The Fragile X Family of Disorders: A Model for Autism and Targeted Treatments

Randi J. Hagerman*,[1,2], Susan M. Rivera[2, 3] and Paul J. Hagerman[4]

[1]*Department of Pediatrics, University of California, Davis, School of Medicine, Sacramento, CA, USA;* [2]*M.I.N.D. Institute, University of California, Davis, School of Medicine, Sacramento, CA, USA;* [3]*Department of Psychology, University of California, Davis, CA, USA;* [4]*Department of Biochemistry and Molecular Medicine, University of California, Davis, School of Medicine, Davis, CA, USA*

Abstract: CGG-repeat expansion mutations of the fragile X mental retardation 1 (*FMR1*) gene are the leading known cause of autism and autism spectrum disorders (ASD). Full mutation expansions (>200 CGG repeats) of the gene are generally silenced, resulting in absence of the *FMR1* protein and fragile X syndrome. By contrast, smaller expansions in the premutation range (55-200 CGG repeats) result in *excess* gene activity and RNA toxicity, which is responsible for the neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS), and likely additional cases of developmental delay and autism. Thus, the *FMR1* gene is causative of a common (autism) phenotype via two entirely different pathogenic mechanisms, RNA toxicity and gene silencing. The study of this gene and its pathogenic mechanisms therefore represents a paradigm for understanding gene-brain relationships and the means by which diverse genetic mechanisms can give rise to a common behavioral phenotype.

INTRODUCTION

Within the past decade a family of disorders has been described in which each phenotypically distinct disorder is caused by expansion mutations of the fragile X mental retardation 1 (*FMR1*) gene. These disorders include fragile X syndrome [FXS, 1], fragile X-associated tremor/ataxia syndrome [FXTAS, 2, 3], fragile X-associated premature ovarian failure [FXPOF, 4], and psychiatric problems including autism and autism spectrum disorders (ASD) associated with both the premutation and the full mutation [5-9]. The characterization of the many phenotypes associated with fragile X mutations has paralleled a greater understanding of the molecular underpinnings for both the full mutation (>200 CGG repeats; lack of the *FMR1* protein, FMRP), and the premutation (55 to 200 CGG repeats; elevated *FMR1* mRNA and RNA toxicity) (Fig. **1**). Because of our expanding knowledge of the molecular and neurobiological changes that occur in FXS, new targeted treatments are being developed that may reverse the cognitive and behavioral changes associated with the disorder [10, 11]. Newborn screening for expanded *FMR1* alleles is in process [12], providing the opportunity for intensive early intervention to improve the outcome of children affected by this mutation. Mutations in the *FMR1* gene can give rise to autism as a component of the broader FXS phenotype, with substantial similarities between the autism associated with FXS and other as-yet-undefined (idiopathic) forms of autism; therefore, we believe that the autism of FXS constitutes a genetic paradigm for the study of the common molecular pathways leading to all forms of autism. Identification of those pathways will facilitate the development of targeted treatments for both FXS and autism.

*Address correspondence to this author at the UC Davis Health System, M.I.N.D. Institute, 2825 50th Street, Sacramento, CA 95817, USA; Tel: (916) 703-0247; Fax: (916) 703-0240;
E-mail: randi.hagerman@ucdmc.ucdavis.edu

OVERVIEW OF AUTISM

Autism is a behavioral disorder that is currently (operationally) defined as a single entity on the basis of the DSM-IV-TR criteria [13]; however, there are many known biological/molecular causes of the autism behavioral phenotype [14, 15]. The molecular tools that can now be utilized in the medical workup of children diagnosed with autism have expanded remarkably in the past few years. Whereas in the past decade we could identify approximately 10-15% of the underlying causes of autism [16, 17], more recent studies have increased this percentage to 20-41% in those instances where a thorough genetic workup includes sophisticated metabolic and molecular studies [15, 18-20].

A recent advance in diagnostic testing includes array comparative genomic hybridization (CGH) analysis for small genomic insertions/deletions, including sub-telomeric deletions [21]. The most common findings in autism identified on array CGH are similar to the high resolution cytogenetic and FISH studies recently reported by Vorstman, Staal *et al*. [22]. These findings include deletion of 2qter, deletion of 22qter, and duplication of 15q11-q13 (Prader-Willi Syndrome/Angelman Syndrome; PWS/AS critical region) [23]. In screening 29 individuals with ASD using 1Mb CGH arrays, Jacquemont, Sanlaville *et al.* [23] found abnormalities in 8 individuals (28%). Subtelomeric deletions of 22q13 were common and were typically associated with developmental disabilities, absent or delayed speech, and other autistic behaviors. This finding led to the discovery of a new autism-associated gene, *SHANK3*, within this region. *SHANK3* encodes a synaptic protein that is critical for proper brain development; point mutations of this gene cause ASD [21, 24].

An additional finding based on array CGH analysis is that duplication of the *MECP2* gene is associated with severe developmental delay and autism [25, 26]. *MECP2* point mu-

1573-3963/08 $55.00+.00

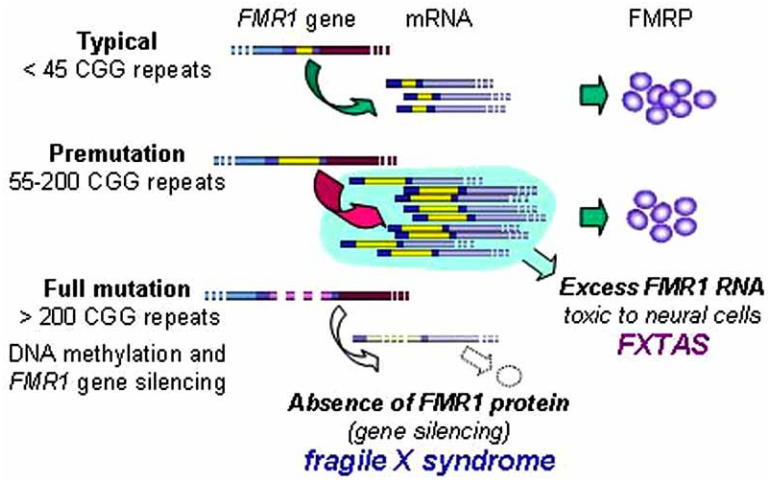

Fig. (1). Schematic of the entirely distinct mechanisms of molecular pathogenesis for fragile X-associated tremor/ataxia syndrome (FXTAS), RNA toxicity due to the elevated levels of expanded-CGG-repeat *FMR1* mRNA; and fragile X syndrome, gene silencing and absence of *FMR1* protein (FMRP).

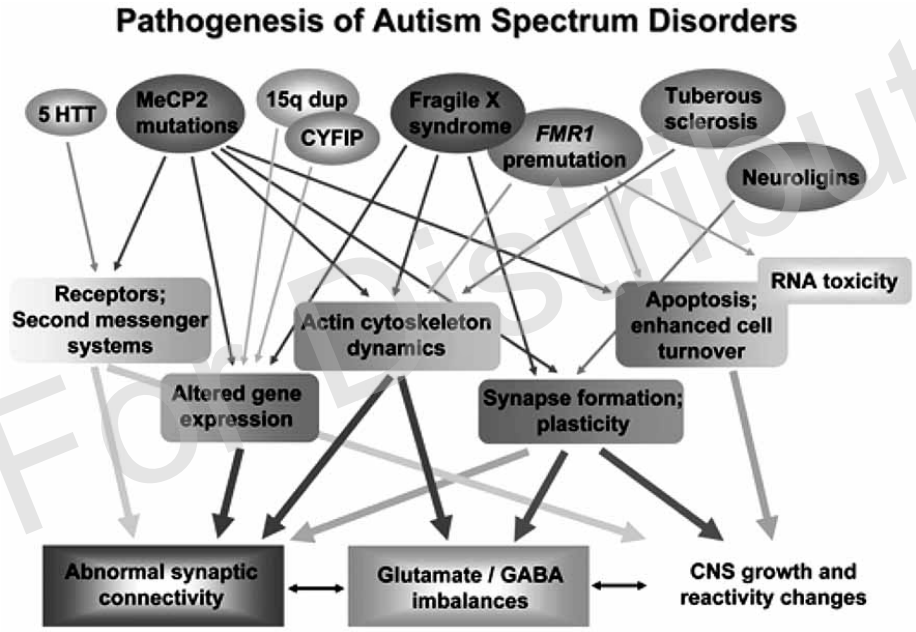

Fig. (2). Multiple genetic causes of autism and the mechanisms through which they act to disrupt neural connectivity and synaptic function.

tations cause Rett syndrome and its associated autistic features, presumably due to the absence of a functional methyl-CpG binding protein (MeCP2). This protein normally silences transcription of specific genes, leading in turn to up-regulation of genes at inappropriate times or locations during development. Although *MECP2* mutations were thought to be lethal in males, we now know that 1 to 2% of males with intellectual disability with an X-linked pattern will have an *MECP2* mutation [27]. The *MECP2* gene also demonstrates expression abnormalities that are associated with autism. Samanco, Nagarajan *et al*. [28] studied the brains of children and adults who had died with autism and found significantly lowered expression of MeCP2 compared to control brains. Therefore, autism can result not only from structural mutations in some genes, but also from expression changes in structurally normal genes [29, 30]. This concept should reveal new diagnostic methods to identify the molecular bases of newly-discovered genetic causes of autism and may also help us understand the layering of genetic changes that can ultimately produce the autism phenotype.

The molecular and neurobiological advances in autism have lead to identification of a variety of genes involved in ASD, which share several categories of function. Such genes can regulate the expression of other genes (e.g., *MECP2*, *FMR1*, *WNT2*, *HOX1A*), alter actin cytoskeleton dynamics (*TSC1*/*TSC2*, *NF1*), affect synapse formation and plasticity (*FMR1*, *NLGN3*, *NLGN4*), operate as components of second messenger systems (i.e., *PRKCB1*, *CACNA1C*), and influence neuronal migration (*RELN*, *LAMB1*, *NrCAM*) [14]. Mutations in genes that guide or facilitate proper neuronal connectivity, and genes involved with either inhibitory (GABAergic) or stimulatory (glutamatergic) synaptic connections are also contributory to autism [31] (Fig. **2**). Although the developmental program for these neural networks

Table 1. Fragile X Involvement

Full Mutation [a]	Premutation in Children [b]	Premutation in Adulthood [c]
ADHD	ADHD	Anxiety
Autism spectrum disorders	Autism spectrum disorders	Depression
Flat feet	Hyperextensible finger joints	POF
Hand biting	Prominent ears	FXTAS [d]
Hand flapping	Shyness	- Tremor
High arched palate	Social Anxiety	- Ataxia
Hyperextensible finger joints		- Neuropathy
Long face		- Muscle pain
Macroorchidism		- Hypothyroidism
Mitral valve prolapse		- Cognitive decline
Mood instability		- Anxiety
Perseverative speech		- Depression
Poor eye contact		- Apathy
Prominent ears		- Dysinhibition
Shyness		
Social anxiety		
Tantrums		

[a] Features in 25-80% of individuals with the full mutation
[b] Features in 10-30% of children with the premutation
[c] Features in 20-25% of adults with the premutation
[d] Occurs in 30-40% in males and 4-8% in females

in the CNS is genetic, environmental influences can have a significant effect on how these systems develop [32]. For example, recurrent seizures, significant birth trauma, inflammatory processes, or environmental toxins can all adversely affect neuronal connectivity with consequent developmental problems or even autism. Conversely, intensive behavioral and/or educational intervention can have a positive influence on development, with mitigation of the symptoms of autism [33]. In this regard, one of the key proteins for translating environmental stimuli to changes in synaptic structure is the *FMR1* protein (FMRP) that is deficient or missing in fragile X syndrome [34, 35].

Fragile X Syndrome

Full mutation alleles of the *FMR1* gene (>200 CGG repeats) are typically methylated, leading to reduction or absence of *FMR1* mRNA and, as a consequence, of FMRP. It is the lack of FMRP that results in fragile X syndrome (FXS). FMRP is an RNA binding protein that is thought to transport a number of mRNAs of other genes to the postsynaptic region; FMRP is also thought to inhibit the translation of these mRNAs. This important role of FMRP in the regulation of synaptic function is likely why its absence leads to ASD. Although the precise molecular mechanisms are still being revealed, absence of FMRP is thought to result in increased (or unregulated) translation of synaptic mRNAs, leading in turn to the upregulation of proteins that influence synaptic function and plasticity [34] (Fig. **2**).

The prevalence of FXS with mental retardation is approximately 1 per 3,600 males in the general population; however, females with FXS usually present with learning disabilities [36-39]. The frequency of full mutation alleles among females in the general population is approximately 1 per 2,700 [40]. The prevalence of the premutation range of 55 to 200 CGG repeats is approximately 1 per 130-260 females and 1 per 300-800 males in the general population [41-43].

Typical physical features of FXS include prominent ears, a long narrow face, hyperextensible finger joints, and macroorchidism in puberty [44, 45]. Approximately 85% of males and 25% of females with the full mutation have an IQ level < 70 [46]. Females with FXS more typically present with learning disabilities, and approximately 40% have a borderline IQ (70-85), although 30% have an IQ in the normal range (>85) [46, 47]. For individuals with a normal or borderline IQ, the presenting features are emotional and behavioral problems; particularly involving shyness, social anxiety and mood instability. Hyperanxiety disorder, social phobia, selective mutism, or ADHD are seen commonly and are part of the behavioral phenotype in those with FXS, whether they have intellectual impairment or not [45, 48] (see Table **1**).

Mutations of the *FMR1* Gene Constitute a Leading Heritable Cause of Autism

Approximately 30% of children with FXS have clinically-defined autism, based on the formal DSM-IV-TR criteria, and an additional 20% have pervasive developmental disability, not otherwise specified (PDDNOS); thus, autism and the broader autism spectrum disorders (ASD) are also part of the behavioral phenotype of FXS [6-9, 49]. Even children with FXS without a diagnosis of ASD typically have poor eye contact, unusual hand mannerisms, perseveration in speech and other features that are components of autism. Although FXS is the most common inherited cause of intellectual disability, milder forms of FXS involving psychological problems and/or learning disabilities without mental impairment are increasingly being recognized. This expanding recognition of the breadth of the phenotype of FXS is facilitating more frequent diagnosis and treatment of FXS [50-52].

In the context of this discussion, it is important to stress that the expansion mutations of the *FMR1* gene constitute a leading *cause* of autism, both in terms of the extremely high association between *FMR1* mutations and autism, and by virtue of the fact that the autism of FXS fully satisfies the DSM-IV-TR criteria. This issue is important, since a large segment of the medical/scientific community still regards "idiopathic" autism as somehow fundamentally distinct from the autism of FXS, or from any other known single-gene disorder with autism as a component of the phenotype. Causation is a remarkably imprecise term when applied to genetic and/or environmental factors that lead to disease phenotypes. In the case of FXS, full mutations of the *FMR1* gene give rise to DSM-IV-TR autism at a rate that is at least fifty-fold greater than in the general population; thus, 49 out of 50 FXS children with autism have autism as a result of the abnormal *FMR1* gene. It is thus formally correct to consider *FMR1* mutations to be a contributing cause of autism just as phenylalanine hydroxylase (PAH) mutations are a contributing cause of the autism associated with PKU. Both *FMR1* and PAH mutations require an additional factor for full expression of the autism phenotype; in the case of PAH mutations, the other factor is a diet containing phenylalanine. In FXS, there is variability in the levels of FMRP and those with the most severe deficit of FMRP are most likely to have autism [6, 8]. However, additional medical problems that affect the CNS, including seizures, additional genetic problems, or CNS structural defects, are also increased in those with FXS and ASD compared to FXS without autism [unpublished results]. An important example of this additive genetic effect to FXS is the Prader-Willi Phenotype (PWP) of FXS [53]. These children with FXS have hyperphagia, obesity, a lack of satiation after meals, small genitalia and a higher rate of ASD than what is seen in FXS generally. Although the critical region for PWS at 15q is normal structurally, a gene located in this region produces a protein, Cytoplasmic Interacting FMR1 Protein (CYFIP) that is dramatically down regulated in those with the PWP compared with normal individuals, and compared to those with FXS without the PWP [53]. CYFIP interacts with Rac and Rho and is important for synaptic function and neuronal migration, so it likely produces other problems that are additive to the autism phenotype in FXS. There may be overlap with other causes in patients with idiopathic autism, particularly those that have obesity or evidence of hypothalamic dysfunction. Indeed, the broader goal of autism research is to define all of the contributing causes of the behavioral phenotype of autism, or the phenotypic spectrum of autism, and in each individual there is likely a layering of genetic causes. As a single gene disorder that has an influence on multiple genes and pathways, FXS provides a valuable model for dissecting the neurochemical pathways that lead to autism.

Commonalities between FXS and Idiopathic Autism

Although there is a remarkably strong association between autism and FXS as well as commonalities across the phenotypes of idiopathic autism and FXS, there is also remarkable heterogeneity in the autism phenotype that occurs in FXS. This heterogeneity, undoubtedly a consequence of additional genetic factors, but perhaps also due to as-yet-unidentified environmental factors. For instance, individuals with the PWP of FXS are usually autistic with hyperphagia, obesity and obsessional thinking about food, whereas FXS individuals without the PWP may have autism with severe anxiety, aggression, or severe mood instability. The allelic variants of the serotonin transporter have been associated with autism and this is now being studied in fragile X [54]. So far we have found a higher rate of homozygosity of the long allele of the serotonin transporter in boys with FXS and aggression compared to those with FXS without aggression.

Psychophysiological studies in FXS have demonstrated hyperarousal of the autonomic system with sensory stimuli, particularly enhancement of the sympathetic response, as measured by the sweat response in electrodermal studies [55, 56], and by decreased vagal tone [57-59]. The enhanced sympathetic response and lack of habituation with stimuli correlates with the degree of FMRP deficit seen in patients with FXS [55]. Interestingly, the coexistence of autism in FXS is correlated with higher arousal and less tolerance to environmental stimuli compared to cases of FXS without autism [60]; thus, hyperarousal and lack of appropriate attenuation with stimuli is associated with the autism subphenotype in FXS. Roberts, Boccia *et al.* [57] demonstrated in preliminary studies that boys with FXS and autism also have decreased vagal tone with transitions compared to boys with FXS without autism. The enhanced arousal in children with FXS is likely related to sympathetic hyperarousal coupled with stress and anxiety and the enhanced release of cortisol [61-63]. A subgroup of individuals with idiopathic autism also have enhanced sensory modulation problems [64, 65]; therefore, FXS represents a useful model for this subtype of autism.

Recent studies of prepulse inhibition (PPI), a measure of frontal gating, have demonstrated deficits in PPI in children with FXS compared to controls [66]. The PPI deficit in FXS demonstrates an inhibitory deficit frontally and may be related to GABA deficits that have been documented in the knock out (KO) mouse model of FXS [11, 67]. The PPI deficits have also been seen in patients with autism who do not have FXS [68]. This again may reflect commonalities in GABA deficits between FXS and idiopathic autism.

A concordant physical feature of FXS and idiopathic autism, seen early on in development, is a large head. Chiu,

Wegelin *et al.* [69] have demonstrated that children with FXS and ASD have a head that grows faster than children with FXS without ASD. This finding parallels the rapid head growth seen in children with idiopathic autism reported by Courchesne, Carper *et al.* [70] and others, i.e., Mills, Hediger *et al.* [71]. Individuals with idiopathic autism and macrocephaly have been investigated genetically and 18% were found to have a PTEN mutation [72]. PTEN is a gene with pleiotropic functions that include regulation of cell growth, suggesting molecular commonalities in the pathways that lead to growth dysregulation in FXS related to hypothalamic dysfunction [45] and PTEN-coupled growth dysregulation.

Seizures have also long been recognized as part of the phenotype in FXS. Wisniewski *et al.* [73] reported that the seizures usually occurred in childhood, although seizures may also occur in adulthood [74]. In a summary of seizures in FXS from several prior studies, Musumeci *et al.* [75] found that approximately 23% (65/285) of males with FXS had seizures. The EEG patterns in patients with FXS and seizures typically include temporal and central spikes that are reminiscent of benign Rolandic spikes [74, 75]. Approximately 50% of children with FXS and without clinical seizures can have these spike wave discharges on the EEG [76]. Whether the presence of seizures is associated with more severe cognitive deficits in FXS is unclear. Our recent work has demonstrated that seizures are more frequent in children who have FXS plus ASD (28% with seizures) compared with FXS without autism (12% with seizures) [unpublished results].

Neuroimaging Studies in FXS and Autism

There is a growing body of work employing neuroimaging techniques in individuals with FXS as well as in those with autism, and a number of structural abnormalities have been observed in each group. In FXS, these abnormalities include hypoplasia of the cerebellar vermis [77-79], increased fourth and lateral ventricles [77-81], larger caudate nuclei [81, 82], and significantly increased thalamic volume in girls [81-83]. In autism, the results have been more variable, owing at least in part to the complications of group heterogeneity and limited sample sizes. Notwithstanding this variability within the autism group, one of the features that emerges most clearly is the presence of reduced volume of cerebellar hemispheres and vermal lobules [84-87] – findings consistent with those observed in FXS. In accord with these neuroanatomical similarities between the two disorders, the anxiety and posterior cerebellar area measures in FXS have distinct associations with subsets of autistic behaviors; specifically, the posterior cerebellar vermis area is negatively correlated with measures of communication and stereotypic/restricted behaviors [88]. Likewise, the severity of stereotypic/restricted behaviors in this study was negatively correlated with the activation ratio (AR; the fraction of normal *FMR1* alleles that are active). The evidence that the size of posterior cerebellar vermis (an area involved in motor function, cognition and sensory perception) is directly associated with subsets of autistic behaviors in FXS underscores the potential benefit of studying the autism of FXS for a broader understanding the neurobiology of autism.

Another brain region that has been consistently implicated in both FXS and idiopathic autism is the parietal lobe. There are now converging lines of evidence indicating that there are abnormalities in parietally-mediated processing in FXS. In a functional neuroimaging (fMRI) study of simple mental arithmetic processing, females with the FXS full mutation exhibited less overall activation than did unaffected individuals during both 2-operand (e.g., 2 + 1 = 3) and 3-operand (e.g., 3 + 3 – 1 = 5) trials [89]. Unlike the unaffected group, participants with FXS did not show increased extent of activation in association with greater task difficulty and were not able to efficiently increase recruitment of their parietal cortex to execute the more difficult, 3-operand addition and subtraction problems. There was a positive correlation between FMRP and activation in left parietal regions (including angular, supramarginal gyrus) during the 3-operand trials, providing evidence of abnormal, parietally-mediated cognitive processing in persons with FXS, and that decreased FMRP production underlies the deficits in mental arithmetic performance experienced by this population.

Evidence for parietal lobe dysfunction in FXS comes from work in early visual processing, which points to a so-called "dorsal stream" deficit. There is growing support for the existence of two parallel streams of processing, extending from V1 to other areas of the cortex: a stream directed ventrally (ventral stream) through the temporal lobe (the "what" pathway) and a stream directed dorsally (dorsal stream) through the parietal lobe (the "where" pathway) [90-93]. The dorsal stream is thought to be crucial in the visual control of action, while the ventral stream is believed to be involved in pattern recognition and object identification. Kogan *et al.* [94] has provided both neurobiological and behavioral evidence that the visual-motor deficits evident in FXS are attributable to a selective dorsal stream deficit. Mazzocco and colleagues [95] examined visuospatial skills as well as mathematical performance in non-mentally retarded girls with FXS. While their experiments were not designed to specifically probe dorsal versus ventral pathway functioning, their results were intriguingly suggestive of deficits tied to dorsal stream processing. When subjects were matched on MA, girls with FXS were significantly worse than both typically developing girls, and girls with Turner syndrome, on (a) math tasks and (b) one sub-test of the Developmental Test of Visual Perception (DTVP-2) – the "position in space" task, which involves identifying which of several similar or identical shapes matches the overall spatial position of a target stimulus. This data adds to the growing body of research suggesting a dorsal stream deficit in FXS.

Recent investigations also point to a general deficit in dorsal stream processing in autism. Much of the evidence for this comes from studies of biological motion processing, with reports of increased thresholds in either coherent motion detection or biological motion perception obscured by "noise" dot stimuli [96-98]. In addition, behavioral performance on a biological motion recognition task (person vs. non-person judgment) was found to be impaired in autism, and correlated strongly with autistic symptomology [99].

Another line of research that supports a dorsal stream deficit hypothesis in autism is the finding of deficits in smooth pursuit eye movements [100], which in schizophre-

nia has been found to be the result of dysfunction in the dorsal stream areas MT/V5 and the middle superior temporal area (MST) [101]. This type of deficit in dorsal stream processing could result in a visual perception bias in those with autism towards the ventral stream. Given that the dorsal stream tends to be specialized for quick processing of global, low spatial-frequency information and the ventral stream for slower processing of local, high-spatial frequency detail, these data suggest a general weakening of the dorsal stream that results in a bias towards the ventral stream in autism. (See [102], for a discussion of the relevance of these findings to FXS).

Overall, these findings lend support to the hypothesis that when autistic behavior and FXS co-occur, the effect may be additive in its impact on development. If substantiated by further research, these results suggest that children with both FXS and autism may be at increased risk for severely compromised development during the early years, and more intensive or specialized approaches to intervention may be warranted.

Animal Models and Targeted Treatments for FXS

Neurobiological studies in the animal models of FXS have demonstrated both glutamate and GABA system abnormalities that are hypothesized to be related to the behavioral and neurological problems in humans with FXS [10, 11, 103-105]. GABA-mediated inhibition is important in the epileptic process because it terminates ictal discharges and limits the spread of hyperexcitability [106]. The loss of FMRP in FXS leads to a dramatic lowering of the seizure threshold resulting in clinical seizures in 20% and spike wave discharges in approximately 50% of patients as previously described [75]. Imbalances in glutamate and GABA systems are also hypothesized in other forms of autism [31].

Recent studies on the mouse model of FXS have demonstrated decreased expression of the $GABA_A$ receptor subunits, particularly the delta subunit [11, 107]. As $GABA_A$ receptors are the major inhibitory receptors in the brain and are involved in processes that are disturbed in fragile X, including seizures, anxiety, insomnia and learning, an agonist for this receptor may have major therapeutic benefits for FXS [67]. Differential expression profile studies in the KO mouse have shown consistent underexpression of the delta subunit of the $GABA_A$ receptor in the cortex in multiple genetic backgrounds of the fragile X KO mice compared to control littermates [107]. Subsequent studies have shown that not only is the delta subunit underexpressed (lower levels of mRNA), but also seven other subunits, including alpha1, 3, and 4; beta 1 and 2; and gamma 1 and 2 [11]. Further studies in the *Drosophila* model of FXS demonstrated approximately 50% reduction in all 3 subunits responsible for the assembly of the GABA receptor in *dFmr1* mutants compared with the wild type strain [11]. Miyashiro *et al*. [108] demonstrated direct binding between FMRP and the mRNA of the delta subunit of the $GABA_A$ receptor, suggesting that FMRP is required for the transport and/or localization of this subunit. Not only is expression disrupted, but also the protein levels of the beta subunit of the $GABA_A$ receptor is reduced in cortex, hippocampus, diencephalon, and brainstem in the KO mouse [109]. It has been found that the ratio between inhibitory (taurine and GABA) and excitatory (aspartate and glutamate) amino acids is decreased in the brainstem, hippocampus and caudal cortex of the KO mouse [110]. There is also electrophysiologic evidence that decreased GABAergic system efficacy in KO mice interferes with cholinergic mechanisms [111]. Therefore, reversal or treatment of this specific deficit in patients with FXS may have beneficial effects in development and in aging. The evidence in animal models of FXS is strong for dysfunction of the $GABA_A$ receptor subunits and there is an opportunity now for translational research utilizing a new neuroactive steroid (epalon) anticonvulsant, ganaxolone.

In mammals, approximately 30-50% of all synapses in the CNS are GABAergic [112]. $GABA_A$ receptors mediate fast synaptic inhibition in the brain and spinal cord. There are many drugs that modulate this receptor, including ethanol and benzodiazepines; and the epalons, including ganaxolone, modulate this receptor through a unique site. Ganaxolone is a synthetic analogue of allopregnanolone, a metabolite of progesterone. However, ganaxolone is devoid of any hormonal activity [113] and has passed through phase I and II trials with demonstrated efficacy in infantile spasms in human infants and young children [114] and an absence of significant toxicity even in young children. The side effects include mild sedation but this is not as noticeable as with benzodiazepines. Controlled studies are planned to assess whether ganaxolone will be an efficacious treatment in children and adults with FXS.

The metabotropic glutamate receptor 5 (mGluR5) antagonists also represent potential therapeutic agents for individuals with FXS [115]. Huber, Gallagher *et al*. [103] demonstrated enhanced long term depression (LTD) in the hippocampus of the KO mouse model of FXS. The enhanced LTD was mediated by the mGluR5 pathway and FMRP normally inhibits the translation of proteins that internalize the AMPA receptors at the synapse, leading to LTD or weakening of synaptic connections [10]. When FMRP is absent or deficient in neural cells, as is the case in FXS, there is enhanced LTD leading to weak synaptic connections. This phenomenon is thought to be responsible for the mental impairment in FXS [10]. The study of the mGluR5 antagonist, MPEP, in the KO mouse model of FXS has led to a decrease in seizures and some improvement in cognition [116]. The study of MPEP and another partial mGluR5 antagonist, lithium, in the *Drosophila* model for fragile X has demonstrated enhanced cognition and reversal of the brain structural abnormalities caused by the KO mutation in *dfmr1* [117]. Studies using mGluR5 inhibitors and lithium in animal models suggest that there may be merit in using such agents to treat patients with FXS, and clinical trials are either underway or are being designed; however, MPEP itself is too toxic for use in humans. The first of the new experimental medications to be tried in FXS is fenobam, an anti-anxiety medication that was recently discovered to be an mGluR5 antagonist [118].

Clinical Involvement Among Carriers of *FMR1* Premutation Alleles

The most remarkable phenotypic development in the fragile X field in the past decade has been the recognition of various forms of clinical involvement in some individuals with the premutation (55 to 200 CGG repeats). Although

premutation carriers were initially considered non-penetrant (clinically unaffected) by the premutation, there are now at least two major forms of involvement among premutation carriers: premature ovarian failure (POF; primary ovarian insufficiency, POI), first reported in 1991 [119]; and the fragile X-associated tremor/ataxia syndrome (FXTAS), reported initially in 2001 [2]. Both disorders only affect a subgroup of premutation carriers, although milder involvement may be common for both the endocrine and neurological problems [120, 121]. In addition to adult involvement among carriers, there is substantial evidence that a subgroup of boys with the premutation have social deficits, autism spectrum disorders, and ADHD [5, 122, 123] (See Table 1). The mechanism of involvement in the premutation is entirely different from the full mutation in that the *FMR1* mRNA levels are substantially increased in the premutation range, leading to an RNA toxic gain-of-function that adversely affects neuronal function and survival [124-126].

POF occurs in approximately 20% of female carriers [4, 127-131]. In a review of screening studies of women who present with POF, 9.5% (9/95; 95% CI: 4.4%-17.2%) of those with familial POF have the premutation and 3.0% (8/267 CI: 1.3%-17.2%) of those with sporadic POF have the premutation [132-139]. Further endocrine studies demonstrate that although approximately 20% of carriers have POF, those carriers that are cycling normally also have endocrine dysfunction [121]. Welt, Smith *et al*. [121] studied 11 normally ovulating premutation carriers (ages 23 to 41 years) and demonstrated a significantly shortened cycle, elevated FSH throughout the cycle (91% with elevations >2 SD above the mean), elevated inhibin B in the follicular phase, and elevated inhibin A and progesterone in the luteal phase, compared to controls. These findings suggest a decreased number of follicles and granulosa cell dysfunction, or decreased cell number in the corpus luteum, compared to controls. In addition, 45% (5 of 11) had a history of infertility as defined by 1 year of unprotected intercourse without a pregnancy. This study demonstrates sub-clinical ovarian dysfunction in premutation females who do not have POF.

A recent study by Sullivan *et al.* [4] involving over 500 women representing a broad range of CGG repeats, from the normal range into the high end of the premutation range, demonstrated a significant, non-linear association between CGG repeat number and prevalence of POF. For those with a repeat <40, the prevalence of POF was 0.9%; for those with 41-58 repeats, the prevalence increased to 2.2%; for those with 59-79 repeats, the prevalence was 5.9%; for those with 80-99 repeats, the prevalence was 18.6%; and for those with $\geq$ 100 repeats, the prevalence decreased to 12.5%. Those authors suggested that individuals with a high CGG repeat number may have some ovarian target cells with a full mutation, or perhaps some cells have early methylation at a lower CGG repeat number, which may protect those cells from the toxicity of the premutation. The Sullivan *et al.* [4] paper also found an overall effect of the CGG repeat size on age of menopause, with low-end CGG repeats demonstrating menopause 2.5 years earlier than controls, and medium- to high-end premutation carriers demonstrating menopause and additional 4 years earlier than low-end carriers. For women who were still cycling, there was a CGG repeat effect on the FSH level, but only when controls and carriers were included. The activation ratio (AR) correlated with FSH levels when all women were included [4].

Other physical problems, which occur in approximately 25% of premutation carriers, include prominent ears and hyperextensible finger joints [140, 141] (See Table 1). However, these features [142] appear to be a mild version of the physical features that occur in those with the full mutation and thus may be related to subtle deficits of FMRP that can occur particularly in the upper range of the premutation [44, 143, 144]. In contrast, POF is not seen in individuals with the full mutation, suggesting that it is also related to the toxic effects of the elevated *FMR1* mRNA, which occurs almost exclusively in the premutation range [124, 145]. *FMR1* is more highly expressed in ovarian follicles compared to other organs [146], which would make it more vulnerable to *FMR1* mRNA toxicity.

The basis for the psychological and emotional problems found frequently in premutation carriers has remained controversial, due to the confounding effects of the stress of raising a child with FXS [147] (See Table **1**). Reiss *et al*. [148] reported no emotional problems in female premutation carriers compared to controls; however, Sobesky *et al*. [149, 150] reported problems of shyness, social anxiety, and depression in approximately 25%. These problems were also seen by Franke *et al*. [151], who compared premutation females, both with and without children with FXS, to control females who had children with autism. Social anxiety and social phobia were significantly higher in carriers compared to controls without the premutation. The rates of depression were similar in women with or without the premutation who had children with developmental disabilities. The study by Johnston *et al.* [83] further established the role of the CGG-repeat expansion by demonstrating a significant association between psychiatric problems and molecular variables. Those authors studied 85 women with the premutation and found that those with greater than 100 CGG repeats had higher rates of depression and interperson sensitivity on the Symptom Checklist-90-Revised [SCL-90-R, 152] than women with CGG repeats of less than 100. They hypothesized that this difference was likely due to a lower level of FMRP in those with a higher CGG repeat level. However, the elevation of mRNA in carriers has a strong correlation to psychological measures in male carriers with obsessive compulsive behavior and overall psychopathology correlating with the degree of elevation of mRNA [153].

A recent report by Hessl, Rivera *et al*. [154] demonstrated a lack of amygdala activation on fMRI to fearful faces in adult males with the premutation compared to age matched controls. This work suggests that the premutation likely interferes with connectivity to the amygdala in premutation male carriers. Similar findings of amygdala dysfunction have been also been reported in patients with ASD [155, 156].

Of all types of premutation involvement, FXTAS has had the greatest clinical impact, since it is a progressive neurodegenerative disorder with a generally late-adult-onset and increasing penetrance with age [124, 157]. The core features of FXTAS are intention tremor and gait ataxia, and are generally accompanied by cognitive decline, autonomic dysfunction, and neuropathy [158] (see Table **1**). Characteristic

radiological features of FXTAS include white matter disease in subcortical and periventricular regions; and symmetric high-signal lesions (T2/FLAIR) in the middle cerebellar peduncles (MCP sign), which consist of axonal tracts traveling from the pontine nuclei to the cerebellum [159].

The neuropathology of FXTAS includes the presence of eosinophilic intranuclear inclusions in neurons and astrocytes throughout the brain and brainstem, with the highest inclusion counts in the hippocampus and elsewhere in the limbic system. Inclusions have also been observed in the anterior and posterior pituitary [160, 161]. Purkinje cells in the cerebellum are lost (remarkably, Purkinje cells are generally devoid of inclusions) and subcortical astrocytes are remarkably activated. The inclusions contain a number of proteins that may be relevant to the disease process, including, Lamin A/C, hnRNPA2, and heat shock proteins, including alpha B crystallin [126]. There is dysregulation of the lamin A/C nuclear architecture as a direct consequence of expression of the expanded CGG-repeat mRNA; this lamin dysregulation alters cell cycle dynamics and may render the neuronal and astrocytic cells more sensitive to oxidative stress [125].

We suspect that the toxic effects of elevated *FMR1* RNA levels may also occur in early childhood, leading to clinical problems in a subgroup of premutation carriers, particularly males. Farzin, Perry *et al.* [5] studied 43 boys with the premutation, 14 boys who presented clinically, compared to their brothers who were identified by cascade testing of the family, and to their typically developing brothers who did not have the premutation. The proband boys with the premutation had a high rate of ADHD (93%) in addition to ASD (73% with half of these boys demonstrating full autism). Eight percent of the non-proband brothers who also carried premutation alleles, and who were identified by cascade testing, satisfied criteria for full autism; moreover, the group as a whole demonstrated shyness and mild social deficits that were more common than the controls [5]. Clifford, Dissanayake *et al*. [9] studied 50 premutation carriers and found that 14% of males and 5% of females met ADOS criteria for ASD. Therefore, premutation *FMR1* alleles can also cause ASD, apparently through a new mechanism of RNA toxicity that is unique to the premutation [124].

Males are more vulnerable to the adverse effects of the premutation mRNA presumably because females are protected by admixture of mRNA from the normal X chromosome. However, recent studies of adult females with the premutation have demonstrated a higher rate of autoimmune problems compared to controls. In a study of 146 female carriers, Coffey, Cook *et al.* [120] demonstrated that 4 to 8% of the older women developed FXTAS and that 50% of those with FXTAS had hypothyroidism and over 40% had fibromyalgia. Muscle aches and peripheral neuropathy were each seen in approximately 30% of adult female carriers. A previous report demonstrated multiple sclerosis in approximately 3% (3/106) of female carriers [unpublished results], and we have recently documented the presence of both MS and FXTAS in a female carrier on neuropathological studies [unpublished results]. It is likely that the elevated mRNA somehow leads to the stimulation of autoimmune dysfunction, particularly in female carriers, perhaps through the production of the increased levels of alpha B-crystallin [162, 163] observed in individuals with FXTAS, or possibly through Toll receptors that respond to mRNA and stimulate inflammation in the CNS [164, 165]. Recent studies in autism also demonstrate enhanced inflammation in the brain that likely interferes with CNS connectivity leading to autism [166]. We hypothesize that autism in premutation carriers is a model for the inflammatory or autoimmune mechanisms associated with idiopathic autism.

The RNA toxicity mechanism leading to FXTAS [review: 124] is now supported by animal research in both premutation mouse and *Drosophila* models, and in neural cell models. The addition of the premutation expansion leads to disease and inclusion formation in both mouse and *Drosophila* [167, 168]. Moreover, Jin *et al*. [167] demonstrated that a 90 CGG repeat, as RNA, results in atrophy in the eye and the formation of inclusions in the *Drosophila* neurons. Willemsen *et al*. [168] described the development of the eosinophilic inclusions in neurons (but not astrocytes) by 20 weeks of age in the premutation mouse model. Neurological problems, specifically motor problems have now been observed in these premutation mice who are aging [169]. Finally, Arocena, Iwahashi *et al.* [125] have demonstrated that many of the neuropathological features of FXTAS, including the formation of intranuclear inclusions, and altered lamin A/C regulation, can be recapitulated in neural cells in culture upon transfection of the cells with an 88 CGG repeat plasmid reporter construct. Cellular dysregulation was only observed when the plasmid was actively transcribing the expanded-repeat RNA, thus establishing the role of the RNA itself in the pathogenic mechanism.

CONCLUSION

In summary, the *FMR1* mutation has generated two different molecular models for autism. In the case of full mutation alleles that give rise to fragile X syndrome, the underlying mechanism is gene silencing and the absence of FMRP, leading in turn to the dysregulation of other genes and proteins related to synaptic plasticity. This mechanism overlaps with other genetic causes of autism that impact the synapse, including mutations in SHANK3, Neuroligins 3 and 4, and MECP2. In the case of premutation alleles, which give rise to POF, FXTAS, and developmental and behavioral problems, including ADHD and ASD, an entirely different molecular mechanism – *FMR1* mRNA toxicity – is operating. However, both molecular mechanisms lead to phenotypic overlap and, indeed, both give rise to autism. Thus, the operation of two separate mechanisms giving rise to a similar autism phenotype indicates that there are likely to be shared molecular or neural cellular pathways that precipitate the complex behavioral phenotype of autism. An excellent conceptual framework for the notion of integrated molecular functions giving rise to similar (autism) phenotypes has been presented by Laumonnier, Cuthbert *et al.* [170]. Those authors discuss the role of multiprotein postsynaptic complexes in human X-linked brain diseases; one of these complexes, the postsynaptic density (PSD), contains over one-thousand distinct protein species. In their representation, mutations in one or more constituent proteins (e.g., shank, homer, or members of the ERK/MAPK pathways), while having diverse functions, all have an adverse effect on the

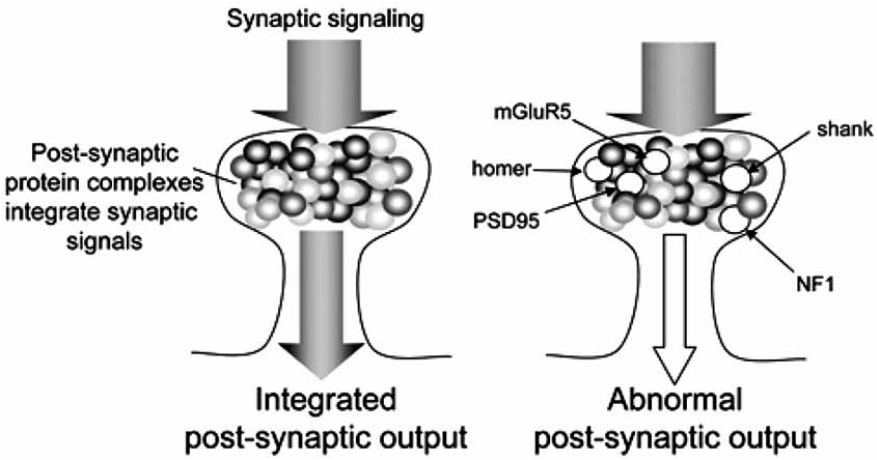

Fig. (3) Multi-protein post-synaptic complexes act to process synaptic receptor activity to produce an integrated post-synaptic output. Such complexes also act as integrators of the effects of specific single-gene mutations to produce common or overlapping phenotypic outcomes (e.g. cognitive impairment, autism).

PSD – the PSD thus becomes an integrator of diverse protein functions that results in the broader cognitive impairment/autism phenotypes (Fig. **3**). Finally, the emerging therapeutic agents for targeted treatment of FXS and FXS-associated autism may turn out to be helpful in other forms of autism. Such hope should stimulate further diagnostic endeavors for fragile X in those who present with ASD and further treatment studies in both fragile X disorders and autism.

ACKNOWLEDGEMENTS

This work was supported by NICHD grants HD036071 and HD02274; Autism Speaks; the Centers for Disease Control and Prevention collaborative agreement U10/CCU925 123; the National Fragile X Foundation; the UC Davis M.I.N.D. Institute; the Clinical Translational Science Center (CTSC) at UC Davis (UL1 RR024146) from the National Center for Research Resources; and Health and Human Services Administration on Developmental Disabilities 90DD 0596. We also thank the families and patients who have participated in our research.

REFERENCES

[1] Hagerman RJ, Hagerman PJ. Fragile X Syndrome: Diagnosis, Treatment, and Research, 3rd Edition. Baltimore. The Johns Hopkins University Press 2002.

[2] Hagerman RJ, Leehey M, Heinrichs W, *et al*. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology 2001; 57: 127-30.

[3] Jacquemont S, Hagerman RJ, Hagerman PJ, Leehey MA. Fragile-X syndrome and fragile X-associated tremor/ataxia syndrome: two faces of FMR1. Lancet Neurol 2007; 6: 45-55.

[4] Sullivan AK, Marcus M, Epstein MP, *et al*. Association of FMR1 repeat size with ovarian dysfunction. Hum Reprod 2005; 20: 402-12.

[5] Farzin F, Perry H, Hessl D, *et al*. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. J Dev Behav Pediatr 2006; 27: S137-44.

[6] Loesch DZ, Bui QM, Dissanayake C, *et al.* Molecular and cognitive predictors of the continuum of autistic behaviours in fragile X. Neurosci Biobehav Rev 2007; 31: 315-26.

[7] Rogers SJ, Wehner EA, Hagerman RJ. The behavioral phenotype in fragile X: symptoms of autism in very young children with fragile X syndrome, idiopathic autism, and other fevelopmental disorders. J Dev Behav Pediatr 2001; 22: 409-17.

[8] Hatton D, Sideris J, Skinner M, *et al*. Autistic behavior in children with fragile X syndrome: Prevalence, stability, and the impact of FMRP. Am J Med Genet A 2006; 140: 1804-13.

[9] Clifford S, Dissanayake C, Bui QM, Huggins R, Taylor AK, Loesch DZ. Autism spectrum phenotype in males and females with fragile X full mutation and premutation. J Autism Dev Disord 2007; 37: 738-47.

[10] Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. Trends Neurosci 2004; 27: 370-77.

[11] D'Hulst C, De Geest N, Reeve SP, *et al*. Decreased expression of the GABAA receptor in fragile X syndrome. Brain Res 2006; 1121: 238-45.

[12] Bailey DB, Jr., Beskow LM, Davis AM, Skinner D. Changing perspectives on the benefits of newborn screening. Ment Retard Dev Disabil Res Rev 2006; 12: 270-79.

[13] APA. Diagnostic and Statistical Manual of Mental Disorders, 4th Edition Text Revision. Washington DC: American Psychiatric Association 2000.

[14] Persico AM, Bourgeron T. Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. Trends Neurosci 2006; 29: 349-58.

[15] Veenstra-Vanderweele J, Christian SL, Cook EH, Jr. Autism as a paradigmatic complex genetic disorder. Annu Rev Genomics Hum Genet 2004; 5: 379-405.

[16] Reddy KS. Cytogenetic abnormalities and fragile-X syndrome in Autism Spectrum Disorder. BMC Med Genet 2005; 6: 3.

[17] Wassink TH, Piven J, Patil SR. Chromosomal abnormalities in a clinic sample of individuals with autistic disorder. Psychiatr Genet 2001; 11: 57-63.

[18] Schaefer GB, Lutz RE. Diagnostic yield in the clinical genetic evaluation of autism spectrum disorders. Genet Med 2006; 8: 549-56.

[19] Steiner CE, Guerreiro MM, Marques-de-Faria AP. Genetic and neurological evaluation in a sample of individuals with pervasive developmental disorders. Arq Neuropsiquiatr 2003; 61: 176-80.

[20] Martin CL, Ledbetter DH. Autism and cytogenetic abnormalities: solving autism one chromosome at a time. Curr Psychiatry Rep 2007; 9: 141-47.

[21] Stankiewicz P, Beaudet AL. Use of array CGH in the evaluation of dysmorphology, malformations, developmental delay, and idiopathic mental retardation. Curr Opin Genet Dev 2007; 17: 182-92.

[22] Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L. Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. Mol Psychiatry 2006; 11: 18-28.

[23] Jacquemont ML, Sanlaville D, Redon R, *et al.* Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. J Med Genet 2006; 43: 843-49.

[24] Durand CM, Betancur C, Boeckers TM, *et al*. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 2007; 39: 25-7.

[25] Van Esch H, Dom R, Bex D, *et al.* Screening for FMR-1 premutations in 122 older Flemish males presenting with ataxia. Eur J Hum Genet 2005; 13: 121-23.

[26] del Gaudio D, Fang P, Scaglia F, *et al*. Increased MECP2 gene copy number as the result of genomic duplication in neurodevelopmentally delayed males. Genet Med 2006; 8: 784-92.

[27] Villard L. MECP2 mutations in males. J Med Genet 2007; 44: 417-23.

[28] Samaco RC, Nagarajan RP, Braunschweig D, LaSalle JM. Multiple pathways regulate MeCP2 expression in normal brain development and exhibit defects in autism-spectrum disorders. Hum Mol Genet 2004; 13: 629-39.

[29] Lopez-Rangel E, Lewis ME. Multiple pathways lead to MeCP2 in the aetiology of autism spectrum-associated neurodevelopmental disorders. Clin Genet 2006a; 69: 22-3.

[30] Lopez-Rangel E, Lewis ME. Further evidence for epigenetic influence of MECP2 in Rett, autism and Angelman's syndromes. Clin Genet 2006b; 69: 23-5.

[31] Belmonte MK, Bourgeron T. Fragile X syndrome and autism at the intersection of genetic and neural networks. Nat Neurosci 2006; 9: 1221-25.

[32] Grossman AW, Churchill JD, McKinney BC, Kodish IM, Otte SL, Greenough WT. Experience effects on brain development: possible contributions to psychopathology. J Child Psychol Psychiatry 2003; 44: 33-63.

[33] Ozonoff S, Rogers SJ, Hendren RL. Autism Spectrum Disorders: A research review for practitioners. Washington, DC: American Psychiatric Publishing, Inc. 2003.

[34] Bagni C, Greenough WT. From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Nat Rev Neurosci 2005; 6: 376-87.

[35] Tassone F, Hagerman RJ, Iklé DN, *et al.* FMRP expression as a potential prognostic indicator in fragile X syndrome. Am J Med Genet 1999; 84: 250-61.

[36] Sherman S. Epidemiology. In: Hagerman RJ, Hagerman PJ, eds. Fragile X Syndrome: Diagnosis, Treatment and Research, 3rd edition. Baltimore: The Johns Hopkins University Press 2002; 136-68.

[37] Crawford DC, Acuna JM, Sherman SL. FMR1 and the fragile X syndrome: human genome epidemiology review. Genet Med 2001; 3: 359-71.

[38] Turner G, Webb T, Wake S, Robinson H. Prevalence of fragile X syndrome. Am J Med Genet 1996; 64: 196-97.

[39] Crawford DC, Meadows KL, Newman JL, *et al*. Prevalence of the fragile X syndrome in African-Americans. Am J Med Genet 2002; 110: 226-33.

[40] Pesso R, Berkenstadt M, Cuckle H, *et al*. Screening for fragile X syndrome in women of reproductive age. Prenat Diagn 2000; 20: 611-14.

[41] Rousseau F, Morel M-L, Rouillard P, Khandjian EW, Morgan K. Surprisingly low prevalance of FMR1 premutation among males from the general population. Am J Hum Genet 1996; 59: A188-1069.

[42] Dombrowski C, Levesque ML, Morel ML, Rouillard P, Morgan K, Rousseau F. Premutation and intermediate-size FMR1 alleles in 10 572 males from the general population: loss of an AGG interruption is a late event in the generation of fragile X syndrome alleles. Hum Mol Genet 2002; 11: 371-8.

[43] Dawson AJ, Chodirker BN, Chudley AE. Frequency of FMR1 premutations in a consecutive newborn population by PCR screening of Guthrie blood spots. Biochem Mol Med 1995; 56: 63-9.

[44] Loesch DZ, Huggins RM, Hagerman RJ. Phenotypic variation and FMRP levels in fragile X. Ment Retard Dev Disabil Res Rev 2004; 10: 31-41.

[45] Hagerman RJ. Physical and Behavioral Phenotype. In: Hagerman RJ, Hagerman PJ, eds. Fragile X Syndrome: Diagnosis, Treatment and Research, 3rd edition. Baltimore: The Johns Hopkins University Press 2002; 3-109.

[46] Bennetto L, Pennington BF. Neuropsychology. In: Hagerman RJ, Hagerman PJ, eds. Fragile X Syndrome: Diagnosis, Treatment, and Research. 3rd Edition ed. Baltimore: Johns Hopkins University Press 2002; 206-48.

[47] de Vries BB, Wiegers AM, Smits AP, *et al*. Mental status of females with an FMR1 gene full mutation. Am J Med Genet 1996; 58: 1025-32.

[48] Mazzocco MMM, Sonna NL, Teisl JT, *et al*. The FMR1 and FMR2 mutations are not common etiologies of academic difficulty among school-age children. J Dev Behav Pediatr 1997; 18: 392-8.

[49] Kaufmann WE, Cortell R, Kau AS, *et al.* Autism spectrum disorder in fragile X syndrome: communication, social interaction, and specific behaviors. Am J Med Genet 2004; 129A: 225-34.

[50] Murphy MM, Mazzocco MM, Gerner G, Henry AE. Mathematics learning disability in girls with Turner syndrome or fragile X syndrome. Brain Cogn 2006; 61: 195-210.

[51] Hagerman RJ. Lessons from fragile X regarding neurobiology, autism, and neurodegeneration. J Dev Behav Pediatr 2006; 27: 63-74.

[52] Solomon M, Hessl D, Chiu S, Hagerman R, Hendren R. A genetic etiology of pervasive developmental disorder guides treatment. Am J Psychiatry 2007; 164: 575-80.

[53] Nowicki ST, Tassone F, Ono MY, *et al.* The Prader-Willi phenotype of fragile X syndrome. J Dev Behav Pediatr 2007; 28: 133-8.

[54] Hessl D, Tassone F, Cordeiro L, *et al*. Brief Report: Aggression and stereotypic behavior in males with fragile X syndrome-moderating secondary genes in a "Single Gene" Disorder. J Autism Dev Disord 2007 [Epub ahead of print].

[55] Miller LJ, McIntosh DN, McGrath J, *et al*. Electrodermal Responses to Sensory Stimuli in Individuals with Fragile X Syndrome: A Preliminary Report. Am J Med Genet 1999; 83: 268-79.

[56] Hagerman RJ, Miller LJ, McGrath-Clarke J, *et al.* Influence of stimulants on electrodermal studies in fragile X syndrome. Microsc Res Tech 2002; 57: 168-73.

[57] Roberts JE, Boccia ML, Bailey DB, Hatton D, Skinner M. Cardiovascular indices of physiological arousal in boys with fragile X syndrome. Dev Psychobiol 2001; 39: 107-23.

[58] Roberts JE, Boccia ML, Hatton DD, Skinner ML, Sideris J. Temperament and vagal tone in boys with fragile X syndrome. J Dev Behav Pediatr 2006; 27: 193-201.

[59] Boccia ML, Roberts JE. Behavior and autonomic nervous system function assessed via heart period measures: the case of hyperarousal in boys with fragile X syndrome. Behavior Research Methods, Instruments, & Computers 2000; 32: 5-10.

[60] Cohen IL. Behavioral profiles of autistic and non autistic fragile X males. Dev Brain Dysfunct 1995; 8: 252-69.

[61] Wisbeck JM, Huffman LC, Freund L, Gunnar M, Davis EP, Reiss AL. Cortisol and social stressors in children with fragile X: A pilot study. J Dev Behav Pediatr 2000; 21: 278-82.

[62] Hessl D, Glaser B, Dyer-Friedman J, Reiss AL. Social behavior and cortisol reactivity in children with fragile X syndrome. J Child Psychol Psychiatry 2006; 47: 602-10.

[63] Hessl D, Rivera SM, Reiss AL. The neuroanatomy and neuroendocrinology of fragile X syndrome. Ment Retard Dev Disabil Res Rev 2004; 10: 17-24.

[64] Rogers SJ, Ozonoff S. Annotation: what do we know about sensory dysfunction in autism? A critical review of the empirical evidence. J Child Psychol Psychiatry 2005; 46: 1255-68.

[65] Philofsky A, Hepburn SL, Hayes A, Hagerman RJ, Rogers SJ. Linguistic and cognitive functioning and autism symptoms in young children with fragile X syndrome. Am J Ment Retard 2004; 109: 208-18.

[66] Frankland PW, Wang Y, Rosner B, *et al*. Sensorimotor gating abnormalities in young males with fragile X syndrome and FMR1-knockout mice. Mol Psychiatry 2004; 9: 417-25.

[67] D'Hulst C, Kooy RF. The GABA(A) receptor: a novel target for treatment of fragile X? Trends Neurosci 2007; 30(8): 425-31.

[68] Perry W, Minassian A, Lopez B, Maron L, Lincoln A. Sensorimotor gating deficits in adults with autism. Biol Psychiatry 2007; 61: 482-6.

[69] Chiu S, Wegelin JA, Blank J, *et al.* Early acceleration of head circumference in children with fragile x syndrome and autism. J Dev Behav Pediatr 2007; 28: 31-5.

[70] Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. JAMA 2003; 290: 337-44.

[71] Mills JL, Hediger ML, Molloy CA, *et al.* Elevated levels of growth-related hormones in autism and autism spectrum disorder. Clin Endocrinol (Oxf) 2007; 67(2): 230-7.

[72] Butler MG, Dasouki MJ, Zhou XP, *et al*. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated

with germline PTEN tumour suppressor gene mutations. J Med Genet 2005; 42: 318-21.
[73] Wisniewski KE, Segan SM, Miezejeski CM, Sersen EA, Rudelli RD. The Fra(X) syndrome: neurological, electrophysiological, and neuropathological abnormalities. Am J Med Genet 1991; 38: 476-80.
[74] Berry-Kravis E. Epilepsy in fragile X syndrome. Dev Med Child Neurol 2002; 44: 724-8.
[75] Musumeci SA, Hagerman RJ, Ferri R, *et al*. Epilepsy and EEG findings in males with fragile X syndrome. Epilepsia 1999; 40: 1092-9.
[76] Musumeci SA, Colognola RM, Ferri R, *et al*. Fragile-X syndrome: A particular epileptogenic EEG pattern. Epilepsia 1988; 29: 41-7.
[77] Reiss AL, Aylward E, Freund LS, Joshi PK, Bryan RN. Neuroanatomy of fragile X syndrome: the posterior fossa. Ann Neurol 1991; 29: 26-32.
[78] Reiss AL, Freund L, Tseng JE, Joshi PK. Neuroanatomy in fragile X females: the posterior fossa. Am J Hum Genet 1991; 49: 279-88.
[79] Mostofsky SH, Mazzocco MM, Aakalu G, Warsofsky IS, Denckla MB, Reiss AL. Decreased cerebellar posterior vermis size in fragile X syndrome: correlation with neurocognitive performance. Neurology 1998; 50: 121-30.
[80] Reiss AL, Patel S, Kumar AJ, Freund L. Preliminary communication: neuroanatomical variations of the posterior fossa in men with the fragile X (Martin-Bell) syndrome. Am J Med Genet 1988; 31: 407-14.
[81] Eliez S, Blasey C, Freund LS, Hastie T, Reiss A. Brain anatomy, gender and IQ in children and adolescents with fragile X syndrome. Brain 2001; 124: 1610-8.
[82] Reiss AL, Abrams MT, Greenlaw R, Freund L, Denckla MB. Neurodevelopmental effects of the FMR1 full mutation in humans. Nat Med 1995; 1: 159-67.
[83] Johnston C, Eliez S, Dyer-Friedman J, *et al*. Neurobehavioral phenotype in carriers of the fragile X premutation. Am J Med Genet 2001; 103: 314-9.
[84] Murakami JW, Courchesne E, Press GA, Yeung-Courchesne R, Hesselink JR. Reduced cerebellar hemisphere size and its relationship to vermal hypoplasia in autism. Arch Neurol 1989; 46: 689-94.
[85] Lotspeich LJ, Ciaranello RD. The neurobiology and genetics of infantile autism. Int Rev Neurobiol 1993; 35: 87-129.
[86] Ciesielski KT, Harris RJ, Hart BL, Pabst HF. Cerebellar hypoplasia and frontal lobe cognitive deficits in disorders of early childhood. Neuropsychologia 1997; 35: 643-55.
[87] Pierce K, Courchesne E. Evidence for a cerebellar role in reduced exploration and stereotyped behavior in autism. Biol Psychiatry 2001; 49: 655-64.
[88] Mazzocco MM, Kates WR, Baumgardner TL, Freund LS, Reiss AL. Autistic behaviors among girls with fragile X syndrome. J Autism Dev Disord 1997; 27: 415-35.
[89] Rivera SM, Menon V, White CD, Glaser B, Reiss AL. Functional brain activation during arithmetic processing in females with fragile X syndrome is related to FMR1 protein expression. Hum Brain Mapp 2002; 16: 206-18.
[90] Ungerleider LG, Mishkin M. Two cortical visual systems. In: Ingle DJ, Goodale MA, Mansfield RJW, eds. Analysis of visual behavior. Cambridge, MA: MIT Press 1982; 549-86.
[91] Van Essen DC, Maunsell HHR. Hierarchical organization of the functional streams in the visual cortex. Trends Neurosci 1983; 6: 370-5.
[92] Sagi D, Julesz B. "Where" and "what" in vision. Science 1985; 228: 1217-9.
[93] Posner MI. Cognitive neuropsychology and the problem of selective attention. Electroencephalogr Clin Neurophysiol Suppl 1987; 39: 313-6.
[94] Kogan CS, Boutet I, Cornish K, *et al*. Differential impact of the FMR1 gene on visual processing in fragile X syndrome. Brain 2004; 127: 591-601.
[95] Mazzocco MM, Singh Bhatia N, Lesniak-Karpiak K. Visuospatial skills and their association with math performance in girls with fragile X or Turner syndrome. Child Neuropsychol 2006; 12: 87-110.
[96] Spencer J, O'Brien J, Riggs K, Braddick O, Atkinson J, Wattam-Bell J. Motion processing in autism: evidence for a dorsal stream deficiency. Neuroreport 2000; 11: 2765-7.
[97] Milne E, Swettenham J, Hansen P, Campbell R, Jeffries H, Plaisted K. High motion coherence thresholds in children with autism. J Child Psychol Psychiatry 2002; 43: 255-63.
[98] Bertone A, Mottron L, Jelenic P, Faubert J. Motion perception in autism: a "complex" issue. J Cogn Neurosci 2003; 15: 218-25.
[99] Blake R, Turner LM, Smoski MJ, Pozdol SL, Stone WL. Visual recognition of biological motion is impaired in children with autism. Psychol Sci 2003; 14: 151-7.
[100] Takarae Y, Minshew NJ, Luna B, Krisky CM, Sweeney JA. Pursuit eye movement deficits in autism. Brain 2004; 127: 2584-94.
[101] Lencer R, Nagel M, Sprenger A, Heide W, Binkofski F. Reduced neuronal activity in the V5 complex underlies smooth-pursuit deficit in schizophrenia: evidence from an fMRI study. Neuroimage 2005; 24: 1256-9.
[102] Rivera SM, Koldewyn K. Unraveling the mystery of motion perception impairments in autism: Some further considerations. Current Psychology of Cognition 2005; 23: 189-97.
[103] Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A 2002; 99: 7746-50.
[104] Kooy RF. Of mice and the fragile X syndrome. Trends Genet 2003; 19: 148-54.
[105] Koekkoek SK, Yamaguchi K, Milojkovic BA, *et al*. Deletion of FMR1 in Purkinje Cells Enhances Parallel Fiber LTD, Enlarges Spines, and Attenuates Cerebellar Eyelid Conditioning in Fragile X Syndrome. Neuron 2005; 47: 339-52.
[106] Carter RB, Wood PL, Wieland S, *et al*. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3alpha-hydroxy-3beta-methyl-5alpha-pregnan-20-one), a selective, high-affinity, steroid modulator of the gamma-aminobutyric acid(A) receptor. J Pharmacol Exp Ther 1997; 280: 1284-95.
[107] Gantois I, Vandesompele J, Speleman F, *et al*. Expression profiling suggests underexpression of the GABA(A) receptor subunit delta in the fragile X knockout mouse model. Neurobiol Dis 2006; 21: 346-57.
[108] Miyashiro KY, Beckel-Mitchener A, Purk TP, *et al*. RNA cargoes associating with FMRP reveal deficits in cellular functioning in Fmr1 null mice. Neuron 2003; 37: 417-31.
[109] El Idrissi A, Ding XH, Scalia J, Trenkner E, Brown WT, Dobkin C. Decreased GABA(A) receptor expression in the seizure-prone fragile X mouse. Neurosci Lett 2005; 377: 141-6.
[110] Gruss M, Braun K. Age- and region-specific imbalances of basal amino acids and monoamine metabolism in limbic regions of female Fmr1 knock-out mice. Neurochem Int 2004; 45: 81-8.
[111] D'Antuono M, Merlo D, Avoli M. Involvement of cholinergic and gabaergic systems in the fragile X knockout mice. Neuroscience 2003; 119: 9-13.
[112] Paredes RG, Agmo A. GABA and behavior: the role of receptor subtypes. Neurosci Biobehav Rev 1992; 16: 145-70.
[113] Monaghan EP, McAuley JW, Data JL. Ganaxolone: a novel positive allosteric modulator of the GABA(A) receptor complex for the treatment of epilepsy. Expert Opin Investig Drugs 1999; 8: 1663-71.
[114] Kerrigan JF, Shields WD, Nelson TY, *et al*. Ganaxolone for treating intractable infantile spasms: a multicenter, open-label, add-on trial. Epilepsy Res 2000; 42: 133-9.
[115] Bear MF. Therapeutic implications of the mGluR theory of fragile X mental retardation. Genes Brain Behav 2005; 4: 393-8.
[116] Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. Neuropharmacology 2005; 49: 1053-66.
[117] McBride SM, Choi CH, Wang Y, *et al*. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. Neuron 2005; 45: 753-64.
[118] Porter RH, Jaeschke G, Spooren W, *et al*. Fenobam: A clinically validated nonbenzodiazepine anxiolytic is a potent, selective, and noncompetitive mGlu5 receptor antagonist with inverse agonist activity. J Pharmacol Exp Ther 2005; 315: 711-21.
[119] Cronister A, Schreiner R, Wittenberger M, Amiri K, Harris K, Hagerman RJ. Heterozygous fragile X female: historical, physical, cognitive, and cytogenetic features. Am J Med Genet 1991; 38: 269-74.

[120] Coffey SM, Cook K, Tartaglia N, *et al*. Expanded clinical phenotype of women with the *FMR1* premutation. Am J Med Genetics in press.

[121] Welt CK, Smith PC, Taylor AE. Evidence of early ovarian aging in fragile X premutation carriers. J Clin Endocrinol Metab 2004; 89: 4569-74.

[122] Aziz M, Stathopulu E, Callias M, *et al*. Clinical features of boys with fragile X premutations and intermediate alleles. Am J Med Genet 2003; 121B: 119-27.

[123] Goodlin-Jones B, Tassone F, Gane LW, Hagerman RJ. Autistic spectrum disorder and the fragile X premutation. J Dev Behav Pediatr 2004; 25: 392-8.

[124] Hagerman PJ, Hagerman RJ. The fragile-X premutation: a maturing perspective. Am J Hum Genet 2004; 74: 805-16.

[125] Arocena DG, Iwahashi CK, Won N, *et al*. Induction of inclusion formation and disruption of lamin A/C structure by premutation CGG-repeat RNA in human cultured neural cells. Hum Mol Genet 2005; 14: 3661-71.

[126] Iwahashi CK, Yasui DH, An HJ, *et al*. Protein composition of the intranuclear inclusions of FXTAS. Brain 2006; 129: 256-71.

[127] Schwartz CE, Dean J, Howard-Peebles PN, *et al*. Obstetrical and gynecological complications in fragile X carriers: a multicenter study. Am J Med Genet 1994; 51: 400-2.

[128] Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, *et al*. Fragile X premutation is a significant risk factor for premature ovarian failure: The international collaborative POF in fragile X study- preliminary data. Am J Med Genet 1999; 83: 322-5.

[129] Murray A, Ennis S, MacSwiney F, Webb J, Morton NE. Reproductive and menstrual history of females with fragile X expansions. Eur J Hum Genet 2000; 8: 247-52.

[130] Vianna-Morgante AM, Costa SS. Premature ovarian failure is associated with maternally and paternally inherited premutation in Brazilian families with fragile X [see comments] [letter]. Am J Hum Genet 2000; 67: 254-5; discussion 6-8.

[131] Sherman SL. Premature ovarian failure in the fragile X syndrome. Am J Med Genet (Semin Med Genet) 2000; 97: 189-94.

[132] Gersak K, Meden-Vrtovec H, Peterlin B. Fragile X premutation in women with sporadic premature ovarian failure in Slovenia. Hum Reprod 2003; 18: 1637-40.

[133] Mallolas J, Duran M, Sanchez A, *et al*. Implications of the FMR1 gene in menopause: study of 147 Spanish women. Menopause 2001; 8: 106-10.

[134] Murray A, Webb J, Grimley S, Conway G, Jacobs P. Studies of FRAXA and FRAXE in women with premature ovarian failure. J Med Genet 1998; 35: 637-40.

[135] Conway GS, Payne NN, Webb J, Murray A, Jacobs PA. Fragile X premutation screening in women with premature ovarian failure [see comments]. Hum Reprod 1998; 13: 1184-7.

[136] Uzielli ML, Guarducci S, Lapi E, *et al*. Premature ovarian failure (POF) and fragile X premutation females: from POF to fragile X carrier identification, from fragile X carrier diagnosis to POF association data. Am J Med Genet 1999; 84: 300-3.

[137] Bussani C, Papi L, Sestini R, *et al*. Premature ovarian failure and fragile X premutation: a study on 45 women. Eur J Obstet Gynecol Reprod Biol 2004; 112: 189-91.

[138] Marozzi A, Vegetti W, Manfredini E, *et al*. Association between idiopathic premature ovarian failure and fragile X premutation. Hum Reprod 2000; 15: 197-202.

[139] Sherman S, Pletcher BA, Driscoll DA. Fragile X syndrome: Diagnostic and carrier testing. Genet Med 2005; 7: 584-7.

[140] Hull C, Hagerman RJ. A study of the physical, behavioral, and medical phenotype, including anthropometric measures, of females with fragile X syndrome. Am J Dis Child 1993; 147: 1236-41.

[141] Riddle JE, Cheema A, Sobesky WE, *et al*. Phenotypic involvement in females with the FMR1 gene mutation. Am J Ment Retard 1998; 102: 590-601.

[142] Loesch DZ, Huggins RM, Bui QM, Taylor AK, Hagerman RJ. Relationship of deficits of FMR1 gene specific protein with physical phenotype of fragile X males and females in pedigrees: A new perspective. Am J Med Genet 2003; 118A: 127-34.

[143] Hagerman RJ, Hagerman PJ. The fragile X premutation: into the phenotypic fold. Curr Opin Genet Dev 2002; 12: 278-83.

[144] Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of *FMR1* mRNA in carrier males: a new mechanism of involvement in fragile X syndrome. Am J Hum Genet 2000; 66: 6-15.

[145] Allen EG, He W, Yadav-Shah M, Sherman SL. A study of the distributional characteristics of FMR1 transcript levels in 238 individuals. Hum Genet 2004; 114: 439-47.

[146] Hinds HL, Ashley CT, Sutcliffe JS, *et al*. Tissue specific expression of FMR-1 provides evidence for a functional role in fragile X syndrome [see comments] [published erratum appears in Nat Genet 1993 Nov; 5(3): 312]. Nat Genet 1993; 3: 36-43.

[147] Lewis P, Abbeduto L, Murphy M, *et al*. Psychological well-being of mothers of youth with fragile X syndrome: syndrome specificity and within-syndrome variability. J Intellect Disabil Res 2006; 50: 894-904.

[148] Reiss AL, Freund L, Abrams MT, Boehm C, Kazazian H. Neurobehavioral effects of the fragile X premutation in adult women: a controlled study. Am J Hum Genet 1993; 52: 884-94.

[149] Sobesky WE, Hull CE, Hagerman RJ. Symptoms of schizotypal personality disorder in fragile X women. J Am Acad Child Adolesc Psychiatry 1994; 33: 247-55.

[150] Sobesky WE, Taylor AK, Pennington BF, *et al*. Molecular-clinical correlations in females with fragile X. Am J Med Genet 1996; 64: 340-5.

[151] Franke P, Leboyer M, Gansicke M, *et al*. Genotype-phenotype relationship in female carriers of the premutation and full mutation of FMR-1. Psychiatry Res 1998; 80: 113-27.

[152] Derogatis LR. Symptom Checklist-90-R (SCL-90-R): Administration, Scoring, and Procedures Manual. Minneapolis: National Computer Systems 1994.

[153] Hessl D, Tassone F, Loesch DZ, *et al*. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. Am J Med Genet B Neuropsychiatr Genet 2005; 139: 115-21.

[154] Hessl D, Rivera S, Koldewyn K, *et al*. Amygdala dysfunction in men with the fragile X premutation. Brain 2007; 130: 404-16.

[155] Baron-Cohen S, Ring HA, Bullmore ET, Wheelwright S, Ashwin C, Williams SC. The amygdala theory of autism. Neurosci Biobehav Rev 2000; 24: 355-64.

[156] Howard MA, Cowell PE, Boucher J, *et al*. Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. Neuroreport 2000; 11: 2931-5.

[157] Jacquemont S, Hagerman RJ, Leehey MA, *et al*. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. JAMA 2004; 291: 460-9.

[158] Leehey MA, Berry-Kravis E, Min SJ, *et al*. Progression of tremor and ataxia in male carriers of the FMR1 premutation. Mov Disord 2007; 22: 203-6.

[159] Jacquemont S, Hagerman RJ, Leehey M, *et al*. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet 2003; 72: 869-78.

[160] Greco CM, Berman RF, Martin RM, *et al*. Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). Brain 2006; 129: 243-55.

[161] Greco CM, Soontarapornchai K, Wirojanan J, Gould JE, Hagerman PJ, Hagerman RJ. Testicular and pituitary inclusion formation in fragile X associated tremor/ataxia syndrome. J Urol 2007; 177: 1434-7.

[162] van Noort JM, van Sechel AC, Bajramovic JJ, *et al*. The small heat-shock protein alpha B-crystallin as candidate autoantigen in multiple sclerosis. Nature 1995; 375: 798-801.

[163] Vojdani A, Vojdani E, Cooper E. Antibodies to myelin basic protein, myelin oligodendrocytes peptides, alpha-beta-crystallin, lymphocyte activation and cytokine production in patients with multiple sclerosis. J Intern Med 2003; 254: 363-74.

[164] Kariko K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA is an endogenous ligand for Toll-like receptor 3. J Biol Chem 2004; 279: 12542-50.

[165] Ma Y, Li J, Chiu I, *et al*. Toll-like receptor 8 functions as a negative regulator of neurite outgrowth and inducer of neuronal apoptosis. J Cell Biol 2006; 175: 209-15.

[166] Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 2005; 57: 67-81.

[167] Jin P, Zarnescu DC, Zhang F, *et al*. RNA-mediated neurodegeneration caused by the fragile X premutation rCGG repeats in drosophila. Neuron 2003; 39: 739-47.

[168] Willemsen R, Hoogeveen-Westerveld M, Reis S, *et al*. The FMR1 CGG repeat mouse displays ubiquitin-positive intranuclear neu-

ronal inclusions; implications for the cerebellar tremor/ataxia syndrome. Hum Mol Genet 2003; 12: 949-59.

[169] Van Dam D, Errijgers V, Kooy RF, *et al.* Cognitive decline, neuromotor and behavioural disturbances in a mouse model for fragile-X-associated tremor/ataxia syndrome (FXTAS). Behav Brain Res 2005; 162: 233-9.

[170] Laumonnier F, Cuthbert PC, Grant SG. The role of neuronal complexes in human X-linked brain diseases. Am J Hum Genet 2007; 80: 205-20.

Received: 07 August 2007 Revised: 20 October, 2007 Accepted: 03 November, 2007