status discussed earlier are common across the genetic studies. Individuals are generally loosely characterized as mentally retarded or normal. The wide range of phenotypic presentation is rarely taken into account. The clearest example of the spectrum of cognitive phenotype is seen in females, who can range from being mentally retarded to having specific cognitive deficits to being apparently

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in the juvenile onset cases (MacDonald, et al., 1993).

15 This is also true of myotonic dystrophy but not of Kennedy disease.

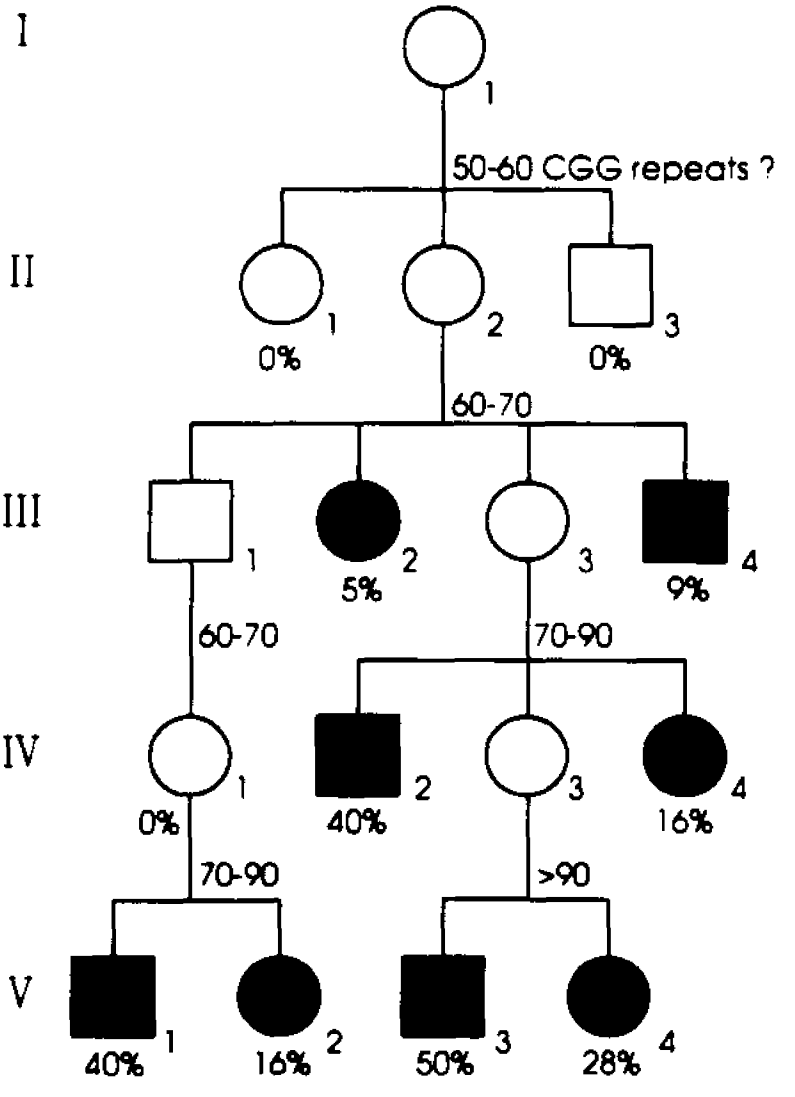

Figure 5.
Pedigree showing relationship between relative risk of being affected and size of CGG repeat As size of CGG insert increases, so does risk of penetrance in the subsequent generation, (from Fu et al., 1991).

unimpaired. The full mutation is associated with mental retardation in males. In females, who have two X chromosomes (one presumably with a functional gene), the effect of the full mutation is less obvious[16]. Rousseau et al. (1991) noted that only 53% of women in their group with the full mutation were mentally impaired. He states that subjects were given a "mental status assessment," but does not specify how. Whether the other 47% of the female group with the full mutation did not differ from matched controls on standardized psychometric measures was not addressed.

The role of genomic imprinting in fra(X) is supported molecularly, as well as cognitively. Even in one case of transmission from an affected male to his daughter, the expansion reduces in length rather than increases, and the daughter is not retarded (Willems et al., 1992). Thus, CGG amplification does appear to be gender specific. Further,

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16

Two other molecular elements, Lyonization and variation due to somatic mutation, may also be related to degree of impairment, through tissue mosaicism. Lyonization, or random inactivation of one of the two X chromosomes in each somatic female cell, results in differential expression of each X chromosome within tissues (or mosaicism). Thus, in female fra(X) carriers, each tissue will be a mixture of cells. Some cells will have the fra(X) chromosome inactivated (and the presumably normal X chromosome active and expressing), while other cells within the same tissue will have the fra(X) chromosome active and expressing (with the presumably normal X chromosome inactivated). Somatic mutation may also cause some variation in gene structure (and presumably gene function) and can lead to mosaicism with respect to the function of the FMR-1 gene in either sex. This would also lead to tissue with a mixture of cells - some with the fra(X) chromosome expressing, and some not. Thus, if only a portion of the cells within any given tissue exhibit the fra(X) mutation, then overall effects of the mutation may be diminished. Degree of cognitive impairment may be correlated with proportion of cells within a tissue (such as the brain) that have the mutated FMR-1 gene.

characterization of 110 female obligate carriers according to inheritance, molecular and unspecified IQ measures suggested that persons with general cognitive impairment ("borderline" as well as mentally retarded) and/or the full mutation were only found in the maternal inheritance group (Smits et al., 1992). Methods for determining IQ were not mentioned, and likely differed across the groups as they were gathered retrospectively. No measures of specific cognitive skills were reported. No comparisons to controls were made. The possibilities of more selective cognitive effects and cognitive effects associated with the premutation were not investigated.

It is the hypermethylation that likely controls expression of the fra(X) gene. In general, methylation is believed to be involved in regulation of many genes. Undermethylation of CpG sequences have been observed in many tissues with known expressing genes, compared to tissues where the gene is inactive (Watson, Hopkins, Roberts, Steitz & Weiner, 1987). The methyl groups could cause conformational changes that might affect DNA repressor (or activator) binding, thus affecting transcription. In fra(X), it is possible that the large CGG expansion may make the area at risk for hypermethylation, since the hypermethylation is observed in conjunction with amplifications over 500 bp. This is true of all tissues studied except fetal chorionic villi samples (Oberle et al., 1991; Sutherland et al., 1991; Yu et al., 1991)[17]. Pieretti et al. (1991) noted that in Northern analysis of mRNA

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[17] Many genes are not methylated early in development. Temporal control of expression is commonly regulated by methylation, and the patterns are heritable (Watson et al., 1987). The amplified CGG region may interfere with this process, resulting in hypermethylation. The product may not be made during critical periods in development.

probed with cDNA of the fra(X) gene, most affected fra(X) males had no mRNA; thus, in these individuals the protein presumably is not being made. However, in a small group of males who had some unmethylated alleles, some mRNA was present. Further, in carriers and normals, mRNA is present. Sutcliff et al. (1992) studied amplification of the CGG repeat, methylation status and presence of FMR-1 mRNA in both human chorionic villi and fetal tissue. In the chorionic villi sample there was evidence of gene transcription, even though marked CGG expansions were observed, they were undermethylated. The fetal sample, however, had both marked CGG amplification and hypermethylation, and no FMR-1 mRNA was detected. As such, it appears that it is the hypermethylation that prohibits transcription.

Regardless of the mechanism potentially controlling the fra(X) gene's transcription, the finding that in affected individuals mRNAs are not being made suggests that it is the lack of the fra(X) gene product that results in the developmental anomalies. What the role of the fra(X) gene product is in development is of preeminent interest. Verkerk et al. (1991) isolated the gene and cloned a complementary DNA sequence. Analysis of mRNA indicates the CGG repeat is transcribed, but whether it is translated is unclear. The FMR-1 protein appears to be unlike other known proteins when compared to sequence samples. However, it does have a consensus peptide sequence for nuclear translocation, so it may be a nuclear protein. Northern analysis of mRNAs in different tissue types indicates expression in many tissues, particularly testes, placenta and brain. In situ hybridization in mouse brian demonstrated diffuse transcription throughout the brain, with granular layers of the hippocampus and cerebellum showing particularly high levels of transcription (Hinds, et al., 1993). Antibodies to the FMR-1 protein have been raised, but to date binding appears to be

nonspecific, so tissue localization is equivocal. As such, understanding of the role of the fra(X) gene product is only just beginning to come to light.

Nonetheless, the product appears to be highly conserved across species. Whatever the FMR-1 protein does, its DNA sequence is found in all mammals studied, as well as chicken, drosophila and yeast (Verkerk, et al., 1992). Warren (1992) has sequenced the mouse FMR-1 protein and found that 98% of the amino acids in the mouse version are conserved in the human sequence. This suggests an evolutionary selection pressure to maintain the protein.

Presumably in humans, lack of the protein results in mental retardation. At minimum, the full mutation is associated with the presence of mental retardation in males. Whether the full mutation is also associated with lesser cognitive deficits is unclear. Similarly, whether persons with the premutation differ from controls on cognitive measures that are more subtle and specific than overall IQ is undetermined. The relationship between the molecular structure of the fra(X) gene and cognition has not been thoroughly investigated.

Again, the difficulty lies in the broad-based classification of "affected" used in most genetic studies. Individuals are characterized generally as mentally retarded or normal by observation or report. Most do not get thorough behavioral and cognitive evaluations. Some have been administered IQ tests and some "affected" persons with borderline functioning have been observed. But even the use of IQ score as a defining marker of phenotype is problematic. IQ scores are very general composite measures of test performance and do not take into account more subtle cognitive characteristics. Low IQ scores may be due to generalized depressed skills or to poor performance in circumscribed areas that effectively

pull the total IQ score down. In addition, tests are frequently not administered in a controlled manner. Measures are gleaned from school records or clinical assessments, and the type of tests used to determine IQ often are not specified and oftentimes vary even within groups. As such, classifications based on subjective report or IQ score provide limited information regarding phenotype. Standardized testing of specific cognitive skills as well as overall IQ test performance is necessary for controlled evaluation.

A number of questions regarding the mutated fra(X) gene and cognition can be addressed. Are there cognitive phenotypic differences between non-retarded persons with the three different states of the gene (normal, premutation, full mutation)? Are changes in molecular structure associated with specific cognitive deficits? Do individuals with a maternally inherited premutation differ from persons with a paternally inherited premutation? Psychometric testing of controls and of nonretarded female carriers of the fra(X) gene grouped according to inheritance can answer these questions. Future studies examining the amount, temporal control, specificity and function of the gene product will offer more information regarding neuropsychological development.

The cognitive phenotype of fra(X) females

One in 700 women is thought to be a carrier of fra(X). Of those, one third are believed affected (Hagerman & Smith, 1983; Sherman et al.,1985). This may be an underestimate, however, since many of the mildly affected women may go undiagnosed. As mentioned previously, the phenotypic presentation is generally less severe than in males, and in some cases may not be readily observed without psychometric testing. Women range from

being apparently unaffected, to having specific cognitive deficits, to being mentally retarded. The roles of inheritance and of gene structure and function on this variable phenotype have not been carefully investigated.

The nature of the cognitive phenotype has been studied. Unfortunately, research examining fra(X) females' intelligence and neuropsychological test performance has generally been fraught with methodological problems. The work has suffered both from poorly delineated subject groups and poorly designed neuropsychological test batteries. Numerous studies have loosely grouped females with fra(X) without taking the issues of inheritance, familial position and (because of its recent discovery) gene structure, into account. Only the more recent studies considered variables such as age and cytogeneticstatus[18]. Further, many studies had no, or inadequate, control comparisons. Most test batteries were chosen to obtain descriptive information and were not hypothesis-driven. As such, interpretation of much of the data is problematic. Nonetheless, some general consistencies do emerge across much of the literature. Most, but not all, studies suggest that not all heterozygotes are affected and those who are affected may have particular difficulties in nonverbal areas. Reviews of the literature have suggested that a particular cognitive phenotype and a specific learning disability may exist in women with fra(X) (Baumgardener, et al., 1992; Miezejeski & Hinton,

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18

Cytogenetic status refers to the cytogenetic visualization of the fragile site on the X chromosome. Cells are grown in a folate deficient medium, fixed, and the number with microscopically observed fragile sites (generally out of 100 cells) is counted. This gives a percent fragility. Cytogenetically expressing cells have been associated with mental impairment and the full Fra(X) gene mutation state (Rousseau, et al., 1991).

1992; Pennington, et al., 1991).

In 1984, Miezejeski, et al. noted that females mildly affected with fra(X) may have different cognitive profiles than those more severely affected and that, in general, their Performance IQ (PIQ) scores on the Wechsler intelligence tests were lower than their Verbal IQ (VIQ) scores. Miezejeski's work examined IQ scores in seven females from two families, and found a VIQ minus PIQ difference of ten or greater in five subjects. The authors note that these findings may be due to familial traits unrelated to fra(X). Hagerman and Smith (1983), reported no reliable IQ scale differences in six fra(X) females. In four school-age girls, relatively lower Wechsler Intelligence Scale for Children - Revised (WISC-R) scaled scores on the Arithmetic, Digit Span and Block Design subtests were noted. Further, they reported attentional difficulties, memory deficits and visual motor coordination problems in both retarded and nonretarded fra(X) females.

In an expanded presentation of their data, Miezejeski et al. (1986) examined Wechsler subtest scores, Wide Range Achievement Test (WRAT) scores, and Benton Visual Retention Test (BVRT) performance in seven nonretarded women (six from the original two families, and one from a new family). Results indicated poor performance on: the Wechsler Arithmetic, Digit Span, Block Design and Object Assembly subtests; the Arithmetic portion of the WRAT; and the recall section of the BVRT. The authors suggested that females from fra(X) families have a profile of cognitive deficit that "may present with developmental learning disability characterized by relative arithmetic and visual spatial deficits" (Miezejeski et al., 1986, pp.408). Unfortunately, their sample size was small, heterogeneous (subjects of various ages, generations, cytogenetic status, and parental inheritance status are included),

and no comparisons to controls are made.

Kemper, Hagerman, Ahmad and Mariner (1986) tested 23 fra(X) females, who ranged in age from 6 to 54 years old, were of variable cytogenetic status, and differed in familial position and parental inheritance. Subjects' FSIQ scores ranged from 48 to 115, and only 10 of the 23 had FSIQs in the average range. No significant VIQ-PIQ differences were observed. To determine whether there was a characteristic cognitive profile for fra(X) girls, 8 school-age fra(X) girls and 8 non-fra(X) learning disabled girls matched for age and FSIQ scores from the WISC-R, were compared on the basis of WISC-R mean subtest scores. A significantly lower score for Block Design was noted for the fra(X) girls. To determine whether the profile for the entire group was similar, the mean discrepancy between Wechsler subtest scores from the total Verbal or Performance means was calculated for 22 fra(X) subjects and 20 learning-disabled female controls. Results indicated a characteristic combination of low Arithmetic, Digit Span, and Block Design subtest scores only for the fra(X) group. The investigators noted that the profile is observed even in normal IQ females, and that it is less marked in the retarded ones because their overall functioning is lower. The authors write, "this distinct cognitive profile suggests that the presence of the fra(X) gene causes a specific set of focal deficits" (Kemper et al., 1986, pp.140). However, because of the mixed grouping of subjects, the possibility that the profile existed in only a subgroup of the fra(X) females was not investigated.

Loesch & Hay (1988) examined cognitive profiles of 113 fra(X) females. Again, the group was not carefully delineated and was comprised of females of varying age, cytogenetic status, familial position, parental inheritance, and degree of impairment. No comparisons to

control subjects were made. Fra(X) females were given the Peabody Picture Vocabulary Test (PPVT) and the Wechsler Block Design subtest. The mean IQ score for Block Design was about 20 points lower than the mean IQ score for the PPVT, and the authors concluded that fra(X) females have better developed verbal than nonverbal skills. Although neither measure is comprehensive enough to support the generalized conclusions, the data again support the finding of poor Block Design performance in fra(X) females.

Grigsby, Kemper and Hagerman (1987) and Grigsby, Kemper, Hagerman and Myers (1990) tested for signs of Gerstmann syndrome in females with fra(X). Subjects were segregated according to cytogenetic status. Twenty fra(X) subjects who were cytogenetically expressing (fra(X)-positive), and 20 fra(X) relatives who were not cytogenetically expressing (fra(X)-negative), were compared to a group of mixed head-injured and learning disabled females. Subjects ranged in age from 8 to 64 years, but the mean ages were similar across groups. On WAIS-R measures, the mean FSIQs differed across the groups, such that the fra(X)-positive group scored significantly lower than the other two groups. Further, the fra(X)-positive group did more poorly than the other groups on the WAIS-R Digit Span, Arithmetic, and Block Design subtests, supporting previous findings. However, it is notable that this profile of poor subtest performance was not found in the fra(X)-negative group, suggesting that the cognitive characteristics of the two groups may be different. In addition, the fra(X)-positive group did significantly worse than the other groups on measures of the four symptoms of Gerstmann syndrome: spatial dyscalculia, dysgraphia, finger agnosia, and right-left disorientation, as well as on a measure of constructional dyspraxia. They did not differ from the other groups on measures of visual agnosia, ideomotor apraxia or aphasia.

Because it was unclear whether the finding of deficits in the fra(X)-positive group were due to lower FSIQ, selected subjects were matched for FSIQ scores, and data from the five fra(X)-positive subjects with the lowest FSIQ scores were dropped from the analysis (Grigsby, et al., 1990). These analyses again indicated that the fra(X)-positive group performed significantly more poorly than the other two groups on measures of dyscalculia, dysgraphia, constructional dyspraxia, finger agnosia and the Wechsler Digit Span and Block Design subtests. Unfortunately, the authors computed multiple t-test comparisons, so the inflated alpha may have affected the legitimacy of the conclusions drawn; more stringently analyzed data might not have revealed so many significant group differences. In addition, the fra(X)-negative control group was a combination of obligate carriers and individuals who may not have been carriers, so whether differences between those two groups exist were not addressed. The authors concluded that their finding of the full Gertsmann syndrome in one third of the fra(X)-positive cases is "striking, given the controversy that has gone on for years over the existence of the syndrome" (Grigsby, et al., 1990). Yet their assessment was not too discriminating; in the 1990 study where FSIQs were matched, no measures other than those believed related to the syndrome were given. Since significantly poorer performance is noted on almost all of them, whether there are cognitive areas in which the fra(X)-positive group is not deficient is unclear. Therefore, the specificity of deficit was not clearly demonstrated. Further, a great deal of variability in the distribution of the Gertsmann signs was observed within the fra(X)-positive group; only one third of the subjects had all of them. Grigsby, Kemper and Hagerman (1992) subsequently examined verbal learning and memory in the same subjects. Again, there was no control for FSIQ group differences. Despite wide VIQ

differences, fra(X)-positive subjects performed no differently from other groups on a paired-associate, a visual-verbal associate, and a delayed recall for word learning test. They did differ, however, on Digit Span and the trials to criterion score for the Word Learning Test. Thus, these data, taken together with those previously published on the same subjects, support an overall deficit in nonverbal skills relative to verbal skills. However, the data should have been analyzed jointly, controlling for the effects of FSIQ. In addition, statistical analyses should have been planned to account for chance occurrence of significant differences among the numerous comparisons.

Borghgraff, Fryns & van den Berghe (1990) examined seven girls, all of whom were cytogenetically expressing (from 2 to 30%), and who ranged in age from 6 to 13 years. A wide range in IQ (from 50 to 135) was observed, and no significant subtest scatter was noted. There was no correlation between percent of cytogenetic expression and IQ. Clinical observations suggested that all seven subjects had attention deficit disorder, and in two there were also signs of hyperactivity. Unfortunately, no measures of how these determinations were made were presented, and there were no comparisons to controls.

Wolff, et al. (1988) studied 14 mothers and one sister of affected fra(X) males, all of whom were of normal intelligence, to determine whether they had specific learning disabilities. The group was mixed (11/15 subjects were not cytogenetically expressing) and parental inheritance was not taken into account. Subjects were compared to 13 mothers and one sister of Down syndrome males and one mother of a male with "nonspecific" mental retardation. This group was chosen because these women, unlike the fra(X) women, were not believed to be carriers of the developmental disorder. Yet, both groups had similar familial

experiences of living with someone who is developmentally disabled. A large battery of neuropsychological tests was administered. The groups did not differ in FSIQ and, unlike other reports, no consistent scatter profiles on individual Wechsler subtest scores was observed. The authors note that they used more stringent criteria for subtest scatter, but the fact that most of their subjects were not cytogenetically expressing may also account for their inability to replicate previous findings. Wolff et al. defined the presence of specific learning disability using grade equivalent criteria and reported 8/15 fra(X) women, but only 1/14 controls had specific learning disabilities. However, their criteria were arbitrary and the areas of poor performance differed across the subjects. Quantitative testing of group differences of academic achievement using raw achievement scores indicated that controls performed better than fra(X) carriers on measures of word recognition, reading and arithmetic. Standard scores were not presented. On neuropsychological measures, controls scored higher than fra(X) carriers on measures of expressive language and auditory-linguistic memory. No significant differences between the groups were found in the areas of receptive language, visual memory, or rapid automatized naming. These findings are notable because they differ considerably from those of other studies. In part this may be due to the fact that the majority of fra(X) subjects were not cytogenetically expressing. Therefore, they should look more like Grigsby et al.'s (1987, 1990, 1992) fra(X)-negative group. The findings may also be somewhat artifactual as the number of comparisons made was numerous, thereby increasing the chances of type I errors.

Another study that also suggested deficits (albeit, different ones) in carriers who are cytogenetically negative was done by de von Flindt, Bybel, Chudley and Lopes (1991).

WAIS-R subtest profiles and performance on a visual memory test were studied. The sample consisted of 13 adult carrier women (two of whom were fra(X) positive). Subjects were age matched to control women with no familial history of mental retardation. Fra(X) carriers scored significantly lower than controls on all Wechsler IQ measures, but the difference may have been due to the controls scoring above average (mean FSIQ, fra(X) = 98; controls = 116). Analysis of subtest scatter using the Sattler method indicated no significant patterns. On the Revised Visual Retention Test, fra(X) subjects did worse than controls on the memory, but not the copy, conditions. This difference remained even when the two fra(X)-positive subjects were removed from the analysis, although the effects of FSIQ was not controlled for. Comparison of the data to norms based on age and intelligence indicated that the fra(X) group (even without the two positive members) performed worse than expected in the memory conditions, but control subjects did not. The authors interpret this as being "evidence of short-term memory and possible limited visual-spatial deficits in our fra(X)-negative carrier women" (de von Flindt, et al., 1991, p. 491). However, neither subject group was well defined. Carriers could have inherited from either parent, and controls were not matched for FSIQ, education or environmental constraints of living with a person with developmental disabilities. Still, this finding does support the possibility that cognitive deficits may exist in women who are not cytogenetically expressing.

In contrast to these findings, Hinton et al. (1991) segregated adult fra(X) females according to cytogenetic status and found visual memory deficits only in the cytogenetically expressing group. Nine fra(X) women who were cytogenetically positive, 15 fra(X) women who were cytogenetically negative and 15 control mothers of children with non X-

linked developmental disabilities were tested on the Wechsler Adult Intelligence Scale-Revised (WAIS-R) and the Benton Visual Retention Test (BVRT). Women did not differ significantly in age, education or VIQ (where the mean for each group fell within the average range). Group differences were noted on measures of PIQ, however, where fra(X) positive women scored significantly lower than controls, but not lower than fra(X) negative women. WAIS-R data were analyzed according to the three WAIS-R factors, to minimize the number of statistical comparisons. Significant differences were found across the groups on the Freedom from Distractibility factor (FFD), but not on the Verbal or Perceptual Organization factors. Pairwise analysis indicated that the fra(X) positive women performed significantly worse than controls on the FFD factor. This is consistent with other reports of poor performance on WAIS-R Digit Span and Arithmetic subtests (since the FFD factor is comprised in part of the standard scores of those subtests). Further, on the 10 second recall condition of the BVRT, group differences were found; fra(X) positive women performed significantly more poorly than controls. No differences were found, however, on the BVRT copy administration. These data suggest that the cytogenetic-positive group may have visual memory and attentional deficits. Yet the measures are far from specific, and may depend on many intact cognitive domains for successful performance. Further, the data support the idea that fra(X) females who are cytogenetically expressing are distinct from fra(X) females who are not. No indication of cognitive impairment was observed in fra(X) women who were not expressing. This is different from the findings of de von Flindt, et al. (1991) and may be due in part to the better control group in this study. Yet, Hinton et al.'s paired comparisons only demonstrated significant differences between the fra(X)-positive and control groups; the

fra(X)-negative group did not significantly differ from either group. Thus, the possibility exists that the fra(X)-negative group performance may fall somewhere in between that of the other two groups, but these data are inconclusive. Unfortunately, inheritance status was not taken into account, so possible differences related to that are not addressed.

The idea that cytogenetically expressing females may differ from carriers who are not cytogenetically positive, was further examined by Brainard, Schreiner, and Hagerman (1991). Seventy four fra(X) women (age range 18 to 68 years), 38 of whom were fra(X) negative and 36 of whom were fra(X) positive were tested. This sample included subjects reported on by Grigsby et al. (1987, 1990, 1992). A strong inverse relationship between WAIS-R FSIQ and the presence of cells with fragile sites was found. Separation according to cytogenetic status resulted in different group profiles. Subjects who were not cytogenetically expressing performed poorly on Digit Span, and well on Vocabulary, Comprehension and Block Design. Subjects who were cytogenetically expressing had low Arithmetic scores and relatively higher Digit Span scores. The authors note that no consistent individual subtest patterns were found. Their main conclusion came from examination of WAIS-R FSIQ scores, and they state that fra(X)-negative women " appear to be cognitively unimpaired and should be considered separately from fra(X)-positive women" (Brainard, et al., 1991, p. 505).

Cianchetti, et al. (1991) studied 149 subjects from 18 families. Of those, 36 were heterozygotes ranging in age from 10 to 77 years and in IQ scores from 20 to 99. All 36 female subjects were given the WAIS-R, and, overall, the mean VIQ was greater than the mean PIQ. There was no significant subtest scatter. All female subjects who were cytogenetically expressing in 5 or more percent of their cells had a FSIQ less than 80, but

there was no other significant relationship between percent fragility and FSIQ. Cianchetti et al. examined cognitive skills more thoroughly in the 14 female heterozygotes whose FSIQ was greater than 80 and compared their performance to both 14 control females and 14 control males, matched on age and IQ and married to persons in families with fra(X). Results on a large battery of neuropsychological tests indicated that the fra(X) female group performed significantly worse than either of the two control groups only on the Ravens Standardized Progressive Matrices (a measure of logical reasoning based on figurative models) and the WAIS-R Digit Span subtest. Groups did not differ on measures of verbal or spatial memory, logical sentences, graphomotor skills, an arithmetic computation test, or the other WAIS-R subtests. Many t-test comparisons were made, so alphas were inflated. The authors suggest that the poor performance on the Ravens "is mainly due to the figurative-spatial aspect of the test," since on "the other test of "pure" logic, the logic sentences, heterozygotes performed well" (Cianchetti, et al., 1991, p. 241). However, the finding that the fra(X) group did not perform differently than controls on the spatial memory test does not support this conclusion. The authors note there are nonsignificant trends suggestive of poorer performance by the fra(X) group on the visual spatial measures. This, taken with the overall VIQ > PIQ difference in the larger sample may be indicative of visual spatial weakness. This interpretation must be tentative, however.

Freund and Reiss (1991) tested 11 cytogenetically expressing fra(X) females on the Stanford-Binet, 4th Ed. Subjects ranged from 6 to 20 years old and from moderate mental retardation to above average intelligence. To generate a cognitive profile, each subject's subtest score was converted to a deviation score, and then the mean deviation for each subtest

was calculated. Data indicated a relative weakness in short-term memory for nonverbal, sequential material and relative strength in short term verbal sequential material. Other areas did not appear to be discriminating, and contrary to some previous findings, no deficits in quantitative ability were observed. The authors suggest that since only one type of short term memory appears to be affected, the data argue for a specific deficit. Yet, whether the deficit is due to poor encoding of visual information or poor memory strategies is unclear. Further, since no control comparisons were made, it is unclear whether this profile might be found in other persons of similar IQ or whether it is specific to females with fra(X).

Steyaert et al. (1992) gave a battery of tests to 11 adult female carriers of fra(X). All subjects had inherited their fra(X) chromosome from their mothers, but the possibility of the group being comprised of two subgroups was not addressed. Subjects were either not cytogenetically expressing (7/11), or had very low percent fragilities (4/11). WAIS-R FSIQ scores were average. WAIS-R subtest analyses, in which mean deviation from individual mean standard score was examined, indicated poor performance only on the Arithmetic and Object Assembly subtests and good performance on the Comprehension subtest. Neuropsychological results were not compared to those of controls or to standardized norms; rather, comparisons were made to statistically derived cut off scores believed to fall below the 25th percentile in the normal population. Results suggested poor performance on visual memory and attentional tasks and good performance on visual orientation and auditory memory tasks. The differences between visual vs. verbal memory are similar to those reported by Freund and Reiss (1991). However, the lack of a control group, the different definition for the experimental group, and the lack of standardized norms for comparison

make interpretation of these data difficult.

Mazzocco, Hagerman, Cronister-Silverman and Pennington (1992) studied neurocognitive phenotype in fra(X) women in a carefully designed study. Ten women who were cytogenetically expressing, 10 women who were obligate carriers but not expressing, and 10 controls who were mothers of children with a non-fra(X) developmental disability were examined. All subjects were adults, and age did not differ across the groups. Subjects did differ on WAIS-R FSIQ, with the fra(X) expressing group being significantly lower than the other groups. Stepwise regression analysis was used to remove the FSIQ effects on subsequent analyses. On tests of achievement (Peabody Individual Achievement Test - Revised), the expressing group was found to perform significantly worse than the other two groups in arithmetic, but not in reading and spelling. On tests of verbal function, nonverbal function and long-term memory, no group differences were observed. However, on measures of executive function, the fra(X) expressing group performed significantly more poorly than the other groups on three of the four measures (Wisconsin Card Sorting Test, Contingency Naming Test, Tower of Hanoi), which the authors interpret as indicative of frontal lobe deficits. They suggest that these frontal lobe deficits may be implicated in the behavioral and emotional difficulties noted in females with fra(X) (e.g. attentional problems, distractibility, shyness, difficulty with transitions (Hagerman & Sobesky, 1989)). As well, they suggest that frontal lobe deficits can also be observed in males with fra(X) and this "may be responsible for some of the unique behavioral characteristics of fragile X mental retardation" (Mazzocco et al, 1992a, p. 1148). Although the findings are of interest, this interpretation may be unwarranted in the absence of control groups of individuals with other developmental

disorders matched for FSIQ. Deficits in executive functions are seen in many disorders and may be more susceptible to impairments in developmental disorders, so that characterization of specificity to fra(X) may not be justifiable. Further, since the skills needed to successfully complete the tasks rely on many different cognitive domains, deficits in more specific areas could present as overall executive function deficits on these measures. On the other hand, the careful design of the study suggests that impaired executive functions indeed may be overriding problems in fra(X). The lack of significant group differences among the nonverbal measures, particularly on the figural short-term memory task, differed from previous reports. These data did not support generalized fra(X) female visual spatial deficits, yet the tasks on which impairment occurred all required attention to visual details to perform correctly, so there may be some visual perceptual involvement. It is notable that the one executive function measure that fra(X) subjects did not do more poorly on, the Stroop color-word test, is verbally, not visually-spatially mediated. The good performance on verbal and long-term memory measures was consistent with most other reports.

In a detailed error analysis of performance on two of the deficient frontal lobe measures, Mazzocco, Hagerman and Pennington (1992) examined problem solving strategies used by the subjects. Performance on the Contingency Naming Test and the Tower of Hanoi indicated that fra(X) expressing women had more difficulty acquiring rules for problem solving, but once acquired could use them appropriately. This is suggestive of Grigsby et al.'s (1992) finding of fra(X) expressing females having more trials to criterion to learn a word memory test than did controls, but being no different on delayed recall of those words. Mazzocco et al.'s data did not indicate an increased number of perseverative or impulsive

responses. Further, subjects were not unable to solve complex tasks, but as demands on attention to the number of details needed to solve them increased, they had difficulty. This may suggest overall attentional difficulties, rather than generalized problem solving deficits. The authors describe fra(X) expressing women as potentially having a lower "threshold for the number of features which can be simultaneously considered" (Mazzocco et al., 1992b, p. 85).

Taken together, the above described studies do support a few generalizable findings. Even though the studies suffer from numerous methodological problems, certain consistencies regarding both the classification of the subjects and the nature of the cognitive profiles emerge. As such, three tentative conclusions can be drawn.

(1) Not all female fra(X) subjects are alike. When subjects are segregated according to cytogenetic status, the groups present differently. Consistent across the studies is the finding that cytogenetically expressing females are more likely to have cognitive deficits. It is less clear whether non-expressing heterozygotes are impaired. Studies that examined well-defined subject groups (Brainard, et al., 1991; Grigsby, et al., 1987,1990, 1992; Hinton, et al., 1991; Mazzocco, et al., 1992) indicate no difference in test performance between cytogenetically negative and well matched control women. Studies with less well defined groups, where the majority of subjects are cytogenetically negative (Cianchetti, et al., 1991; de von Flindt, et al.,1991; Steyaert, et al., 1992; Wolff, et al., 1988) have suggested that mild deficits may exist in non-expressing women. It is difficult to determine, from these studies, whether there is an overriding effect of the cytogenetically positive females in the group, whether controls are poorly matched, or whether there is in fact some mild variable

phenotype.

One problem inherent in these studies is the legitimacy of using cytogenetic status as a defining variable. Since measures of chromosomal fragility are subject to variation (the number of cells observed to have the fragile site is never 100%, it can range from 2 to 60%), and have been demonstrated to be found both in persons with no cognitive deficits (estimates from 10 to 40% of presumed unaffected females show some fragile cells), and lacking in some persons with deficits (Webb, 1992), this may not be the best way of segregating subjects. Chudley, et al. (1983) suggested that both age and intelligence are negatively correlated with the frequency of cytogenetic expression; as either age or intelligence increases, the number of expressing cells decreases. Yet many studies have not found a significant IQ - expressing cell correlation (e.g. Borghgraff, et al., 1990; Cianchetti, et al., 1991); although the presence of cells with fragile sites and low FSIQ has been reported (Brainard, et al., 1991). In addition, in males no IQ - percent fragility correlation has been found (Webb, 1992). Cytogenetic analysis is not a definitive measure. Further, the functional role of fragile sites is unknown. A more theoretically sound way of segregating subjects might be by mode of inheritance, so the influence of gender of inheritance could be tested. A more definitive way of classifying subject groups might be via molecular testing. It remains clear from the work investigating fra(X) females that they vary - some have cognitive deficits and some may not. How they are classified may better determine their differences.

(2) The nature of the cognitive deficits in the fra(X) female appears to be nonverbal. This finding is supported by all the studies reviewed, except that by Wolff, et al. (1988). Wolff, et al. suggested that fra(X) females may have specific verbal learning disabilities, but

the limitations of this study were discussed earlier, and the lack of replication of their findings makes these data less compelling. The remainder of the studies indicate that deficits in the fra(X) groups were on tests of abstract, nonverbal skills. Tests of language function were relative strengths for the fra(X) group. This is not to suggest that all verbal skills are completely intact in the fra(X) females. Rather, in terms of relative cognitive skills, the data consistently indicate that verbal skills are less likely to be as affected as are other skills. Grigsby, et al. (1992) offer the best demonstration of this; even with lower VIQ scores than controls, the fra(X)-positive group did not differ from them on specific measures of verbal learning and verbal memory. Thus, it is likely that fra(X) does have a generalized effect of depressing intellectual skills, but within that context, language mediated skills are better maintained.

(3) The more specific areas likely affected include visual spatial perception, short term nonverbal memory, attentional processes, arithmetic and abstract reasoning, although clear delineation of the nature of these cognitive deficits in fra(X) females is difficult.

Findings of poor performance on the Wechsler Block Design subtest (Grigsby, et al., 1987,1990; Hagerman & Smith, 1983; Kemper, et al., 1986; Loesch & Hay, 1988; Miezejeski, et al., 1986) may suggest possible visual-spatial, organizational deficits, although some authors have argued that it may be more representative of deficits in executive functioning (Pennington, et al., 1991; Mazzocco et al., 1992a). Findings of deficits on the WAIS-R Digit Span subtest (Grigsby, et al., 1987,1990,1992; Hagerman & Smith, 1983; Kemper, et al., 1986; Miezejeski, et al., 1986) may be indicative of short term memory deficits or problems of attention. Poor performance on visual-spatial short term memory tests (de

von Flindt, et al., 1991; Freund & Reiss, 1991; Hinton, et al., 1991; Miezejeski, et al., 1986; Steyaert, et al., 1992) could result from deficits in any or all of the areas of visual-spatial perception, short term memory or attention. Difficulties on abstract reasoning tests (Cianchetti, et al., 1991; Mazzocco, et al., 1992a,b) could also result from a variety of problems, including deficits in visual-spatial, attentional, or executive function skills. Likewise, problems with arithmetic (Grigsby, et al., 1987, 1990; Hagerman & Smith, 1983; Kemper, et al., 1986; Mazzocco, et al.,1992a; Miezejeski, et al., 1986; Steyaert, et al., 1992; Wolff, et al., 1988) may be indicative of weakness in attention, abstract visualization or problem solving skills. Clinical observation of poor attention (Borghgraff, et al., 1988, Hagerman & Smith, 1983) may contribute to any of these problem areas, but has not been objectively measured.

Thus, across the test measures affected, some generalized skills may be implicated. However, some data exist suggesting that, in some cases, fra(X) females are not deficient in these areas. For example, attention and memory skills have appeared to be strong on measures of verbal or auditory memory (Freund & Reiss, 1991; Grigsby, et al., 1992; Steyaert, et al., 1992), and Mazzocco, et al. (1992a) found no difference between fra(X) subjects and controls on an abstract memory measure. Good visual-spatial skills were noted in some studies (Cianchetti, et al., 1991; Mazzocco, et al., 1992a), and the lack of deficits on the Wechsler Block Design subtest or Perceptual Organization factor have been noted by many (Cianchetti, et al., 1991; de von Flindt, et al., 1991; Hinton et al., 1991; Steyaert, et al., 1991; Wolff, et al., 1988). Similarly, reasoning skills appear to be intact as evidenced by consistently good performance on the Wechsler Similarities subtest and on a test of sentence

logic (Cianchetti, et al., 1991).

These discrepancies in specific findings and in their interpretation across the studies may be due, in part, to the lack of carefully chosen measures. These problems may be addressed by designing a hypothesis-driven battery of neuropsychological tests. Although some studies have taken this approach (Grigsby, et al., 1987,1990; Mazzocco, et al., 1992a), in general, studies have been designed merely to obtain descriptive information about the population.

Based on the information available, a number of questions regarding the specific cognitive phenotype of the fra(X) female can be addressed. Are there specific deficits in any or all of the areas of visual-spatial perception, memory or attention? Can tests be administered to tease out the relative contribution of each of these skills? Are some skills relatively intact? Administration of a battery of neuropsychological tests chosen to focus on these areas can potentially answer these questions.

Specific Questions to be Addressed

1) Does parental origin of the fragile X gene affect cognitive and genomic expression in female offspring?

Specifically, are women who inherit from males unaffected (have no cognitive deficits) and are some, but not all, women who inherit from females affected (have some cognitive deficits)?

2) Are mild cognitive impairments in females with fra(X) associated with genomic structural changes?

Specifically, does an association exist between independently derived measures of gene state and intellectual function in nonretarded fra(X) females, as evidenced by performance on standardized psychometric tests?

3) Do some nonretarded female carriers of fra(X) (a subgroup of those with maternal inheritance) have specific areas of cognitive impairment?

Specifically, are areas of abstract memory, attention and visual-spatial skills relatively weak and areas of language and verbal memory relatively strong in affected female carriers?

Mode of Inheritance Influences Behavioral Expression and Molecular Control of Cognitive Deficits in Female Carriers of the Fragile X Syndrome

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Abstract

The effect of mode of inheritance on expression of fragile X syndrome (fra(X)) was investigated in nonretarded female carriers. Examination included cognitive and molecular measures. A priori predictions about cognitive impairment and size of an unstable region of DNA containing a CGG repeat on the X chromosome were tested in age and education matched heterozygotes grouped according to parental inheritance.

Nine carriers with a maternal fra(X) chromosome, 11 carriers with a paternal fra(X) chromosome and 15 control mothers of children with non X-linked developmental disabilities were tested. Inheritance was established through DNA linkage analysis. Cognitive skills were assessed using the Wechsler Adult Intelligence Scale-Revised and the Benton Visual Retention Test. Molecular status was assessed by Southern blot analysis of genomic DNA digested with Eco RI and Eag I, and probed with StB 12.3.

Results supported the inheritance models' predictions. Heterozygotes who inherited the fra(X) from their fathers appeared to be a homogeneous group. They were indistinguishable from controls on cognitive measures and all had genomic insertions of <500 base pairs. In contrast, heterozygotes who inherited the fra(X) chromosome from their mothers appeared to be made up of two subpopulations. They were as a group deficient in measures of attention and visual memory, but not other measures, with scores of some women consistently below the other subjects. Further, they had some members with >500 base pair inserts. Thus, parental origin of the chromosome appears to influence fra(X) expression on both a behavioral and molecular level.

Mode of Inheritance Influences Behavioral Expression and Molecular Control of Cognitive Deficits in Female Carriers of the Fragile X Syndrome

Current theories about the nature of the fragile X syndrome (fra(X)) suggest that the mode of inheritance of the chromosome may play a crucial role in determining whether an individual is affected. Models based on retrospective examination of pedigrees have focused on differences observed between paternal and maternal inheritance. One model posits that X inactivation and resultant "imprinting" are necessary for fra(X) expression (Laird, 1987). Another model suggests that a premutation involved in a recombination event is the basis for the heritable differences (Pembrey, et al., 1985). Yet another model suggests unequal crossing over leading to amplification of a pyrimidine-rich sequence could contribute to fra(X) expression (Nussbaum et al., 1986). Each of these models, although differing in their molecular mechanisms, predict three possible outcomes for female fra(X) carriers: 1) Females who inherit their fra(X) chromosome from an unaffected nonpenetrant male should not be affected (as the X has been neither inactivated nor recombined). 2) Females who inherit their fra(X) from their mothers should fall into two groups: a. Those who will not be affected, (where presumably the X has not been imprinted or recombined) and b. those who will have cognitive deficits, (where the X may have been either inactivated or recombined).

Recent findings have demonstrated that size of an unstable region of DNA containing

a CGG repeat on the X chromosome is related to both transmission and expression of fra(X) (Oberle et al., 1991; Yu et al, 1991). Transmitting males and females both have a CGG repeat of 150 to 500 base pairs, while affected individuals have a much greater amplification (ranging from 500 to over 3000 base pairs) as well as hypermethylation of an adjacent region. The specific mechanism which leads to the CGG amplification is still unclear. Models emphasizing the contribution of inheritance in fra(X) would predict three possible molecular outcomes for female carriers, which parallel behavioral predictions. Those who carry a paternally inherited fra(X) chromosome should only have the smaller CGG amplification (and likewise, be unaffected). Females who have a maternally inherited fra(X) chromosome should fall into two groups: 1. Those with the small insertion (who are likely unimpaired cognitively) and 2. those with the greater DNA amplification (who likely will have cognitive deficits).

To test these hypotheses, nonretarded female carriers of fra(X) were grouped according to parental origin of the fra(X) chromosome and tested on cognitive measures. The effect, if any, of mode of inheritance on specific cognitive skills was assessed. In addition, the molecular status of the fra(X) repeat was determined for these women. Emphasis was placed on whether the inheritance models' predictions could be substantiated, not on elucidating their underlying mechanisms. We report here preliminary findings of an ongoing study

Method

Nine fra(X) carrier women who inherited the affected chromosome from their mothers, 11 fra(X) carrier women who inherited the affected chromosome from their fathers and 15 control mothers of children with non X-linked developmental disabilities were tested.

Inheritance was established through DNA linkage analysis and investigation of extended family pedigrees where available. Because most (17 out of 20) of the fra(X) carrier women were mothers of children with fra(X), controls were chosen to balance any possible stress factors that might be related to raising a child with developmental disabilities. Groups did not differ significantly in age or years of education, as indicated in Table 1. Furthermore, no subject was mentally retarded. Three subjects in the maternal inheritance group were cytogenetically expressing; cell induction used the FUdR and folate deficient methods (Jenkins, Lele, Krawczun, Gross, Duncan & Brown, 1988).

Table 1. Subject Characteristics

	Control	Paternal (X)	Maternal (X)
# Subjects	15	11	9
Age (years) X ± SD	36.67 ± 2.97	39.18 ± 4.89	36.00 ± 14.98
Yrs Education X ± SD	14.23 ± 2.05	15.18 ± 2.14	13.29 ± 2.69
Number with Fragile Sites	- - -	0	3
Range of % Positive	- - -	- - -	4 - 28

Each subject was assessed using the Wechsler Adult Intelligence Scale-Revised

(WAIS-R) and the Benton Visual Retention Test (BVRT). The WAIS-R is an intelligence test made up of various subtests thought to measure a range of cognitive skills. Data from the WAIS-R were analyzed using full scale IQ and by grouping the subtests into 3 factor scores that are thought to measure verbal, visual-spatial organizational and attentional skills (Kaufman, 1990). The 3 factors are : (1) Verbal Comprehension (comprised of the Information, Similarities, Vocabulary and Comprehension subtests), (2) Perceptual Organization (comprised of the Block Design and Object Assembly subtests), and (3) Freedom from Distractibility (comprised of the Arithmetic, Digit Span and Digit Symbol subtests).

The BVRT is a test of visual-spatial memory. In the recall condition, subjects are shown 10 figures for 10 seconds each and then asked to draw the figure from memory. In the copy condition, subjects are merely shown figures and asked to copy them while they are present. This allows for comparison between perceptual/constructional skills (needed for both conditions) and memory/attentional skills (needed mainly for the recall condition). Genomic molecular analysis was done on the fra(X) carriers according to the method described by Rousseau et al. (1991). Genomic DNA was digested with Eco RI and Eag I and the resulting fragments were analyzed by Southern blot with the probe StB 12.3. Analysis was only done on those subjects for whom we had DNA samples; this decreased sample size to 7 women in the paternal inheritance group and 6 women in the maternal inheritance group. Experimenters were "blind" to the inheritance condition of the samples.

Data were analyzed using nonparametric statistics because of the a priori hypothesis that one group (those who inherited from females) would not be normally distributed. Rather,

the models predict a bimodal distribution for this group. Multiple between-group-comparisons with Mann-Whitney U tests using the Bonferoni modification to control for type I probability errors were run. Analyses of distribution of data were performed using Kolmogorov-Smirnov and chi-square tests.

Results

No differences were found between groups on full scale IQ ($p > .05$). However, distribution of the IQ scores suggests that the maternal inheritance group may in fact be comprised of two groups (Figure 6). Our current data set is too small to demonstrate that the maternal and paternal inheritance data are distributed differently, as the models predict (Kolmogorov-Smirnov test of two independent samples, $p>.05$). Yet, quantal analysis indicates that IQ distribution of the maternal inheritance group is significantly different from the other groups. All controls and subjects in the paternal inheritance fra(X) group have IQ scores within or above 1 standard deviation of the normal mean IQ, while 4 out of 9 in the maternal inheritance group have IQs more than 1 standard deviation below the normal mean (chi-square = 13.05, $p < .01$).

More refined analysis of the WAIS-R scores indicated that the Freedom from Distractibility factor was the most discriminating measure; the maternal inheritance group

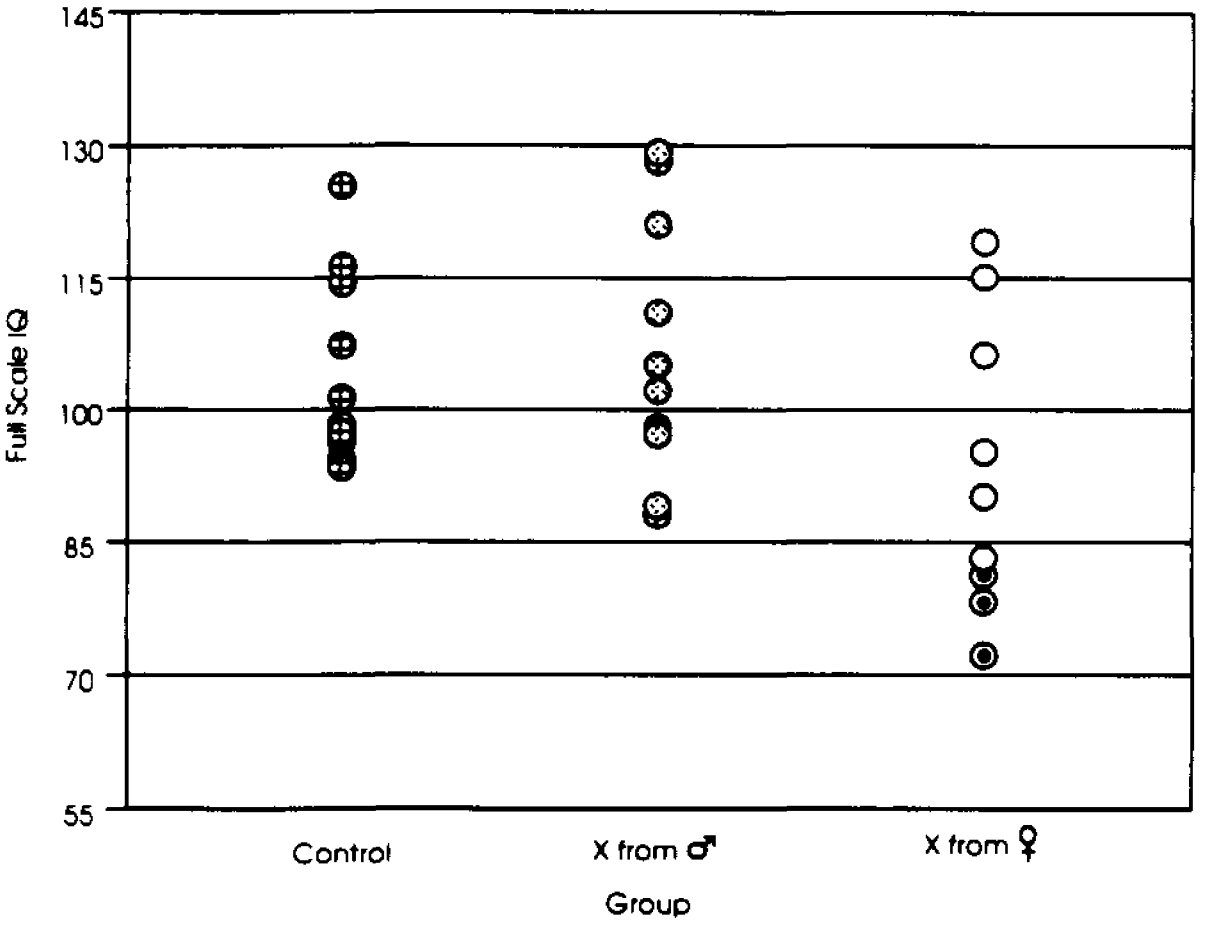

Figure 6.
Data show the distribution of WAIS-R Full Scale IQ across the groups. Although group differences did not reach significance, they approach it. Note that across the three groups most data points fall within one standard deviation of the expected mean for the normal population (100±15), but in the maternal inheritance group, 4 out of 9 points fall below this. Superimposed dots indicate cytogenetically positive subjects.

performed significantly more poorly on this measure than did the other two groups (Us = 21 and 17, respectively, ps < .01). Further, quantal analysis of the distribution of scores for this measure was significant; three scores from the maternal inheritance group fell below 1 standard deviation from the normal mean while none from the other groups did (chi-square = 9.50, p >.01). No differences were observed between the maternal inheritance group and the other groups on the Verbal Comprehension or Perceptual Organization factor measures (ps > .05). Quantal analysis on these factors also did not reach significance (ps > .05). Fra(X) women with paternal inheritance did not differ from controls on any measure (ps > .05). Across the factors, the paternal inheritance group and control groups fell within and above the mean $\pm$ 1 standard deviation expected of the general population (Figure 7). These data suggest that the maternal inheritance group, as a whole, performed worse than the other groups on tasks sensitive to attentional skills.

On the BVRT all groups performed similarly, and at ceiling, in the copy condition; that is, all groups could draw the figures correctly while looking at them. However, while the BVRT recall performance of paternal inheritance group did not differ from controls (U = 78.5, p > .05), the maternal inheritance group performed more poorly than controls (U = 26, p < .01). Women who inherited the fra(X) chromosome from their mothers were less likely to remember and draw correctly a figure they had been shown for ten seconds (Figure 8). Thus, the maternal inheritance fra(X) group appears to have deficits related to visual spatial memory, but not perceptual or graphomotor skills. Genomic molecular analysis of the

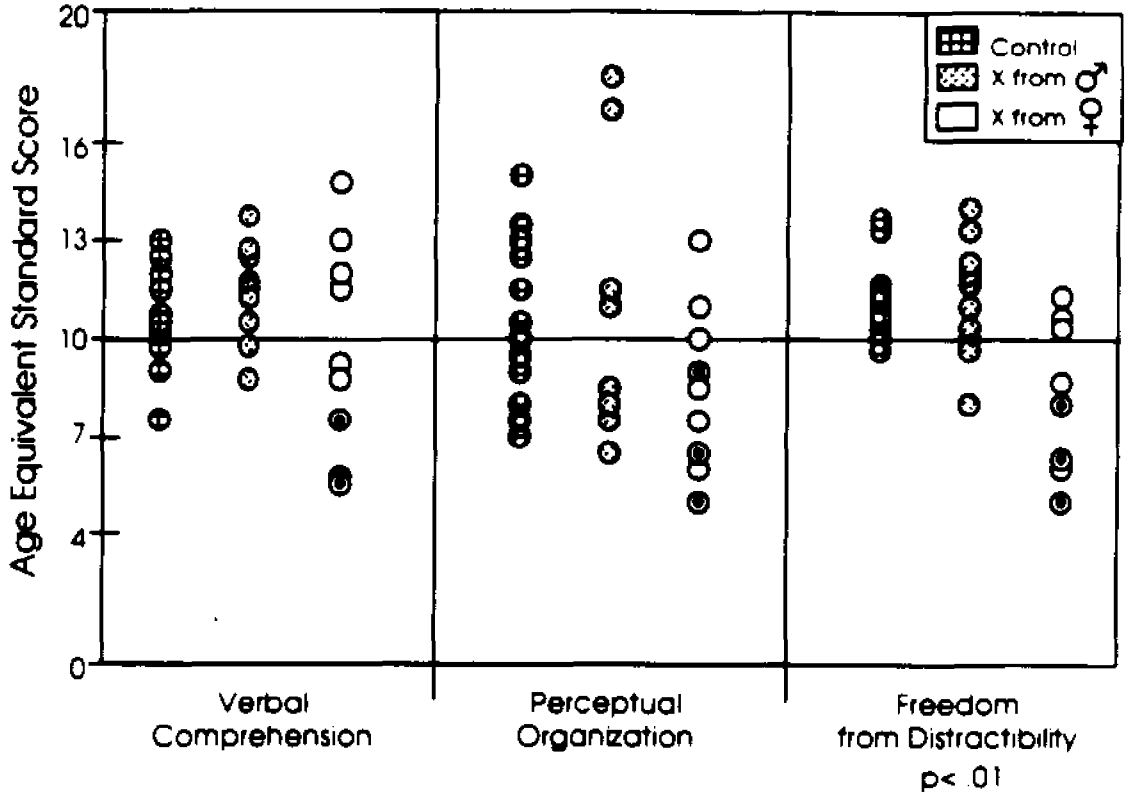

Figure 7.

Data show the distribution of the different WAIS-R factor scores. Women who inherit the fra(X) from their fathers do not differ from controls on any measure. Women in the maternal inheritance group perform significantly more poorly than the paternal inheritance group and controls on the WAIS-R Freedom from Distractibility measure ($p < .01$), but perform similarly on other WAIS-R factor scores. Superimposed dots indicate cytogenetically positive subjects

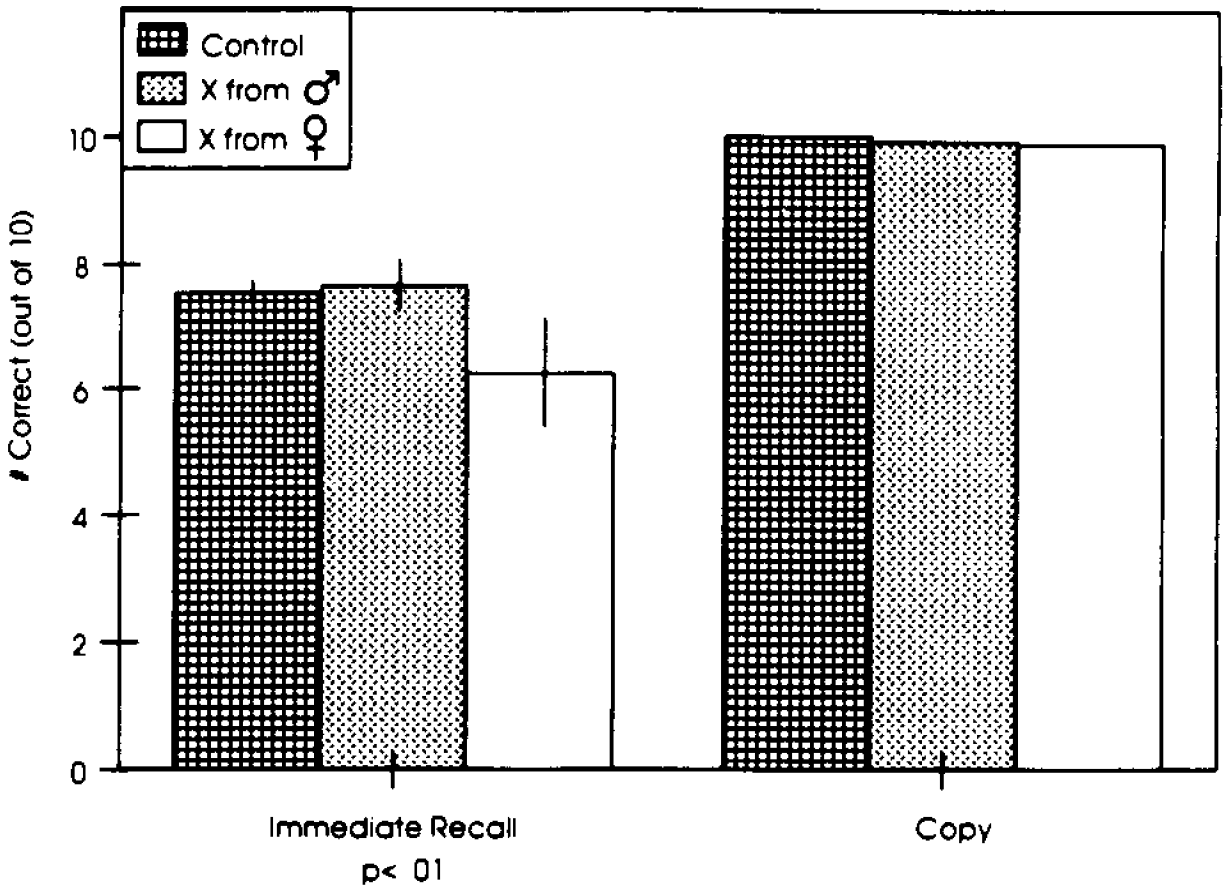

Figure 8.
Women who inherit the fra(X) from their mothers perform significantly more poorly than controls on the 10 second exposure, immediate recall condition of the BVRT ($p < 01$) Women who inherit the fra(X) from their fathers do not differ significantly from either group No differences in performance are observed in the copy administration. Data are presented as mean $\pm$ standard error number correct out of 10.

fra(X) carriers indicates amplification of the CGG repeat in all subjects (Figure 9). Examination of the autoradiograph shows all subjects have bands representative of the normal active X (2.8 kb) and the normal inactivated X (5.2 kb) band (except subject 2 in the maternal group), as well as additional bands indicative of amplification in the fra(X) chromosome. For the paternal inheritance fra(X) heterozygote group, the band $\geq$3 kb is seen in all subjects above the normal active X (2.8 kb) band, and is thought to be related to their carrier status. This $\geq$3 kb band is also found in 3 members of the maternal inheritance group Three others in the maternal inheritance group show the >6 kb band above the normal inactive X (5.2 kb) band, indicative of the > 500 base pair insert. This is the band found in affected fra(X) individuals. The three women with the >6 kb band were the three subjects with the lowest overall IQ, although they were not mentally retarded. These women were also the only three cytogenetically positive subjects in the study. No subject in the paternally inherited group had the >6 kb band. Analysis of the distribution of the >6 kb band indicated statistically significant differences between the groups (chi-square = 5.33, $p < .05$).

Discussion

Differences in cognitive performance and genomic insertion size between women with paternally versus maternally inherited fra(X) chromosomes suggests that inheritance may be a key factor in fra(X) expression. Our preliminary data support the predictions of different genetic models of fra(X). The models predict, on the basis of mode of inheritance, which heterozygotes are likely to be cognitively impaired and have the >500 base pair insert.

Our data indicate, in a relatively small sample of nonretarded female carriers, that

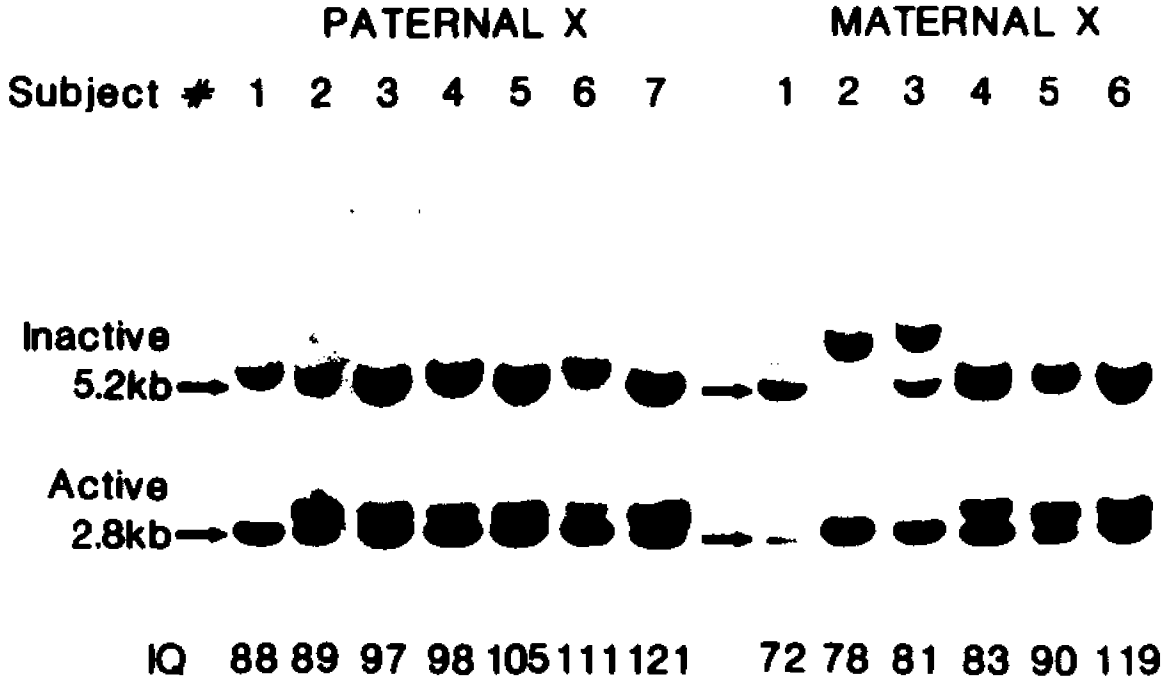

Figure 9.
Southern blot analysis of fra(X) heterozygote DNA digested with EcoR I and Eag I and probed with StB12.3. Bands at 2.8 kb and 5.2 kb represent normal active and normal inactive X chromosomes, respectively. Segregation by mode of inheritance indicates differences in size of CGG amplification observed. All paternally inherited fra(X) women show a $\geq$ 3 kb band indicative of inserts <500 bp. Three women in the maternal inheritance group (4-6) also show the 3kb band. Three other women in the maternal inheritance group (1-3) have bands at >6 kb, indicative of much larger CGG amplifications as well as methylated restriction sites. Women 1-3 also are cytogenetically positive and have the lowest overall IQs.

heterozygotes who inherited the fra(X) from their fathers: (a) were indistinguishable from controls on cognitive measures, (b) had genomic insertions of <500 base pairs, and (c) were not cytogentically positive. In contrast, heterozygotes who inherited the fra(X) chromosome from their mothers: (a) were as a group deficient in measures of attention and visual memory, with scores of some women consistently below the other subjects, (b) had some members with >500 base pair inserts and (c) had some cytogenetically positive members. Furthermore, the 3 subjects with the lowest IQ were those subjects with the >500 base pair insertion and were cytogenetically positive. Thus, the maternal inheritance group appeared to be made up of 2 subpopulations. This segregation is of particular interest in this nonretarded population, suggesting the mutation may contribute to subtle cognitive deficits, in addition to mental retardation.

The nature of the cognitive deficits observed appear to be of two types: attentional and visual memory. There is evidence from other sources of attentional involvement in more severely affected individuals with fra(X) (Borghgraef et al., 1990; Bregman et al., 1988, Chudley & Hagerman, 1987; Hagerman & Smith, 1983). In addition, the Freedom from Distractibility factor of the WAIS-R is comprised, in part, of two subtests (Arithmetic and Digit Span) that have been found to be impaired in fra(X) heterozygotes (Brainard et al., 1991; Kemper et al., 1986; Miezejeski et al., 1986). Similarly, visual memory has been found to be deficient in some fra(X) carriers (de von Flindt et al., 1991; Hinton et al., 1991). More generalized visual spatial deficits have also been reported in fra(X) carrier females (Cianchetti et al., 1991; Grigsby et al., 1990). Therefore, whether our observed visual memory deficits are due to memory deficits, poor visual spatial skills or attentional problems has yet to be

partialed out. More sensitive neuropsychological measures included in our ongoing study will give a clearer understanding as to which specific cognitive processes, if any, are involved in this syndrome.

Our present data suggest that among nonretarded female carriers of fra(X) differences in cognitive function can be explained, in part, by inheritance. Much of the previous work has tended to group all heterozygotes together irrespective of IQ, age, cytogenetic status or inheritance, resulting in very heterogeneous samples. Those who have segregated according to cytogenetic analysis (Brainard et al., 1991; Chudley et al., 1983; Hinton et al., 1991; Mazzocco et al., 1991) have found differences between groups. However, because cytogenetic expression is not a definitive marker for heterozygotes, we felt segregation by inheritance to be a more theoretically sound method for defining the groups. Differences in psychological disability have been examined according to inheritance models (Reiss et al., 1989), but our study is the first to look at cognitive skills in this way. In addition, many studies of fra(X) women have had no, or inadequate, controls, so observed differences may have been exaggerated. Our study indicates clearly that some fra(X) carrier women do not differ from well matched women.

Although our findings do not discriminate between the proposed mechanisms controlling heritable differences (X inactivation vs. recombination), they do indicate that the parental origin of the fra(X) chromosome affects fra(X) expression. This appears to be true at both the molecular and behavioral levels. An expansion of this study with an increased number of subjects, more sensitive neuropsychological measures and further molecular analyses will better address these issues.

Cognitive and Molecular Aspects of Fragile X

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Running head: COGNITIVE & MOLECULAR ASPECTS OF FRA(X)

Abstract

The relationships among parental origin of the fragile X gene, gene structure and specific cognitive deficits were evaluated in nonretarded adult female fragile X carriers to determine whether: 1) origin influences gene structure and cognitive function, 2) mild cognitive impairments are associated with altered gene structure, and 3) specific cognitive domains are affected. Results indicated 56% of women with maternally-inherited, but none with paternally-inherited, fragile X showed large genomic structural alterations and selective deficits on measures of visual attention. Thus, molecular status of the fragile X gene appears to be linked to parental origin and may selectively affect specific cognitive skills.

Cognitive and Molecular Aspects of Fragile X

Fragile X is the most common form of inherited mental retardation and is associated with a known mutated gene on the X chromosome whose function is, as yet, unknown (Verkerk et al., 1991). Fragile X has a number of intriguing characteristics that include a variable phenotype, an unusual inheritance pattern, and an unstable genomic sequence. These features, which range from changes at the DNA level to changes in behavior, make fragile X ideal for the study of molecular contributions to the development of cognition.

Fragile X presents with a wide range of phenotypic expression, particularly among females. Males with fragile X syndrome are usually mentally retarded and often show autistic-like behaviors. Females are generally less severely affected and range from being mentally retarded to having specific cognitive deficits to being carriers without any apparent cognitive impairment. Among these higher functioning persons, the neuropsychological consequences of the mutation can be studied. Previous studies of females with fragile X have suggested specific cognitive profiles may exist (e.g. Freund & Reiss, 1991; Kemper et al., 1986; Mazzocco et al., 1992; Miezejeski et al., 1986), yet subjects were not characterized according to inheritance or gene state. Study of mildly impaired fragile X women offers a unique opportunity to examine the relationship of a single gene to specific aspects of cognition.

Although on the X chromosome, fragile X does not conform to typical X-linked inheritance rules (Sherman et al., 1984, 1985). Only about 80% of males with the fragile X syndrome gene are affected; the other 20% are unaffected nonpenetrant males who may

transmit the fragile X syndrome gene to their daughters. Among women who are carriers, about one third show some phenotypic expression. Theories have postulated that parental origin of the gene, or "imprinting," may account for these unusual features (e.g., Laird, 1987).

Analyses of pedigrees of large fragile X families suggested that predictions regarding the role of inheritance on subsequent phenotype could be made. All affected males must inherit the fragile X gene from their mothers. Females, on the other hand, can inherit the fragile X gene from either their mother or their father. Assuming that males expressing the full fragile X phenotype do not reproduce, females who inherit fragile X gene from a male will inherit it from a nonpenetrant transmitting male. Sherman (1984, 1985) noted that all daughters of nonpenetrant males appear to be unaffected, while some, but not all, of the daughters' daughters are affected. Yet, the designation of "affected" was made subjectively; skills were not measured psychometrically, and as such, level of function may have been incorrectly determined, especially for those with more mild impairments. Nevertheless, these retrospective analyses lead to specific derived predictions regarding inheritance of fragile X in females: 1) Women with paternal fragile X gene inheritance should be unaffected, and 2) Women with maternal fragile X gene inheritance should fall into two subgroups - some should be unaffected, while some should be affected

In a study of psychiatric profiles, Reiss, Freund, Vinogradov, Hagerman & Cronister (1989) found a subgroup of women with maternal inheritance to be more impaired than the other subjects. Cognitive skills have not been similarly studied. Smits et al., (1992) did a retrospective analysis of fragile X females' IQ scores, and also found only those with maternal inheritance were mentally retarded. However, scores were gleaned from past clinic records

and, as such, were derived using a variety of measures. Further, no specific cognitive skills and no controls were studied.

Prospective grouping of nonretarded fragile X and control women followed by standardized psychometric testing can better test the predictions.

The recent discovery and analysis of the fragile X syndrome gene, Fragile X Mental Retardation - 1 (FMR-1) (Verkerk et al., 1991), offered insight into the underlying mechanisms of the unusual inheritance pattern. The gene has an expansion of an unstable CGG repeat segment that is associated with both carrier (nonpenetrant) and affected phenotypes (Rousseau et al., 1991; Fu etal., 1991). In unaffected carrier males, the CGG repeat region is expanded by about 100-500 base pairs more than normals, and the expansion does not appear to affect the function of the FMR-1 gene (Hinds et al., 1993; Pieretti et al.,1991). This has been described as a "premutation," as it is structurally, but presumably not functionally, different from the normal FMR-1 gene (Oberle et al., 1991; Sutcliffe et al., 1992). In affected, mentally retarded individuals, the CGG region is expanded by 500 to 3,000 base pairs. In addition, there is an hypermethylation of both the CGG repeat and an adjacent upstream promoter ("CpG island") area (Rousseau et al., 1991). Together, these structural changes, referred to as the "full mutation," appear to prevent expression of the FMR-1 gene (Rousseau et al., 1991; Fu et al., 1991). This, in turn, is associated with mental retardation. Whether it is also associated with lesser cognitive impairments is unclear Among women with the full fragile X mutation, 53% were noted to be mentally impaired (Rousseau et al., 1991). Yet, careful assessment of cognitive skills was not performed Behavioral classification may have been imprecise and women with subtle cognitive

involvement may have gone undetected.

Thus, in fragile X, a single gene defect can be studied both by examining its behavioral consequences and its molecular basis. Specifically, neuropsychological study of nonretarded females with the fragile X gene enables investigation of the roles of inheritance, gene structure and the cognitive domains that are more susceptible to the FMR-1 gene's mediating effects.

The current study addresses three separate, yet related, questions: (1) does parental origin of the fragile X gene influence phenotype? (2) are mild cognitive deficits related to structural genomic changes? and (3) are there specific cognitive domains that are affected? Preliminary studies lacking in statistical power offered positive but inconclusive answers to all three questions (Hinton, Dobkin, Halperin, Jenkins, Brown, Ding, & Miezejeski, 1992). The results presented here, (with increased sample size, better characterized control subjects, and a more comprehensive battery of neuropsychological tests), clearly demonstrate that mode of inheritance influences both molecular and behavioral expression of the FMR-1 gene, and that FMR-1's function appears to influence specific cognitive domains.

Method

Subjects

Thirty two nonretarded women with fragile X were grouped according to parent of origin of their FMR-1 gene; 16 had paternal fragile X gene inheritance (PI) (mean age = 39.60 $\pm$ 7.92 years), and 16 had maternal fragile X gene inheritance (MI), (mean age = 31.53 $\pm$ 13.22 years). Inheritance was established through DNA linkage analysis. In addition, 18 controls (Co) (mean age = 35.68 $\pm$ 8.01 years) were tested. The controls were mothers or

sisters of persons with Down syndrome (a nonheritable developmental disability) or noncarrier female relatives of males with fragile X. Since fragile X women were identified through mentally retarded probands (either sons or brothers), controls were chosen who had familial experience with a developmentally disabled person, without the heritable contribution. All subjects were at least 16 years old and had a Wechsler Adult Intelligence Scale - Revised (WAIS-R) (Wechsler, 1981) Full Scale IQ (FSIQ) of 70 or greater. Informed consent was obtained from all subjects prior to testing.

Molecular Analysis

All fragile X subjects' DNA was tested according to the method described by Rousseau et al. (1991). Genomic DNA was digested with both Eco RI and Eag I restriction endonucleases. The resulting fragments were analyzed by Southern blot probed with StB 12.3, and examined by autoradiography. Testing was done "blind" to inheritance status.

Neuropsychological measures

In addition to the WAIS-R, a battery of neuropsychological tests was administered to each subject. Preliminary data indicated that the MI group performed more poorly on a measure of visual-spatial memory, the Benton Visual Retention Test (BVRT) (Benton, 1974), than did PI or Co groups (Hinton et al., 1992). Therefore, the current neuropsychological test battery was designed to assess the particular skills necessary for successful completion of the BVRT (i.e., attention, visual-spatial ability, memory and graphomotor skills) to determine more specifically the nature of the cognitive deficit present in affected females. The test measures included: the recall and copy conditions of the BVRT (Benton, 1974); the verbal and visual indices of the Wechsler Memory Scale - Revised (WMS-R) (Wechsler,

1987); the Benton Line Orientation Test (BLOT) (Benton, Hamsher, Varney & Spreen, 1983); the Hooper Visual Organization Test (HVOT) (Hooper, 1958); and a computerized continuous performance test (CPT). All measures were standardized except the CPT. The CPT involved the presentation of 4-digit numbers for a stimulus duration of 20 msec with an inter-stimulus interval of 1.5 seconds. Subjects were asked to monitor the numbers and respond whenever one 4-digit number was immediately followed by exactly the same 4-digit number. The test ran for approximately 12 minutes and had 400 stimuli. Successful completion of a shorter preliminary trial was necessary to demonstrate comprehension of the task before testing. The entire test battery was administered by the same person (VJH) and took about three hours to complete.

Data Analysis

To determine whether women carriers of the fragile X gene who are not mentally retarded may have FSIQ scores that differ from controls, and whether this was related to mode of inheritance, all subjects were administered the WAIS-R. By definition, only those who scored above 70 (2 SD below the mean of 100) on FSIQ were included into the study. Fragile X subjects were grouped according to parental origin of their fragile X gene. An arbitrary cut-off score of 1 SD below the mean (85) was set, and the number of subjects in each group who scored in the "gray" area of 1 to 2 SD below the mean was determined.

To determine whether molecular changes could differentiate mildly affected women with fragile X from unaffected carriers, and if such changes were related to inheritance, a chi-square analysis examining the relationship, if any, between CGG expansion and FSIQ was run

To determine whether FMR-1 affected specific cognitive domains in addition to

generalized intellectual function, group performance on the different measures was compared. The MI group was divided into two distinct subgroups: women who were indistinguishable from controls on both FSIQ and molecular measures (MI1), and women with both FSIQs $\leq$ 85 and CGG expansions of more than 500 bp (MI2). The four subject groups (Co, PI, MI1, MI2) were then compared on all test measures using ANCOVAs (controlling for FSIQ and age) followed by post-hoc Tukey HSD pairwise comparisons. Women in the MI2 group were generally younger than those in the other groups reflecting an ascertainment bias; they were more likely identified for inclusion in the study because they had a mentally retarded brother rather than a son.

Results

Inheritance

As shown in figure10, the distribution of FSIQ scores across the three groups supports the inheritance predictions. The FSIQ distributions for the Co and PI groups do not differ (Kolmogrov-Smirnov, $p > .05$) and no FSIQ scores in these groups falls more than one standard deviation (SD) below the mean (i.e. 85). The MI group distribution, however, differs from the other two groups (Kolmogrov-Smirnov, $p < .05$), and appears to be comprised of two subgroups. Seven data points (44%) cluster around and above the mean of 100, while nine (56%) fall between 1 and 2 SDs below the mean.

Gene Structure

Figure 11 shows an autoradiograph of a Southern blot analysis done on a representative sample. All subjects in the PI group showed the molecular "premutation"

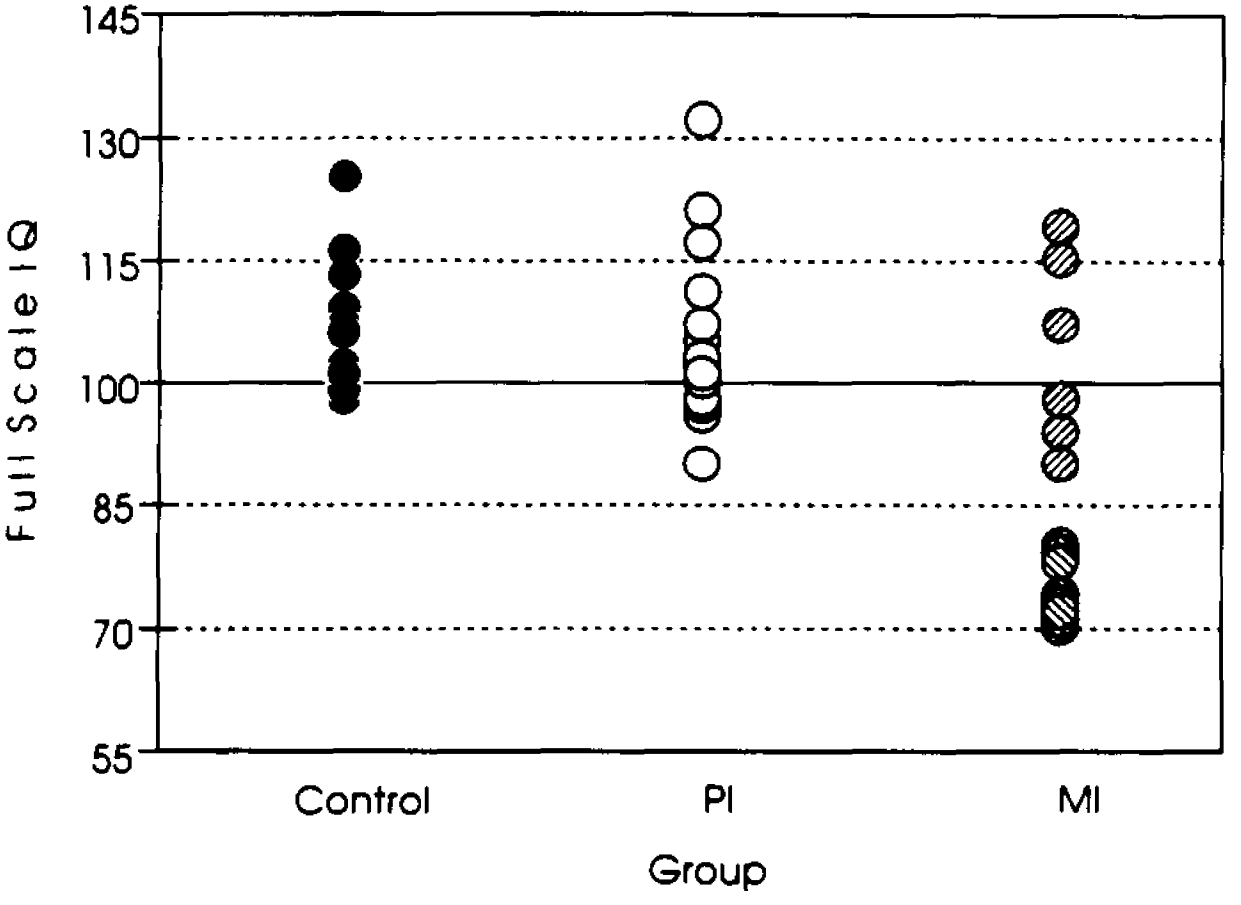

Figure 10.
Distribution of WAIS-R Full Scale IQ scores by group. Each dot represents one subject's score.

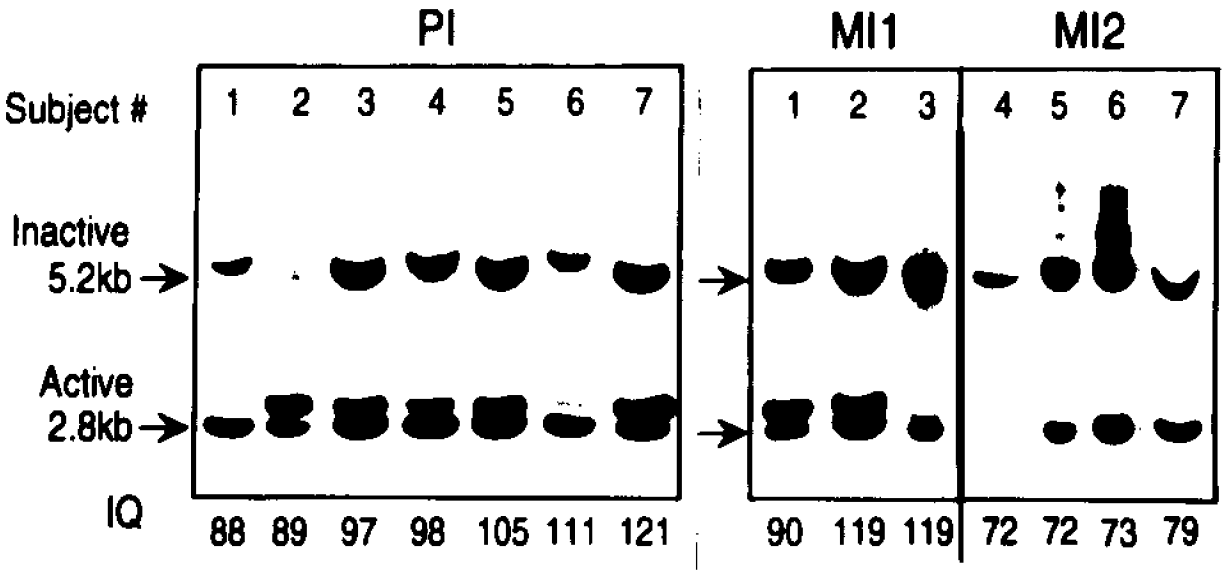

Figure 11.
Southern blot analysis of a representational subgroup of subjects' DNA digested with EcoR I and Eag I and probed with StB12.3 (9). Bands at 2.8 kb and 5.2 kb represent normal active and normal inactive X chromosomes, respectively. Segregation by mode of inheritance indicates differences in size of CGG amplification observed. All PI fragile X women look similar and show a $\geq$ 3 kb band indicative of inserts <500 bp. The MI group, however, can be separated into two subgroups. MI1 is comprised of 7 of the 16 MI women who had the 3kb band, (e.g. subjects 1-3). Nine other MI women (MI2) have bands at >6 kb, indicative of much larger CGG amplifications as well as methylated restriction sites, (e.g. subjects 4-7).

phenotype of fragile X carriers; each had an abnormal band of approximately 3,000 bp (3kb) indicative of a 150-500 bp CGG repeat. The MI group again appeared to be made up of two subgroups. Seven of the MI subjects showed the carrier "premutation" profile, and were indistinguishable from the PI group. However, nine MI subjects had the "affected," "full mutation" profile by molecular measures; each had a >6 kb band indicative of a >500 bp amplification of the CGG repeat and hypermethylation.

Notably, all subjects with the >500 bp CGG expansion had FSIQ scores more than 1 SD below the mean, whereas none of the subjects with the "premutation" profile did (Figure 12). Thus, structural differences on the molecular level occurred only in women who inherited the fragile X gene from their mothers and were associated with mild cognitive impairment.

Cognitive Profile

Specific cognitive deficits were found only in the MI2 group; no differences were observed between the Co, PI and MI1 groups on any of the measures (Table 2). After controlling for FSIQ and age, MI2 women performed significantly more poorly than the other groups on the BVRT, the visualindex of WMS-R, and the CPT, suggesting selective deficits in visual-spatial memory and visual vigilance. MI2 women did not differ from the other groups on measures of verbal memory (Verbal index of WMS-R) or visual-spatial skills that did not require sustained attention or memory (BLOT, HVOT). On the CPT, subjects did not make significantly more inappropriate responses (false alarms) than the other groups; yet, they missed significantly more target stimuli. Thus, the MI2 group appear inattentive to visually presented stimuli. Furthermore, their inattention did not appear to affect aurally

Association Between Genomic Structural Changes and Mild Cognitive Deficits in Women with Fragile X

CGG Expansion

FSIQ	< 500 bp	> 500 bp
> 85	7	0
< 85	0	9

Figure 12.

All MI women with FSIQs ≥ 1 SD below the mean had CGG expansions greater than 500 bp

Chi-square test of independence of two variables (IQ and CGG expansion size) = 16, ($p < .01$)

Table 2

Subjects' Performance Across Neuropsychological Measures

TEST	Co(x+sd)	Pl(x+sd)	MI1(x+sd)	MI2(x+sd)	F	p	Group Difference
Benton Visual Retention							
10 sec recall/# errors	2.42±154	3.56±2.25	4.14±2.54	11.44±3.84	7.01	p<.05	Co=Pl=MI1<MI2
Copy/# errors	0.56±0.23	0.12±0.34	0.29±0.49	2.00±1.58	4.23	p<.05	Co=Pl=MI1<MI2
Wechsler Memory Scale R.							
Verbal Index	104.74±11.00	97.87±10.73	103.42±18.13	84.00±20.16	.70	NS	
Visual Index	113.26±12.64	107.56±13.67	106.29±10.83	67.22±11.74	5.67	p<.05	Co=Pl=MI1>MI2
Benton Line Orientation	25.47±3.01	23.69±4.14	27.47±3.01	13.22±7.64	2.57	NS	
Hooper Visual Org. Test	27.07±2.99	26.79±2.08	27.07±2.99	21.61±3.33	2.31	NS	
Continuous Performance T.							
# Errors	7.76±5.65	5±3.50	10.86±8.76	65.11±50.72	3.58	p<.05	Co=Pl=MI1<MI2

NS = Not Significant

presented material nor did they appear to have a generalized memory problem; MI2 women had no trouble relating back details of a story read to them or remembering pairs of associated words on the WMS-R. Likewise, the MI2 group did not appear to have generalized visual-spatial deficits; on visual stimuli that could be named (e.g., HVOT) MI2 performance did not differ from the other groups. Impaired performance was observed only when MI2 women were asked to attend to and recall visually presented material.

As the Verbal and Visual indices of the WMS-R were designed to assess similar memory processes with different stimulus content, these data were analyzed further. A significant Group, Measure interaction (4 X 2 ANOVA, $F(3,47) = 7.95$, $p < .01$) (Figure 13) was found. Thus, although the MI2 group is performing more poorly than the other three groups, MI2 performance on the Visual Index is significantly worse than on the Verbal Index. The other groups perform similarly on both indices.

Discussion

In summary, the data from this study indicate that: 1) Mode of inheritance influences both behavioral and molecular expression of FMR-1; only a subgroup of women who inherited the FMR-1 gene from their mothers were affected; 2) Changes in the genomic structure of FMR-1 is present in all fragile X females with mild cognitive deficits. Even in nonretarded subjects, lower IQ scores were associated with greatly expanded CGG regions; and 3) The cognitive deficits observed are in specific domains. After controlling for age and overall intellectual functioning, visual attention was found to be impaired, while verbal, general memory and semantically encodable visual-spatial skills remained intact.

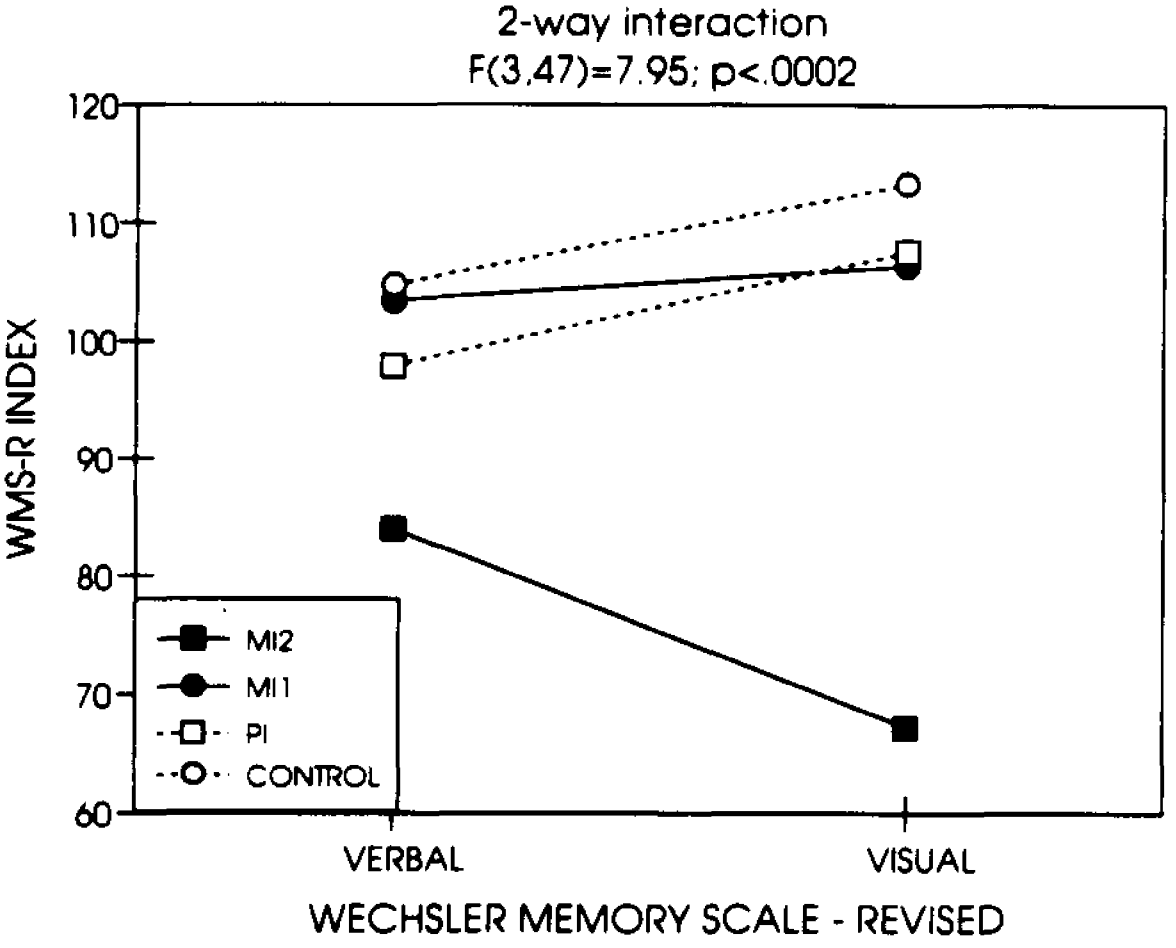

Figure 13

Significant interaction of a 2 X 4 ANOVA (WMS-R Index X Group)

Taken together, these data support a specific gene-cognition-inheritance relationship. Parental origin of the FMR-1 gene appears to affect the likelihood of the full fragile X mutation to occur. The structural changes associated with the full mutation in turn prevent expression of the gene (Hinds et al., 1993; Pierretti et al., 1991). Consequently, no FMR-1 product is made. This may affect the structure, and hence the function, of the central nervous system. In males who lack the FMR-1 product, the developmental behavioral outcome is mental retardation. In females, the developmental outcome is generally less pronounced, and most are not mentally retarded. This is probably due to the effect of the second, non-mutated X chromosome, which has a functional FMR-1 gene and likely makes some gene product. Although random X-inactivation may contribute to the variable phenotypic expression observed in women (so there are likely different dose effects from woman to woman), impaired functioning of one FMR-1 gene clearly results in atypical development. It is notable that the impairment appears to be specific. Thus, a lesion on the molecular level appears to have specific neuropsychological consequences.

The findings of a specific cognitive profile in female carriers of fragile X are consistent with other research on fragile X females. Freund and Reiss (1991) have described abstract visual but not verbal memory deficits. Mazzocco (1992) noted strengths in verbal memory and deficits in executive functions, where the deficient tasks were those that required attention to visual material. Steyaert, Borghgraef, Gaulthier, Fryns & Van den Berghe, (1992) also noted deficient performance on a visual memory task but not an auditory one. More generalized attentional and visual-spatial deficits, with verbal skills as relative strengths, have

similarly been noted in numerous studies looking at WAIS-R subtest profiles (e.g. Kemper et al., 1986; Miezejeski et al., 1986). Although previous reports of cognitive skills have not grouped fragile X women together by mode of inheritance or gene state, deficits in particular cognitive domains were noted even in heterogeneous groups.

The FMR-1 gene appears to preferentially mediate effects on some cognitive domains over others. How this occurs is unknown. Presumably, the FMR-1 product contributes to normal development of the central nervous system and without it, or with less of it, the nervous system cannot operate optimally. FMR-1 is transcribed diffusely throughout normal mouse brain, with the densest transcription observed in the granular layers of the cerebellum and hippocampus (Hinds et al., 1993). In 8 and 9 week old normal human fetuses, FMR-1 mRNAs are expressed in many types of proliferating and migrating cells of the nervous system, and by 25 weeks are most strongly expressed in the nucleus basalis magnocellularis and hippocampus (Abitol, Menini, Delezoide, Rhyner, Vekemans & Mallet, 1993). As such, lack of transcription in these areas may have the most detrimental effects on cognition in individuals with Fragile X. The role of the FMR-1 product is unknown. It may contribute to regulating levels of cAMP (Beery-Kravis and Huttenlocher, 1992), and hence may affect some of the cellular controls of learning (e.g., Kandel, 1982). The FMR-1 product may also contribute to normal structural development of the CNS. Although no gross brain malformations have been observed in fragile X, neocortical dendritic spines appear to be longer and thinner than in normal controls (Hinton, Brown, Wisniewski & Rudelli, 1991), and cerebellar vermis size appears to be decreased (Reiss, Alyward, Freund, Joshi, & Bryan, 1991). Yet these anomalies have both been found in other developmental disabilities, so

neither is likely specific to FMR-1 function. The possibility that FMR-1 is involved in the neurodevelopment of particular structures or systems that mediate specific cognitive skills is intriguing, yet to date there are still no conclusive data to support this.

Fragile X is a striking example of a change in the most fundamental genetic starting point in development which alters the most complex endpoint of definable cognitive traits in humans. Similar to other neurodevelopmental disorders such as neurofibromatosis-1 or phenylketonuria, the one-to-one relationship between gene and distinct behavioral characteristics can be established. The complex intervening steps and the diverse role of environmental influences in development remain unknown. Nevertheless, it is clear that structural changes at the molecular level are linked to specific neuropsychological deficits in women mildly affected by fragile X.

Discussion

Understanding of the developmental neuropsychology of female carriers of the fra(X) gene offers insight into the possible physiological substrates underlying some aspects of cognition. It is a genetic approach to the study of brain-behavior relationships. The differential effects observed on cognitive profiles can be inferred to be due to varying amounts of a specific gene product. Variation from controls on cognitive measures may be either a direct result of the lack of the product or due to developmental adaptive responses of the CNS to that selective loss. Thus, this approach to cognitive development is similar to the study of the consequences of early lesions to the CNS, but on a molecular level. By effectively manipulating one variable in physiological development, the study of a single gene disorder such as fra(X) provides an optimal model for the study of developmental neuropsychology.

This dissertation examined the role of inheritance and gene structure on expression of cognitive skills in females carriers of fra(X). Previous work in fra(X) allowed for the formulation of three specific hypotheses. Retrospective pedigree analysis of large families with fra(X) suggested that passage by a woman was necessary, but not sufficient, to have affected offspring (Sherman et al., 1984, 1985). However, this was untested. Molecular analysis demonstrated that the structure of the fra(X) gene found in mentally retarded persons differed from that found in fra(X) carriers (Rousseau, et al., 1991). Whether there were structural changes in persons mildly affected with fra(X) was unclear. Evaluation of cognitive skills in females with fra(X) suggested that some cognitive domains may be more susceptible

to impairment than others (for reviews see Baumgardener, et al., 1992; Pennington, et al., 1991; Miezejeski & Hinton, 1992). Yet, most studies examined poorly defined subject groups and indiscriminate test batteries, so the characteristics of the profiles were not clearly delineated.

Thus, this dissertation proposed three hypotheses to be tested in non-retarded females with the fra(X) gene: 1) Mode of inheritance will affect phenotype, 2) Structure of the fra(X) gene will determine whether cognitive impairment will be observed, even in mildly impaired women, and 3) The nature of the cognitive skills affected will be nonverbal. The findings of the dissertation supported each of the hypotheses.

First, with psychometric testing, it is clear that gender of inheritance is influential on the expression of cognitive phenotype, even in nonretarded fra(X) women. Cognitive deficits were observed only when the fra(X) gene was inherited from a woman, and in only about half of those cases. Prospective grouping according to gender of origin of the fra(X) gene, followed by careful intellectual testing, supported the inheritance models' predictions.

Second, structural changes in the fra(X) gene representative of the "full mutation" were observed even in persons with mild cognitive impairment determined through standardized assessment. The "premutation" state of the gene was not associated with any cognitive impairment. Furthermore, independent testing of genomic DNA samples indicated that the "full mutation" was only observed in samples of maternal origin. All persons in this study with mild intellectual impairment (e.g. $\geq$ 1 SD below mean FSIQ) also showed the large CGG expansion and concomitant hypermethylation of their fra(X) gene.

Third, careful segregation of the subjects based on inheritance and gene structure

indicated that selective cognitive deficits were observed only in the group of women who inherited the fra(X) gene from their mothers, had IQs less than 85, and had the "full mutation". This group could be distinguished from the other subjects both on the basis of FSIQ and gene state. Neuropsychological testing of these women indicated they had visual-spatial and attention deficits, above and beyond their generalized intellectual impairment. Their memory and verbal skills appeared to be relative strengths. A summary of ongoing work from a number of different laboratories also note similar cognitive profiles (Baumgardner, Freund, Hinton & Mazzocco, 1992). In addition, these data indicate women who had the "premutation" and were not intellectually impaired, showed no selective deficits on testing. Other recent work also supports this conclusion (Reiss, et al., submitted 1992). Further, gender of inheritance of the "premutated" fra(X) gene did not appear to affect cognitive phenotype.

These data offer convincing support of what was previously believed, but untested. However, the study has some limitations.

Limitations of the work

Because of the constraints on subject recruitment (persons had to be drawn from families where genetic information existed on three generations in order to characterize gender of inheritance through DNA linkage analysis), sample sizes were not large. Larger samples would have added considerable power to the statistical analyses. Nevertheless, the complete lack of group variation on the FSIQ/gene structure association is striking, even with the small subject number. The difficulties are more apparent in the interpretation of

performance on the specific test measures. With increased subject number test performance could have been analyzed in more detail; comparisons of scores on individual subtests could have been made, thus offering more insight into group cognitive profiles. The MI1 group had only seven members, yet a total of eight ANCOVAs were run. When the number of dependent variables exceeds the number of subjects in one of the groups, the statistical reliability of the findings may be somewhat suspect. Yet, specific predictions were made prior to testing and those predictions were confirmed, giving more credence to the data. Further, all findings, except the copy condition of the BVRT, remained significant after Bonferoni analysis. Thus, the findings are likely real representations of the fra(X) populations, even though the sample is small.

An additional related methodological problem is the fact that subjects were not truly independent, as many were members of extended families where more than one person was tested. The 32 fra(X) subjects came from 17 families. The seven MI1 subjects came from five families, the nine MI2 subjects came from six families, and the 16 PI subjects came from 10 families. As such, the possibility exists that group differences may be due to familial characteristics unrelated to fra(X). Yet, in some cases, controls are built in; for example, a mother and daughter may be assigned to separate inheritance groups because each inherited the fra(X) gene from a different gender. Some families have members in more than one group. Thus, one family has members in each of the three fra(X) groups, one family has members in both the MI1 and PI groups, and one family has members in both the MI2 and the PI groups. In addition, one MI1 subject had a control sister who is not a carrier, and one PI subject had a control non-carrier daughter. The distribution of familial subjects across the

groups makes similarities in group test performance less of a concern. Further, when the number of degrees of freedom was adjusted to reflect the number of families in the study rather than the number of individuals, the results remained the same. As there was no change in the F values that reached statistical significance after controlling for lack of independence of data points, the reported findings are believed to be robust. In addition, this study is subject to ascertainment bias. Because of the rigorous inclusion constraints, and because fra(X) women tend to be identified through a mentally retarded male proband in their family, these data may not be representative of the general female fra(X) carrier population. Many carriers likely go unidentified, and may have a different presentation of fra(X) than those who are clinically referred for diagnostic testing. Even among those females who have been diagnosed, agreement to participate in a research study that may be considered by some to be demanding and anxiety provoking may be reflective of only a portion of the population. Unfortunately, tallies of the number of persons contacted for participation and the number of refusals were not maintained. As such, the findings of this study are only representative of the people tested; they may not be generalizable to the entire female fra(X) population.

Another limitation of this study is that subjects were given neuropsychological evaluations without the examiner being blind to their inheritance status. This might result in biased evaluations. The difficulty was due again to the constraints of the inclusion criteria; the complexity of determining who to recruit necessitated the careful screening of subjects' pedigrees, and the experimenter was involved in both this and the testing. The neuropsychological tester was blind, however, to the molecular measure. Further, the molecular status was determined by independent investigators who were blind to both

inheritance and cognitive status. Thus, measures were determined independently that corroborated the findings, so concerns of bias are less marked.

Other problematic areas are related to the difficulty of defining "specificity " "Specificity" is used to describe a number of things that, although related, are distinct and easily confused. These include: 1) test measures, 2) functional skills, 3) performance, 4) underlying etiology , 5) neurological substrates, and 6) the definitive nature of the cognitive profile. For all of these, certain caveats must be noted before assuming "specificity "

Specificity of Test Measures

Understanding of the nature of the cognitive skills of the different subject groups is only as good as the tests chosen to measure them. Reasoning that specific deficits underlie poor performance on some test measures, but not others, assumes a "specificity" of the measures that may not be valid. Measures for this study were chosen on the basis of face validity (they look like they measure the skills of interest), and data implicating performance on them to be deficient in certain types of neurological impairment. Ideally, tests should assess circumscribed skills and be comparable in difficulty. Yet, for most tests, successful performance involves multiple functional skills, so deficient performance could be attributed to problems in any or all of those areas.

Preliminary data indicated that women with a maternally inherited fra(X) gene performed more poorly on the BVRT than did those with a paternally inherited gene or controls. BVRT performance relies on attention to the stimuli, visual-spatial comprehension, memory, and graphomotor skills. The subsequent neuropsychological test battery was chosen

to tease out the relative contribution each of those areas, and did to some extent Yet, the CPT, which was intended mostly as a measure of attention, has memory aspects to it; correct responding relies on memory of the previously seen number. Likewise, the memory tests involve attention; memory can only be tested if the stimuli were attended to in the first place. Since only outcome performance is measured, determining what specific skills contribute to that outcome may be difficult. Tests designed to have components that are similar in nature, but distinct in content, allow for a better determination of test "specificity"

The verbal and visual indices of the WMS-R were the only measures used that were constructed with the goal of being equivalent, yet different, in mind. Because of this, these data were analyzed further. A significant group by measure interaction was found, (2 X 4 ANOVA, $F(3,47) = 7.95$, $p<.01$), (see Figure 13). Thus, although the MI2 group is performing more poorly than the other three groups, MI2 performance on the Visual Index is significantly worse than on the Verbal Index. The other groups perform similarly on both indices. As such, given the established "specificity" of the measures, the MI2 group's differential performance on them lends strength to the conclusion that they have "specific" deficits. This is further supported by other research that also finds verbal memory stronger than visual memory in fra(X) females (Freund & Reiss, 1991; Steyaert et al., 1992).

Functional Specificity

Neuropsychological tests are not given to determine how subjects perform on them, but rather to gain an understanding of the subjects' underlying functional skills. Thus, because of the pattern of test results, these data suggest that the MI2 group has functional deficits in

the areas of visual-spatial skills and attention. These findings are in agreement with much of the previous research in females with fra(X). Yet, visual-spatial and attentional deficits are broad symptoms that offer little in terms of understanding functional "specificity."

Luria wrote, "the most important section (in neuropsychological evaluation) is the detailed psychological analysis of the structure of the disturbance and the elucidation of the immediate causes of collapse of the functional system or, in other words, a detailed qualification of the symptom observed," (Luria, 1973, p. 35). Analysis of the "specificity" of functional systems involves determining the distinct contributions to a general skill. Visual-spatial and attentional abilities are not unitary constructs. Detailed understanding of what is impaired in these functional systems in fra(X) females with the full mutation can direct the design of remediation programs. Stimuli in visual tests can be broken into two types: verbal/symbolic and configural (Lezak, 1983). These data suggest that the nature of the visual processes affected in the MI2 group are of the configural type; MI2 performance did not differ from controls on the HVOT, a visual test that can be verbally encoded. However, on tests of abstract visual stimuli (e.g. WMS-R Visual Index, BVRT) the MI2 group scored lower than controls. Other research with fra(X) females also supports these findings. For example, poor performance has been noted on the WAIS-R Block Design subtest (which is abstract) but not on the Object Assembly or Picture Completion subtests (which are meaningful) (Grigsby, et al., 1987, 1990; Hagerman & Smith, 1983; Kemper, et al., 1986). Thus, learning strategies relying on verbal cuing may be helpful when teaching girls with the full fra(X) mutation.

Visual functions can be subdivided into: visual inattention, visual recognition, visual

organization, visual interference, and visual scanning (Lezak, 1983). Because MI2 group performance was not impaired on the BLOT, visual recognition may be intact Likewise, visual organization may also be intact (at least for verbal/symbolic material), since the MI2 group did no differently from controls on the HVOT. However, this dissertation cannot attempt to determine the relative contributions of each of these different aspects of visual spatial skills. Rather, these data offer a starting point for later study. Future comparisons of affected fra(X) females' performance on tests known to be sensitive to the different visual areas can offer more detailed information about the functional "specificity" of the effects of the full fra(X) mutation.

Attention can be subdivided into sustained attention and selective attention (Halperin, 1991; Halperin, McKay, Matier & Sharma, in press). The CPT is generally accepted as a measure of sustained attention, since subjects must maintain focus on the stimuli for the duration of the task. Yet, deficits in sustained attention can only be noted with changes in CPT performance over time. These data indicated that the MI2 group performed significantly more poorly than the other groups on the CPT. Yet, although the MI2 group made significantly more errors than the other groups, the distribution of errors across 4 equal blocks of time did not change. This suggests the MI2 group may have a performance, but not a sustained attention, deficit. Further, error analysis indicated the MI2 group had significantly more missed responses than the other groups, but not more false alarms. This may be more indicative of inattention than impulsivity. The nature of these performance deficits may be related to many things including scanning, encoding, recognition or response deficits. More detailed dissection of the impairment to better determine the functional "specificity" of

attention must await future study. Yet, some observed attention deficits in fra(X) girls may be addressed by increasing exposure to material, both by lengthening viewing time and with repeated presentation.

Performance Specificity

The performance "specificity" of the MI2 group was determined by comparisons to performance of higher functioning persons. Since most of the normative data on the measures have been collected from people without developmental disabilities, how apparent "specificity" of performance in the normal population can be equated with apparent "specificity" of performance in a developmentally disabled population is unclear. As such, future work should include an IQ-matched control comparison for the MI2 group. Even though the effects of IQ were statistically controlled for, it is still entirely possible that lower functioning persons have a different overall cognitive profile than higher functioning persons Taking away the effects of IQ indicated the MI2 group still performed relatively more poorly in certain areas, but maybe all persons with lower IQ do relatively worse in certain areas than do persons with higher IQ. Matching subjects on IQ and giving them the same battery of tests could better determine whether lower functioning fra(X) women have "specific" performance deficits. Further, if subjects were grouped by known etiology as well, then it would be potentially possible to determine "specificity" of profiles.

Etiological Specificity

The idea of "specificity" of profile suggests that a pattern of test performance may be

indicative of the underlying etiology. Yet, this may be hard to establish, since the cognitive presentation in mild forms of phenylketonuria, neurofibromatosis-1, and Turner's Syndrome are not all that dissimilar from that of females with fra(X). Although generalized depressed intellectual skills are observed with persons more severely affected by these disorders, more mild presentations all show visual-spatial, attentional and executive function deficits (de Sonneville, Schmidt, Michel & Batzler, 1990; Eldridge, et al., 1989; Silbert, Wolff & Lillienthal, 1977; Welsh, et al., 1990). Also, verbal skills appear to remain relatively intact in the mild forms of these disorders. As such, there may be little profile "specificity" related to etiology. It is intriguing that these disorders of known etiology look more like each other than they do the majority of learning disabilities of unknown etiology. This offers the possibility that mild genetic disorders may be more likely to affect certain cognitive domains over others.

There are a number of possible theoretical explanations for this Some cognitive domains may be more susceptible to developmental impairment than others because of their fundamental nature. For example, measures of executive functions rely on the integrity of many skills. Therefore, a variety of different underlying deficits in development could result in poor performance on these tests. This is similar to Vygotsky's rule of 'dynamic localization' as described by Luria: "a lesion of a particular part of the brain (or in these cases, a genetic disturbance) in early childhood ha(s) a systemic effect on the higher cortical areas superimposed above it" (Luria, 1973, p.33). Implicit in the name "executive functions" is the idea that they are such higher cortical functions.

Another possibility is that the nervous system may try to adjust or adapt to the genetic

imbalance as best it can. This is similar to the idea of "crowding," as is observed in developmental compensation for early gross neurological injury. For example, in cases where the left hemisphere has been removed in infancy, children have been shown to develop relatively good language skills, yet do poorly on visual-spatial measures. It has thus been argued that the preservation of language may be at the expense of visual-spatial skills, because of a limited amount of available nervous tissue (Vargha-Khadem, et al. 1985). Similarly, in mild genetic disorders, hypothetical developmental compensatory mechanisms may exist that preserve some skills (perhaps those with more evolutionary selection pressure, such as verbal skills in humans?) at the expense of others. Perhaps those skills that get the greatest emphasis during development may be more likely to be preserved.

It is notable that disorders of different etiologies may look very similar in their cognitive phenotype. Testing IQ-matched subjects grouped according to etiology on the same battery of tests could offer insight into the "specificity" of cognitive profiles.

Neurological Specificity

Determining whether MI2 subjects have "specific" neural involvement is more problematic. With localized CNS lesions, persons can be shown to perform differentially on tests of presumably different skills. Thus, "specific" lesions (affecting a circumscribed area) can lead to "specific" deficits (affecting circumscribed skills). Yet, the reverse cannot always be inferred. "Specific" deficits may not be due to "specific" brain lesions. The causal factors of poor performance for the injured, but otherwise normally developed, CNS and the abnormally developed CNS may be very different.

In fra(X), the mutation may have generalized effects on the nervous system. There may be a diffuse lack of the product. The effects of this during development may be far ranging and unfocused.

On the other hand, potentially, the fra(X) mutation could affect only circumscribed areas. There are at least two theoretically possible mechanisms for this. The gene may be preferentially expressed in certain areas, so lack of its product will be more detrimental to those areas than others. Another possibility is that in females, where the effects of the disorder are milder and presumably more "specific," X inactivation may play a key role. In each cell in females only one X chromosome is expressed. Somewhere around the third gestational week in human female development, one of the two X chromosomes is randomly inactivated. After inactivation, the genes on that X chromosome are no longer expressed and the products are no longer made. The active X chromosome then does the work for that cell and its descendants. Presumably, brain areas that develop from precursor cells where the mutated gene is on the inactivated X should function normally, as the normal gene will be being expressed. Brain areas that develop from cells with the mutated gene on the active X chromosome, however, will be lacking in the product. Thus, potentially, whole structures of the brain could be randomly either affected or not. In fra(X) it is possible that X inactivation is not random, as selective X inactivation has been demonstrated in rapidly dividing somatic cells (leukocytes) (Rousseau, Heitz, Oberle & Mandel, 1991). Whether a similar selection could occur in development of the CNS is unknown. Yet, if possible, some brain areas might be preferentially affected over others. If this were the case then, the analogy to localized brain injury might not be all that far fetched.

This could, theoretically, be tested. The data from this study suggested females who have the "full mutation" show selective deficits in abstract visual memory and attentional skills. Neuropsychological work studying persons with specific brain lesions indicates that injury to the parietal association areas, hippocampus and possibly the frontal lobes can present similarly. Thus, it could be hypothesized that those brain areas may be involved. PET scan studies looking at metabolic activity in different brain areas in both fra(X) and control females could indicate whether these areas were preferentially affected. Post mortem studies examining fra(X) gene state in tissue samples from different localized brain areas could indicate whether the X chromosome with the mutated gene was preferentially inactivated. Further, once a specific antibody to the fra(X) gene product is made, brain immunocytochemistry studies could determine if there is a selective lack of that product in certain areas of fra(X) brains. Understanding of the possible relationship between "specific" brain area and "specific" deficit allows for the formulation of testable hypotheses.

Definitive Specificity of the Cognitive Profile

Another quandary in the understanding of "specificity" of cognitive profile has to do with individual variation. Although group data present as having characteristic overall deficits in certain cognitive domains, there was considerable variation in performance within the groups. The MI2 group had the greatest variance of any of the groups. Further, although their group means were substantially lower than those of the other groups on many measures (e.g. BLOT), the amount of variation within the MI2 group prohibited them from reaching statistical significance. Contributing factors to individual variation are likely due to an

immense range of environmental effects, including other genetic material, intra- and extra-cellular influences, familial environment and educational opportunity. Determining the spectrum of contributions may seem daunting since "the central nervous system integrates and filters the dictates of genes in ways that for the most part are inaccessible to experiment" (Scheller & Axel, 1984, p.116). Further, if X inactivation is truly random, it may contribute to individual variation. It is theoretically possible that different persons will have different brain areas that develop from precursor cells with the FMR-1 mutation on the active X chromosome. Thus, different women could have different underlying neural involvement, presenting as different phenotypes.

Individual test performance may not fit the established pattern of overall cognitive profile. As such, diagnosis by test performance will likely never be definitive, yet it may prove to be a useful screening technique for further molecular testing. To determine individual subject's profiles, the effects of IQ were regressed from scores from each of the measures. The mean and standard deviation of the standardized residuals from the control group were obtained on each measure. Cut-off scores were then arbitrarily set at 1 1/2 standard deviations from the control group's mean. The number of subjects from each of the other three groups who scored below (or above when the measure was number of errors) the 1 1/2 SD cut-off was determined. Individual profiles were then assessed looking at relative performance on each measure. None of the ninewomen in the MI2 group showed the exact profile characteristic for the group. Thus, there may be wide variation, and little evidence of definitive "specificity," in individual cognitive profiles.

Nonetheless, it is exactly that variation in presentation that is the essence of complex

traits, such as cognitive skills. Variation is what makes individuals distinct. It is the crux of the problem of cognitive development - to determine how, when brains are all made of basically the same material, each individual's mind is unique.

From Gene to Cognition

This dissertation started out with the premise that grouping persons according to a known etiology, particularly a single gene disorder, could offer insight into some of the causal determinants of cognition. This premise is still held. The innumerable contributions of both genetic and environmental influences, and the dynamic nature of development, must be emphasized. "Development is not strictly governed by an unalterable plan locked away in the genome; rather, developmental phenomenon are initiated by genomic events that depend in turn on epigenetic influences. This means that unraveling the relative contributions of genetic and epigenetic factors to particular developmental events is inevitably frustrated by the fact that genes influence the developmental environment - and that developmental environment influences genes," (Purves & Lichtman, pp.357).

Again, study of single gene disorders offers the advantage of holding one variable constant (e.g., lack of that gene product). Reliable differences in phenotype between persons with the single gene defect and those without can be reasoned to be related to that defect. Thus, a causal chain can be established. Throughout the chain, on a myriad of levels, expression of the phenotype can be controlled. As such, there is no direct one-to-one mapping of gene to cognitive trait. A wide variety of phenotypic presentations, within the constraints of the system with the single gene defect, are likely to be observed.

Yet, the chain can be potentially followed. A gene codes for a product which may affect the structure of the nervous system and hence the function of the nervous system, ultimately affecting behavior (Figure 14). The impact of a missing gene product can be noted at different developmental points. Thus, functional differences due to the lack of specific product, secondary consequences and adaptive developmental responses of the CNS can be observed. Differences can be assessed between the systems with the defect and those without throughout development. The result is a model situation for examining cognitive traits with a neuroscience reductionist approach.

Even in fra(X), where there is a limited amount of information about the disorder, such an approach can be attempted. With time, gaps in the causal chain hopefully will be filled in.

The full mutation in fra (X) develops from the premutation. This appears to occur only with passage from the mother. What causes the original premutation is perplexing; to date, no new mutations have been noted. Rather, the premutation has been traced back in all the pedigrees tested, and has been shown to be carried for multiple generations without developing into the full mutation (Smits, et al., 1993). Fra (X) may be one of the most common single gene disorders in humans, with 1/850 persons carrying the premutation. It has been proposed that because the full mutation is essentially lethal (because generally it is not carried past its appearance in males), the prevalence of the premutation may represent some sort of evolutionary selection pressure to maintain it. Perhaps there is some unknown advantage to maintaining a hypervariable sequence of nucleotides.

As per the central dogma of molecular biology, genes are transcribed into RNA and

FRAGILE X AS A MODEL FOR DEVELOPMENTAL NEUROSCIENCE

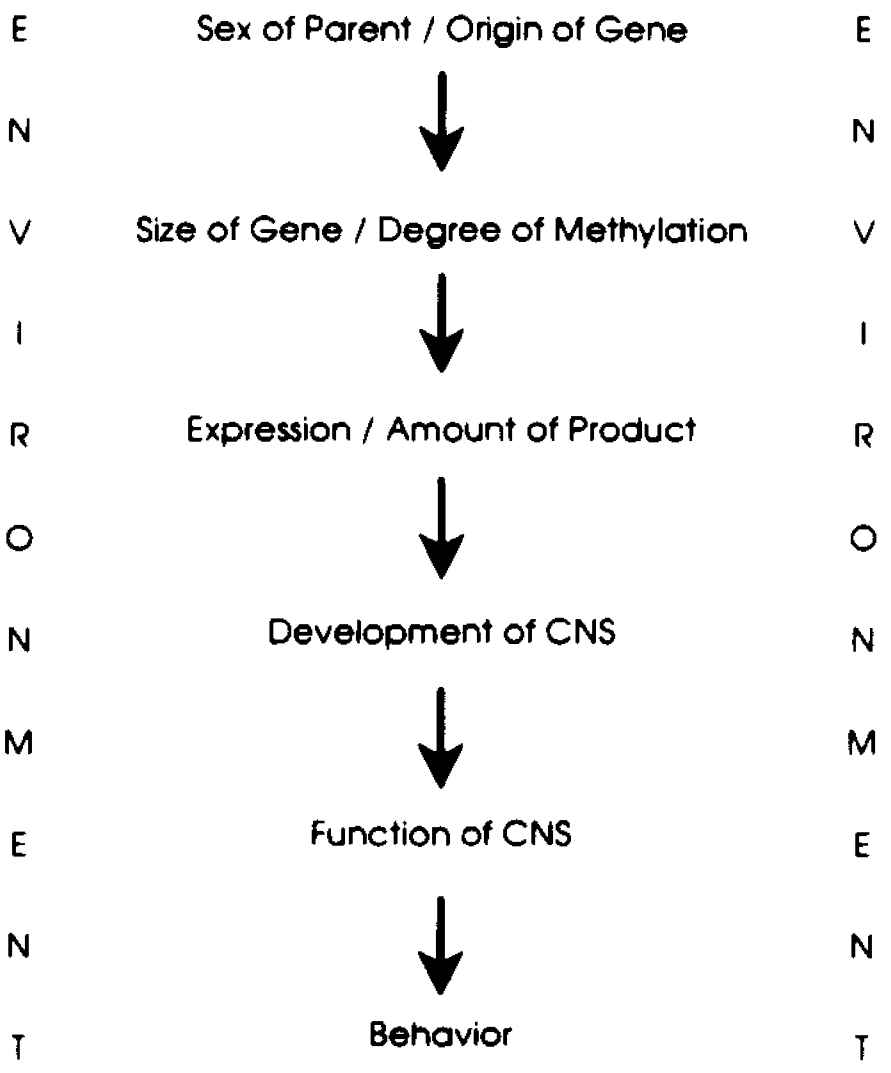

Figure 14.

RNA is translated into amino acid sequences, or proteins. Multiple control mechanisms occur at each level. In fra(X), no transcript of the gene is observed, except in chorionic villi samples (Oberle, et al., 1991; Sutherland, et al., 1991; Yu, et al., 1991). Potentially, then, it may be the lack of gene transcription, or impairment in the developmental control of transcription, that results in the phenotypic presentation. In mouse models with the normal FMR-1 gene, mRNAs have been found. Of note, is that there appears to be a variety of alternative splicing of the mRNAs, so to date, potentially 12 different products are coded for by the FMR-1 sequence (Nelson, 1992). As such, the effect of the gene may be wide ranging, affecting a number of different, yet related, proteins each with a potentially distinct functional role. Research examining tissue specificity of the transcript has suggested that the gene is transcribed diffusely throughout the brain (Hinds, et al., 1993). Neocortical distribution of the transcript shows the molecular layer to be the least dense, suggestive of more neural than glial involvement. The densest transcription is observed in the granular layers of both the hippocampus and the cerebellum (Hinds, et al., 1993). Thus, in fra(X) it is possible that lack of the gene product in these areas may have the most detrimental effects on cognition. Both of these areas may contribute to associative learning (e.g. Thompson, 1986), and the hippocampus has been repeatedly implicated in memory in mammals (Squires, 1992). Results from this study and others demonstrating poor performance on abstract visual memory tasks may be related to impaired functioning of these CNS structures.

Unfortunately, in fra(X), less is known about the next step, translation of the RNA to protein. To date, no specific antibodies have been raised to the presumed protein products, so localization of those products has not been possible. However, even at this stage, multiple

controls could be applied, thus resulting in greater variability of end effect. Potential modifications to the formed amino acid sequences would allow the products to serve different functions. This possibility is being actively studied. The functional role of the FMR-1 protein likely will be determined in the near future.

Very little is known about the FMR-1 protein's role in the structural development of the CNS. Gross pathological examination of fra(X) brains showed no severe abnormalities or malformations. Morphometric comparison of neocortical association areas in three male fra(X) brains examined post mortem indicated no difference in neuronal number when compared to age-matched control brains (Hinton, Brown, Wisniewski & Rudelli, 1991). This is notable because in some developmental disorders (e.g. Down Syndrome) decreased neuron counts have been found (Ross, Galaburda & Kemper, 1984). However, the dendritic spines of neocortical pyramidal cells appeared to be irregularly long and thin on qualitative examination. This latter finding is similar to that observed in other mental retardation syndromes (Marin-Padilla, 1972; Purpura, 1974). Thus, although this finding is not specific to fra(X), it may have a functional consequence. If the surface area of the neuron is increased, the distance from post-synaptic receptor to axon within a neuron may also increase. Increasing the distance the post synaptic potential must travel could possibly decrease its magnitude. The end result could potentially affect the likelihood of generating an action potential. Thus, this microscopic structural change might interfere with neuronal firing. The functional consequence might be overall general intellectual impairment. Unfortunately, neocortical tissue from different circumscribed areas was not examined and compared, so no speculations regarding neural involvement in specific cognitive functions can be made.

Radiological examination of fra(X) brains has demonstrated decreased cerebellar posterior vermis size (Reiss, Alyward, Freund, Joshi & Bryan, 1991). This is also observed in autistic persons (Courchesne, Yeug-Courchesne, Press, Hesselink & Jernigan, 1988), so it is not believed to be specific to fra(X). Further, work examining the functional role of the cerebellum suggests it is more likely involved in motor than higher cognitive skills. Thus, decreased cerebellar size may relate to motor stereotypies observed in some fra(X) individuals, but may not contribute to the relatively subtle and specific cognitive deficits observed in this study.

Functional consequences of the fra(X) gene mutation may be related to levels of cAMP (Berry-Kravitz & Huttenlocher, 1993). Comparisons of levels of cAMP in fra X individuals and those matched for IQ demonstrated that cAMP levels are depressed in fra(X). cAMP has been implicated in a wide variety of cellular functions, including cellular studies of associative learning (e.g. Kandel, 1982). Reduced cAMP levels in fra(X) can potentially affect a number of processes. To date, these are the most likely known cognitive functional consequences of lack of FMR-1 gene product. Ongoing PET scan studies will offer insight into more specific metabolic differences related to cognitive function.

The Fra(X) Female: More Subtle Effects

Most studies examining the effects of the fra(X) mutation have studied persons severely affected with fra(X) (e.g., those with mental retardation), to get the most dramatic results. Women who are mildly affected have not been as thoroughly examined. In women, the contributions of two X chromosomes and the role of X inactivation allow for a very

different phenotypic presentation of the disorder. Whereas in the male with the full fra(X) mutation there is likely no gene product made and the functional consequences stem from that; in the female, there is likely some product, though probably less than in unaffected persons. Thus, in females the general effects observed in males with the full fra(X) mutation may be dampened. Alternatively, areas more susceptible to impairment may be preferentially affected in females. The potential exists to study the manifestation of subtle effects and to determine whether there are specific differences (on every, or any, level) in women with the full fra(X) mutation. Speculation about the diverse genetic and environmental contributions to cognitive development can then be made.

Conclusion

These data demonstrated a relationship among inheritance, gene structure and cognitive skills in women with the fra(X) mutation. By taking a neuropsychological approach, the dissertation investigated the functional consequences of a molecular lesion to the development of the nervous system, and, hence, to cognition. This work attempted a tentative, yet tremendous, leap from the "building blocks of life" to the "mental blocks of cognition" The possibility of causally relating changes on two such conceptually distinct levels is overwhelming. The work brushed against some of the most fundamental issues of philosophy of mind and neuroscience. If "consciousness is a physical property of the brain" (Searle, 198?), then understanding the molecular mechanisms that dictate the brain's physical properties should shed some light on the nature of consciousness. The dissertation offered a glimmer of insight into the relationship between changes on the level of a single gene and predictable, relatively subtle, effects on cognition.

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