Preface

Although it seems clichéd to say so, we live in a time of great discovery. With the remarkable advances in molecular genetics and genomics, and the Human Genome Project essentially completed, the feasibility of establishing meaningful genotype–phenotype correlations for complex human neurobehavioral disorders is within our reach.

In recent years, molecular geneticists have cloned, among others, genes producing Huntington's disease, spinal cerebellar ataxia, myotonic dystrophy, the fragile X syndrome (FXS), FRAXE (the "other" fragile X disorder), α-thalassemia mental retardation (ATR-X syndrome), neurofibromatosis types 1 and 2, tuberous sclerosis 1 and 2, and Rett syndrome. Researchers have also identified many of the genes in regions containing microdeletions that are associated with other neurobehavioral disorders, e.g., Prader–Willi/Angelman syndromes, Williams syndrome, and velo-cardio-facial syndrome (del22q11). Other genes associated with nonsyndromal X-linked mental retardation (MRX) have also been identified.

At the phenotypic end of these disorders, the development, refinement, and standardization of psychometric, clinical, and neuropsychological instruments have led to greater precision in the quantitative assessment and evaluation of cognition deficits and behavioral dysfunction. Among other neuroimaging techniques, functional magnetic resonance imaging (fMRI) now permits noninvasive access to brain function during the performance of various cognitive tasks. The development of animal models to emulate cognitive–behavioral features associated with many human genetic mutations, e.g., α-calcium-calmodulin kinase II, FXS, and Rett syndrome, also permit us to examine neurobiological and neurophysiological functions, as well as neuroanatomical structures that could not have been previously investigated.

The time has come to weave the various molecular genetic, genomic, neurophysiological, and neurobehavioral threads together into a cohesive fabric of human genes, brain, and behavior. The goal of *Genetics and Genomics of Neurobehavioral Disorders* is to provide the reader with a clear and comprehensive account of how genetic abnormalities, neurobiology, and neuropsychology work in concert to manifest cognitive–behavioral dysfunction.

To achieve our objective, we have divided *Genetics and Genomics of Neurobehavioral Disorders* into four distinct parts. In the first we present an

introduction and overview of neurobehavioral disorders. Chapter 1 introduces neurobehavioral disorders from an historical prospective. Chapter 2 considers the neuroanatomical aspects of neurogenetic disorders, and Chapter 3 examines animal model strategies to investigate cognitive–behavioral deficits. The fourth chapter discusses the utility of examining behavioral phenotypes to investigate the pathway between genes and behavior.

The second part of the text is devoted to autosomal disorders that produce neurobehavioral dysfunction. Chapter 5 explores the genetics and pleiotropic phenotype of neurofibromatosis type 1. Chapter 6 is devoted to the cognitive–behavioral phenotype in Prader–Willi syndrome and Angelman syndrome and the genes in the deleted region that seem to affect specific functions in PWS/AS. The seventh chapter examines tuberous sclerosis 1 and 2 and genes recently discovered that cause these disorders. Chapter 8 investigates the behavioral phenotype in del22q11 (velo-cardio-facial syndrome), the psychopathology associated with the disorder, and the genes known to be deleted from the region. In Chapter 9, Williams–Beuren syndrome and genes in the deleted region on chromosome 7 known to be associated with the disorder are presented. The chapter on myotonic dystrophy (Chapter 10) describes the phenotype and the difficulties in teasing out the psychopathology associated with the disorder from what may be produced by the mutation itself.

The third and fourth parts consider X-linked disorders in which syndromal and nonsyndromal forms of XLMR are present. First, the nonsyndromal forms of X-linked mental retardation are presented in Chapters 11 and 12. Chapter 11 is a comprehensive examination of all known genes that produce syndromal and nonsyndromal XLMR (three of which are discussed in Part IV). Chapter 12 is the first comprehensive account of the genotype and phenotype in FRAXE, the "other" fragile X mutation. In Part IV the final three chapters are devoted to the three major syndromal forms of XLMR. In Chapter 13, α-thalassemia mental retardation (ATR-X) syndrome is described and both gene and gene function are reported. Chapter 14 is a comprehensive account of the fragile X syndrome and the fragile X mutation. Chapter 15 discusses Rett syndrome, an X-linked disorder primarily affecting females.

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Neuroanatomical Considerations Specific to the Study of Neurogenetics

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1. INTRODUCTION

There is a great need to understand the fundamental bases of complex behaviors such as language, memory, attention, music, emotion and affect, mathematical thinking, executive functions, visual cognition and mental imagery, and consciousness. These behaviors arise from intricate, developmental, and on-line interactions between genes and environment, having their ultimate effects at the molecular level. This understanding is difficult to achieve, as the interrelationships between genes and environmental factors that control the serial and parallel molecular events that build, adapt, and maintain the extremely complex neural structures that support these behaviors are great. The ultimate promise of neurogenetics research is the understanding of at least part of the molecular basis of behavior, which has to do with the influence of hard-wired genetic factors. As before in the history of this field, the study of disorders, in this case genetic disorders, is a reasonable start.

Identification of the genes and downstream events that lead to mental retardation and affective disorders will doubtlessly be invaluable in the diagnosis, treatment, and even prevention of human genetic disorders, with the desirable added effect of shedding light on the normal biology of behavior and cognition. There is a dearth of information about the participation of specific brain regions—and combinations thereof—in complex behaviors, which provides the opportunity for linking genes to behavior via the study of the brain. Thus, the brain represents the halfway point between genes and behaviors, and the first challenge is to understand how the brain is built from the functions of genes and their interactions with the early environment. At the same time, it is increasingly possible to link brain and behav-

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ior, the other half of the trajectory. In addition to the traditional analysis of effects of focal brain injury, this is accomplished by using techniques of modern cognitive neuroscience, including structural imaging as well as activating and mapping techniques, which permit a more complete picture of the participation of the neural components involved in behavior. These, coupled with advances in cellular, molecular, and systems neurobiology using whole animal and tissue models, optimistically helps to round off the knowledge necessary for going from genes, through brain, to behavior.

Decades of research have revealed that the interaction between gene and brain can be quite complex and nonlinear. Furthermore, the effect of (aneuploidy) haploinsufficiency for even a single gene can have dramatic and widespread effects on brain structure and function. Neuroanatomical differences associated with neurobehavioral disorders resulting from genetic abnormalities encompass virtually every morphologic anomaly imaginable, from the microcephaly of Down syndrome, through the specific neuronal migration anomalies associated with the 7p13.3 deletion associated with the Miller–Dieker malformation, to the relatively targeted striatal atrophy of Huntington's disease. It cannot be assumed that a smaller brain is bad or a larger brain size (or portion thereof) is advantageous, as normal variation and some pathological conditions demonstrate. The writer Anatole France, for instance, seems to have had a small brain. Conversely, the fragile X syndrome is associated with increased brain volume in the presence of significant behavioral anomalies. Further, the possible mechanisms by which a gene may exert its influence on the brain are numerous. For example, a gene may produce a protein with a direct role in synaptic transmission during on-line execution of behavior, may be required for building a specific structure during neural development at a critical time point, or may be a transcription factor responsible for the expression of other genes. Thus, a single change in the molecular structure of a gene could, in principle, produce myriad downstream neuroanatomical effects that, at first glance, have no apparent relationship to one another.

Equally daunting is interpreting the relationship between neuromorphology and behavior. Most studies investigating the neural substrates of behavior show that even a "simple" cognitive function or emotion can be immensely complex in its degree and pattern of brain involvement when compared to elementary sensory and motor processes. Further, unlike those neurologic diseases in which the symptoms are motoric or sensory, cognitive behavior often involves more widespread brain loci with significant individual variability. For example, it is not uncommon to find a brain lesion that produces cognitive loss in one patient and a different loss or nothing at

all in another. Conversely, it is not uncommon to see similar behavioral profiles in two patients with different brain lesions. Thus, determining how and which of several behaviors is linked to a specific lobe, convolution, or cytoarchitectonic region can be problematic. Then there is the effect of learning and the environment, which modify the effects of lesion and change the expression of genes. A given language, for instance, because of its peculiar phonological properties, may be more or less resistant to the effects of genes that cause dyslexia, or may modify the details in aphasia-producing brain lesions. Or, a longer experience with formal education may modulate the time of clinical onset of Alzheimer's disease in a given patient.

Despite intellectual and methodological obstacles toward understanding the genetic impact on brain and behavior, the advent of modern neuroscience has brought impressive advances to the field of neurobiology. Improvements in cellular and molecular methods, such as patch clamping, high-resolution microscopy, hybridization, and cloning, have provided the well established fields of histology and cellular and molecular neuroscience with new tools to elaborate on their discoveries. The ongoing characterization of genetic sequences has allowed construction of probes that react with brain tissue with increasingly greater specificity, as well as construction of mouse models for genetic disease. In addition, the invention of positron emission tomography (PET) and structural and functional magnetic resonance imaging (MRI and fMRI) have allowed the in vivo investigation of brain structure and function in cognitive and behavioral disorders, including neurodevelopmental disorders, in addition to increasing our knowledge of normal brain function.

This chapter is an attempt to explore several neuroanatomical considerations specific to the examination of neurodevelopmental disorders. We describe herein several approaches toward a common goal: the discovery of the connections between gene, brain, and mind. In our presentation, we review some of the current advances in the field, discuss advantages and disadvantages of each approach, and try to provoke new thinking about how to proceed in this area of research.

2. NEUROGENETIC SYNDROMES

Genetic syndromes with well defined etiologies provide an excellent opportunity for examining the contributions of genetics to behavior and brain development. Unlike most psychiatric conditions, the behaviors associated with known syndromes can be traced to a reasonably uniform etiology. Often, the behavioral phenotype of a neurogenetic syndrome is the result of a microdeletion of a very small number of genes that is fairly consistent

from one affected individual to the next; or, in some cases, can be traced to a single gene mutation. Although the most straightforward single-gene syndromes can result in complex and extensive neuroanatomical anomalies, research on neurogenetic conditions represents one of the most direct ways for looking at human gene–brain–behavior relationships. The following syndromes provide examples of the diversity of genetic mechanisms, behavioral phenotypes, and neuromorphology found within this field.

2.1. Down Syndrome

As a result of its relatively high prevalence and distinct cranio-facial features, Down syndrome (DS) is perhaps the most widely recognized genetic syndrome *(1)*. DS is almost always caused by a complete trisomy of chromosome 21 that results from a non-disjunction event, usually with a maternal origin *(2)*. Occurring once in approx 800 live births, DS is the most common genetic cause of mental retardation. In addition to low IQ scores, problems related to memory, language, speech, and motor coordination are frequently reported *(3–6)*.There is now a renewed interest in DS because persons with this condition are at an increased risk for developing Alzheimer-like dementia beginning at a young age.

Geneticists have been able to estimate that chromosome 21 contains only 225 genes *(7)*. However, the genes that are involved in the cognitive phenotype have not yet been identified; multiple genes may be involved. DS has a distinct neuroanatomical phenotype. Postmortem studies indicate that microcephaly and brachycephaly are common in DS *(8)*. MRI studies suggest disproportionate volume reductions in the cerebellum, beyond the decrease in general intracranial volume *(9)*. When examining neuroanatomical differences in greater detail, specific reductions are found in the frontal and temporal lobes *(10)*. Hand measurements (rather than computer or automated measurements) have found significant reductions in the superior temporal sulcus and hippocampus *(11,12)*. Preservations in subcortical tissue and parietal–occipital tissue also are seen *(13,14)*.

The neuroanatomical profile of DS appears to conform to its behavioral phenotype. Selective decreases in frontal lobe volumes have been associated with the characteristic mental retardation seen in DS affecting executive functions. Temporal lobe and hippocampal reductions can be linked to deficits in language and memory. Decreases in the cerebellum are seen to underlie the motor control problems and hypotonia typical of DS. In contrast, the relative preservation of parietal–occipital tissue may be related to the relative sparing of visual–spatial ability in this condition. In addition, preservations in subcortical tissue conform to embryological results in DS

that indicate that brain abnormalities in DS do not begin until the third trimester of pregnancy, after the formation of subcortical structures has already taken place *(8)*.

Interestingly, histological investigations reveal that even before the end of the second decade of life persons with DS commonly have neuropathological features that are similar to those of Alzheimer disease. Young subjects with DS often display amyloid(A)-β42-containing neuritic plaques typical of much older patients with Alzheimer disease *(15,16)*. A postmortem study of 100 subjects with DS found that 56% had amyloid plaques or plaques and neurofibrillary tangles; all subjects older than 30 years showed evidence of amyloid plaques *(17)*. Subjects with DS overexpress amyloid β protein as early as 21 gestational wk of age *(18)*. DS subjects typically exhibit progressive mental deterioration in the third and fourth decades of life, and there is good reason to believe that, as in Alzheimer disease, the dementia in DS is in part caused by excessive amyloid β protein deposition in the brain. However, in DS, unlike Alzheimer disease, this excess reflects the presence of the extra copy of the amyloid precursor protein gene on chromosome 21.

Investigations in DS introduce several issues that are commonly encountered in neurogenetics research. First, because the exact genes responsible for the syndrome are not yet known, the molecular mechanisms responsible for cellular and ultimately brain abnormalities remain a mystery, which makes interpretation of abnormal morphology difficult. Part of the behavioral phenotype may reflect abnormal brain structure formation, and part of it may result from subsequent changes in the brain because of additional acquired damage. Second, because the neurobehavioral phenotype of DS encompasses several cognitive and behavioral domains, and its neuroanatomical profile includes significant differences in several regions, linking a specific behavioral feature (i.e., language difficulty) to the morphology of a single neuroanatomical structure (i.e., temporal lobe) can be quite challenging. There is the problem typical of all developmental disorders, whether genetic or acquired, by which normal organization of function, for instance, cerebral laterality, cannot necessarily be invoked, as the developing brain is apt to change markedly in response to a change in one of its components. As a result, standard localization of function may be bypassed. The challenge in DS remains trying to identify genes that alter the development of the brain, genes that modify maintenance of brain structure throughout life, and genes affecting the formation of other organs, the malfunction of which could affect brain integrity. Each change in structure thus obtained and combinations of changes need to be studied in terms of effects on behavior.

2.2. Williams Syndrome

Williams syndrome (WMS) is a rare (1/20,000 live births) and fascinating neurogenetic condition that typically results from an unequal recombination during meiosis prior to conception *(19,20)*. The consequences of this event are that persons with WMS have only one copy of approx 20 genes in the 7q11.23 region of chromosome 7. The resulting phenotype presents a broad spectrum of unique physical and behavioral characteristics. The physical features of WMS include distinct craniofacial features, hypercalcemia in infancy, widely spaced teeth, strabismus, and narrowing of the vasculature, particularly supravalvular aortic stenosis (SVAS) *(21)*.

However, what is perhaps most interesting in WMS is a truly unusual profile of behavioral features *(22)*. The cognitive hallmark of WMS is a dissociation between a seemingly relatively preserved linguistic ability and profoundly impaired visual–spatial ability. In addition, a preserved social drive, and oddly, an enthusiasm for and love of music characterize WMS. Increased anxiety and attentional problems also are common in this condition *(20,23)*.

As with DS, research into the underlying neuroanatomical features of WMS reveals patterns of alteration concordant with our current understanding of functional neuroanatomy and the behavioral phenotype of WMS. Although both autopsy and MRI studies have shown that the overall brain size of persons with WMS is substantially decreased relative to typically developing controls, certain regions are relatively spared *(24–26)*. As expected from the observation of preserved language and musical abilities in this condition, the temporal lobe, specifically the superior temporal gyrus (STG), is relatively preserved in volume. In addition, the cerebellum is preserved in volume, and, on average, is of similar size compared to typically developing individuals *(25–27)*. Given recent studies implicating the cerebellum in higher cognitive and social abilities *(28,29)*, disproportionately increased cerebellum may be related to the hypersociability seen in this condition. In contrast, regions of the brain that play a large role in visual–spatial ability (i.e., parietal and occipital lobes) are disproportionately decreased compared to expectations based on total cranial volume.

More detailed investigations of WMS also have been performed on a few autopsy specimens, which allows for a much higher resolution of cortical anatomy than that permitted by MRI studies *(24,30)*. Gross examination of the WMS brain shows that there is an overall decrease in brain weight, with parietal and occipital hypoplasia common. Other than focal changes suggestive of immaturity of development, no consistent differences were found in the cytoarchitectonic organization of the cerebral cortex of subjects with

WMS. Motor and sensory association areas are easily identifiable by architectonic features typical of these areas. However, at the histological level, changes are seen in cell packing density and cell size suggesting abnormal neuronal development and connectivity.

The shape of the WMS brain also is unique. Overall, the brains of subjects with WMS are dolichocephalic and have some anomalous gyral patterns. The most consistent gross anatomic observation is a foreshortening of the dorsal central sulcus *(24)*. Unlike most typical brains in which the central sulcus extends fully to the interhemispheric fissure, in WMS the central sulcus usually terminates prematurely on the dorsal, but not ventral end. The second common shape difference is a bilateral forshortening of the parieto–occipital region, effectively a curtailment in the superior–inferior dimension posteriorly in the telencephalon.

Gross morphological differences observed in autopsy specimens have been supported by several recent structural MRI studies that confirmed in larger samples autopsy findings of abnormal central sulcus morphology, posterior curtailment, and anomalous gyri *(31–33)*. Observations made on necessarily small numbers of autopsy specimens direct attention to specific brain areas that can be assessed in large numbers of living subjects. MRI provides highly automated, in vivo evidence with sample sizes that provide more statistical power that can commonly be obtained in autopsy studies. Conversely, observations made using MRI can lead to more detailed studies in autopsy specimens at the architectonic and histological levels. We have found that this cross-level combination of histology, gross anatomical observation, and MRI analyses is a productive strategy for furthering neurogenetics research.

Despite the relatively small size of the WMS deletion region, several genes have likely roles in brain development or synaptic functioning. For example, the gene *STX1A* encodes for *syntaxin1A*, a member of a gene family that has role in neurotransmitter release *(34)*. A second gene, *LIM-kinase1*, has been shown to play a role in growth cone formation and axon guidance *(35,36)*, which may partially underlie the abnormal white matter volume demonstrated by MRI in WMS. Hemizygosity for *LIM-kinase1* has been correlated with visual–spatial impairment for both subjects with WMS and subjects with microdeletions of only the elastin (*ELN*) and *LIM-kinase* genes *(37)*. Another gene in the WMS critical region, *FZD9* (formerly known as *FZD3*, the human homologue of *Drosophila's* frizzled gene), is expressed strongly in adult brains and appears to play a key role in global brain development *(38)*. *FZD9* is a putative receptor for the *Wnt* gene family, which encode for secreted signaling glycoproteins and are known to be involved in

controlling early cell development, tissue differentiation, segmentation, and dorsal–ventral polarity *(39)*.

Neuroanatomical studies on WMS suffer from many of the same methodological limitations that are seen in DS research. Specifically, the broad array of neuroanatomical differences seen in WMS make interpretation of relationships to genetics and behavior difficult. Fortunately, there are many fewer genes in the critical WMS deletion region than in DS (about 20 compared to >200), although several of these have prominent roles in brain development. In addition, as with other developmental disorders of known genetic origin, WMS is a rare condition that can lead to difficulties in gathering statistically powerful results, particularly for studies requiring tissue samples. Finally, as with other mental retardation syndromes and developmental disorders affecting emotional behavior, the noisy and relatively stressful environment of the MRI lab can be a barrier to research.

Study of the WMS neuroanatomical phenotype also raises the question of how to interpret relative involvement in neurodevelopmental conditions. For example, although the STG is relatively preserved in WMS, can it be assumed that this volume preservation is related to the relative preservations in language in this condition? First, there is a strikingly phrenological quality to this form of reasoning, whereby volume of brain tissue is assumed to be causally related to quality of performance. Second, this argument assumes that the superior temporal gyrus in WMS serves the same function as in normal individuals. Third, regional measurements may assume a greater degree of functional localization than is evident from contemporary studies using activation approaches, such as functional MRI and PET. On the other hand, focal measurements provide clues for focusing other types of studies, and it is only through convergent evidence derived from various methodologies that a clearer picture of structure–function relationships begins to emerge.

2.3. Fragile X Syndrome

In the field of neurogenetic conditions, fragile X syndrome (FXS) is somewhat unique in that the primary genetic cause of the disease has been traced to the inactivation of a single gene. Affecting approx 1/4000–6000 live births, FXS is the most common form of inherited mental retardation resulting from a known gene *(40)*. The physical characteristics include macroorchidism, large ears, and a long face *(41)*. A distinct neurobehavioral phenotype, which differs between males and females, is present. Males with FXS are typically quite affected, with mild to severe mental retardation and learning disability. Deficits are present in short-term memory speech and

language, and stereotypic behaviors also are typical *(42–44)*. In addition, boys with FXS often have autistic features such as social withdrawal and gaze aversion *(42–45)*. Although females heterozygous for FXS generally have a similar phenotype compared to males with the disorder, their problems are typically less severe and more variable *(46–49)*.

FXS is one of the recently characterized family of genetic disorders caused by trinucleotide repeat expansions. In FXS, the expansion of a (CGG)*n* trinucleotide sequence ultimately produces methylation in the first exon of the 5' end of the *FMR1* gene, which in turn inactivates gene expression through transcriptional silencing *(50)*. Although the function of FMRP, the protein product of *FMR1*, is not yet understood, its structure suggests that it binds to RNA and can enter the nuclear envelope and therefore may possibly regulate mRNA transcription *(51)*.

Postmortem studies on brain structure in FXS have been instrumental in understanding how a genetic defect in *FMR1* leads to cognitive and behavioral problems. Interestingly, gross morphological examinations report macrocephaly and increased brain weight in FXS *(52)*, which is unusual in genetic conditions. *In situ* hybridization studies for *FMR1*-mRNA and immunohistochemistry and Western blot studies for FMRP have localized the regions within the body that typically express the *FMR1* gene. Not surprisingly, *FMR1* is expressed in brain tissue during normal human development. *FMR1*-mRNA is highly expressed in fetal CNS tissue at 8–9 mo of gestation, particularly in the telencephalon *(53)*. As development continues, there is evidence that expression of *FMR1*-mRNA becomes more specific. Abitbol et al. found that at 25 mo of age, *FMR1* mRNA is most strongly expressed in deep structures (hippocampus, putamen, diencephalon), ventricular and subventricular areas, the neocortical plate, and the cerebellum. Similarly, monoclonal antibodies to FMRP bind strongly to adult brain tissue *(54)*. In cerebellar tissue, Purkinje cells were most reactive. Cerebral tissue showed FMRP expression most prominently in the cytoplasm and proximal regions of dendrites and axons.

Histological studies of the brain have consistently shown abnormalities of neuron structure in FXS. Specifically, the dendritic spines in brains of persons with FXS are longer and thinner when compared to the "mushroom shape" of mature spines seen in typically developing individuals *(52,55–58)*. Long, thin spines in FXS resemble the immature spines of healthy controls and indicates that FMRP may play a role in synaptic development. This hypothesis is supported by observations that dendritic spines are more densely packed in FXS, which suggests a failure of natural synaptic pruning during dendrite formation *(56)*. A recent study found that FMRP interacts

with two other proteins, CYFIP1 and CYFIP2 *(59)*. Although the precise functions of these proteins are not yet known, recent studies have shown that CYFIP1 interacts with other proteins (members of the Rho family of GTPases) that have roles in the dynamic reorganization of the actin cytoskeleton *(60)*. They also play a role in the formation and maintenance of dendritic spines *(61)*. Thus CYFIP1 may be the important link between FMRP and the observed neuromorphological changes seen in FXS.

Imaging studies have allowed a new perspective on the global effects of the fragile X mutation. In addition to macrocephaly, MRI samples had the statistical power to detect morphological differences in localized regions of the brain. The hippocampus, in particular, has been shown to be larger in FXS *(62,63)*. Two studies that specifically examined the posterior fossa found decreases in the size of the posterior vermis in both males and females (particularly lobules 6 and 7) compared to normally developing controls and persons with nonspecific mental retardation *(64–66)*. Conversely, relative increases were seen in the caudate nucleus, thalamus, and lateral ventricular volumes *(67)*.

How these anatomic changes relate to the genetic, molecular, and behavioral characteristics of FXS is still unclear. Mostofsky et al. have found significant correlations between the size of the posterior vermis and verbal (Partial regression coefficient [pr^2] = 0.150; $p < 0.01$) and performance ($pr^2 = 0.099$; $p < 0.05$) IQ in 37 females with FXS *(66)*. Two functional imaging studies provide additional evidence of the neural substrates of the FXS behavioral phenotype. During tests of visual–spatial working memory, Kwon et al. found that whereas 15 typically developing female control subjects had increased activation in the inferior and middle frontal gyrus and superior parietal and supramarginal gyrus as task difficulty increased, 10 subjects with FXS did not *(68)*. Subjects with FXS also performed worse than controls during the more difficult tests of working memory. Further, Menon et al. found significant correlation between both FMRP expression and activation ratio (fraction of cells with the *FMR1* gene active) and activation bilaterally in the middle frontal gyrus (right $r = 0.71$, $p = 0.022$; left $r = 0.81$, $p = 0.004$), right inferior frontal gyrus ($r = 0.69$, $p = 0.027$), and the right supramarginal gyrus ($r = 0.7$, $p = 0.024$) *(69)*.

Because of excellent research on genetic, molecular, neuroanatomical, neurophysiological, and behavioral levels, FXS is a prime example demonstrating the promise of neurogenetic investigation. FXS, however, presents several difficulties and mysteries of its own. Unlike DS and WMS in which extra or missing genes usually appear within the genome *de novo*, the genetic mechanism that primarily causes FXS (CGG trinucleotide repeat expansion)

is not clear cut. Inactivation of *FMR1* generally occurs when the number of (CGG)*n* repeats exceeds 200; however, typically developing individuals have approx 5–50 repeats. As the number of repeats increases, so too does the probability of transcriptional silencing. When an individual has 50–200 repeats they are considered to have a premutation. Most studies agree that the premutation is not associated with cognitive and psychiatric problems, but there is some evidence that large premutations may indeed have an abnormal effect *(70)*. Thus, the existence of a premutation, particularly combined with the sex-linked nature of FXS and its differential effect on males and females, changes a relatively "ideal" single-gene disorder into a more challenging family of conditions.

2.4. FMR1 Knockout Mouse: Example of Animal Models in Neurogenetics

The *FMR1* knockout mouse was generated to study FXS under highly controlled experimental conditions and is an excellent example of the power of this type of research. The *FMR1* gene shares 97% homology between mice and humans *(71)*, and this loss-of-function mouse model has become a valuable tool for understanding the *FMR1* mutation. Since its creation in 1994 *(72)*, studies have shown that the *FMR1* knockout mouse has similar neuropathological findings and physical anomalies when compared to persons with FXS. Like males with FXS, male knockout mice have enlarged testes, learning deficits, and hyperactivity *(72)*. Differences in learning, as assessed by a water maze task, seem to be relatively mild in these mice *(73,74)*. Fisch et al., 1999 studied the *FMR1* knockout mouse for learning capacity. In an operant conditioning paradigm, older and naive mice could learn to discriminate visual from auditory stimuli, even when the task was quite difficult, raising questions about this mutant mouse's suitability as a cognitive–genetic model. In addition, recent studies have demonstrated that the *FMR1* knockout mouse has an increased likelihood for audiogenic seizures and startle responses to loud noises when compared to wild-type mice *(75,76)*. Given that persons with FXS have increased sensitivity to sensory stimuli (which may be associated to autistic-like behavior) *(71,77)*, audiogenic seizures in the *FMR1* knockout mouse may be related to abnormal auditory processing.

Equally intriguing are investigations into the neuropathology of the *FMR1* knockout mouse. As in FXS, dendritic spine abnormalities have been reported *(78,79)*. Specifically, these mice have significantly longer, more immature dendritic spines than wild-type control mice. There is also some evidence of increased spine density in the *FMR1* knockout. These findings

suggest that *FMR1* is necessary for normal pruning and development of dendritic spines, and is yet another similarity between FXS and the murine FMR1 model.

Thus far, abnormal dendritic morphology is the only confirmed neuroanatomical feature of the *FMR1* knockout mouse. Although the learning deficits in this mutant mouse would suggest that *FMR1* plays a role in long-term potentiation (LTP), no differences compared to control mice were found when hippocampal slices were stimulated electrically *(80,81)*. This finding is in contrast to experiments using other types of knockout mice that also perform poorly in water mazes but do show differences in LTP when compared to control mice *(82,83)*.

Although experiments using the *FMR1* knockout mouse provide a wealth of new data on the nature of FXS, several limitations are also apparent. First, the mechanism of *FMR1* inactivation differs between it's the mouse model and its human counterpart; whereas FXS typically results from a CGG trinucleotide expansion, the *FMR1* knockout mouse was created using homologous recombination *(72)*. Second, the *FMR1* gene homologue in mice is not identical to *FMR1*, raising the possibility that it may have a different function. However, two studies provide evidence that the murine homologue has a similar role as *FMR1*. A study using antibodies against human FMRP found that binding occurred with a high specificity for mouse neurons *(84)*. Glial cells were not labeled. The second study used a yeast-artificial chromosome (YAC) containing the human *FMR1* gene in an attempt to "rescue" *FMR1* knockout mice from the affected phenotype *(85)*. Interestingly, the presence of human FMRP in the mouse was able to prevent some alterations in physical development and produced anxiety reduction, although other behavioral problems arose as a result of *FMR1* overexpression.

From the neuroanatomical and behavioral perspectives, the *FMR1* knockout mouse raises several questions. Despite striking similarities with the fragile X phenotype at the cellular level, no global structural changes have been observed in the mouse *(86)*. This is a matter of concern given the relatively robust findings of macrocephaly in FXS, as well as the findings in the hippocampus, posterior fossa, and thalamus. Similarly, the FMR1 mouse model is unlikely to explain some of the typically human aspects of higher cognition affected in FXS, such as language and social communication problems.

3. CONCLUSION

The study of genetic contributions to cognitive and behavioral disorders is having some success and is likely to proceed at a quick pace increasing research interactions among clinicians, psychologists, and neuroscientists.

It is likely that correlations will be discovered between genetic defects and specific anomalies in brain structure and behavior. What is likely to be more problematic will be the quick unraveling of the relationships between normal gene function and normal behavior. Complete understanding of intervening structure and development of the brain, as well as the myriad environmental influences, are likely to make this job a slow one over the next decades.

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