Secondary Medical Diagnosis in Fragile X Syndrome With and Without Autism Spectrum Disorder

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This study examined whether secondary medical diagnoses that affect CNS function (i.e., seizures, malformations, or genetic disorders), are more likely to occur in individuals with fragile X syndrome (FXS) and autism spectrum disorder (FXS + ASD) or FXS alone. Ninety males (3–25 years) with FXS or FXS + ASD were evaluated for secondary medical diagnoses by medical history and examination. A significant difference in the incidence of medical problems was found between patients with FXS + ASD (38.6%) and FXS alone (18.2%, P < 0.05). Medical problems that affect the CNS are more likely to occur in those with FXS + ASD and it is probable that additional brain dysfunction associated with these medical problems enhance the risk of autism in those with FXS. © 2008 Wiley-Liss, Inc.

Key words: fragile X syndrome; autism; PDDNOS; autism spectrum disorder; seizures; Prader–Willi like phenotype

INTRODUCTION

Fragile X syndrome (FXS) is the most common inherited cause of mental retardation, affecting an estimated 1 in 3,600 males in the general population [Sherman, 2002]. The phenotype of FXS includes intellectual disability, cognitive and behavioral impairments of varying degree, and physical anomalies such as large ears, long face, macrocephaly, and macroorchidism [Lachiewicz et al., 2000; Hagerman, 2002]. Behavior characteristics frequently reported include social avoidance, anxiety, poor eye contact, perseverative behavior, hyperarousal to sensory stimuli, distractibility, irritability, hyperactivity, repetitive motor behaviors, and inflexibility [Hagerman, 2002; Artigas and Brun, 2004; Hatton et al., 2006].

FXS is caused by an unstable mutation in the fragile X mental retardation 1 (FMR1) gene. Expansion of a trinucleotide (CGG) repeat (> 200) in the 5' untranslated region of the gene leads to gene silencing and a lack or deficiency of the FMR1 protein (FMRP), which causes the physical, cognitive, behavioral, and emotional characteristics of FXS [Tassone et al., 1999]. Normal individuals have 5–44 CGG repeats that are stably transmitted to the next generation. Alleles harboring 45–54 CGG repeats are named “gray zone” because instability in transmission to the next generation is common [Nolin et al., 1996]. With CGG repeat sizes in the premutation range (55–200 repeats), the allele usually expands to the full mutation (> 200 repeats) when passed from a mother to her children. A mixed pattern of full mutation and premutation alleles is termed mosaicism.

The association between autism and FXS was first reported in 1982 [Brown et al., 1982] and is one of the most recognized behavioral abnormalities observed.
in males and some females with FXS. Two to six percent of children with autism will have the FMR1 mutation [Wassink et al., 2001; Estecio et al., 2002; Reddy, 2005] and approximately 30% of children with FXS are diagnosed with autism [Bailey et al., 2001a; Rogers et al., 2001; Kaufmann et al., 2004]. An additional 20–30% of patients with FXS have Pervasive Developmental Disorder—Not Otherwise Specified (PDDNOS) [Harris et al., 2008]. Studies have shown that the presence of autism with FXS is associated with language and social deficits in addition to a lower IQ compared to children with FXS without autism [Bailey et al., 2001a; Kaufmann et al., 2004; Philofsky et al., 2004]. IQ has also been documented to decline with age in most males with FXS [Wright-Talamante et al., 1996]. A decline is also seen in individuals with Down syndrome who develop Alzheimer disease with age [Kittler et al., 2004], but not in the typical population [Zigler et al., 1984].

The autism that occurs in FXS has been hypothesized to be related to a secondary genetic problem because the degree of FMRP deficit does not correlate with the presence of autism when IQ is controlled [Rogers et al., 2001; Loesch et al., 2007]. Some individuals with FXS have a recognizable second genetic abnormality or an additional medical diagnosis, such as epilepsy, that is a known cause of CNS dysfunction. We hypothesize that individuals with FXS and autism will have a higher number of secondary medical diagnoses including genetic disorders that affect the CNS compared to those with FXS alone. A retrospective analysis was conducted to determine if the prevalence of secondary medical diagnoses is different in individuals with FXS and autism spectrum disorder (ASD, including PDDNOS and autism) compared to individuals with FXS alone.

**MATERIALS AND METHODS**

**Participants**

Included in this retrospective analysis and chart review were 90 male participants with FXS, ages 3–25 years, who were seen sequentially at the University of California at Davis M.I.N.D. Institute and had an Autism Diagnostic Observation Schedule—General [ADOS-G, Lord et al., 2000] as part of the evaluation between January 2001 and July 2005. Subjects and parents/caregivers signed an IRB approved consent form. All the participants had either a full mutation or mosaicism by FMR1 DNA testing. Each individual was evaluated for autism or PDDNOS using the ADOS-G and the Diagnostic Statistical Manual—Fourth Edition criteria for Autistic Disorder [DSM-IV, APA, 1994]. Individuals who met the criteria for autism or PDDNOS were considered in the FXS + ASD group. There were 57 individuals with FXS + ASD and 33 individuals with FXS alone. All participants were evaluated for medical or genetic problems through a medical history and a detailed examination for minor anomalies by a developmental and behavioral pediatrician with expertise in genetic disorders and fragile X syndrome (RJH). If asymmetries were seen on neurological examination, an MRI was done. A description of the participants is presented in Table I.

**Measures**

All patients completed the ADOS-G [Lord et al., 2000] and were evaluated using the DSM-IV criteria for Autistic Disorder [APA, 1994]. When disagreements occurred between the two measures a consensus was reached by a team conference including a developmental and behavioral pediatrician, psychologist, social worker, and genetic associate, who are all experienced in the diagnosis of autism. Patients also underwent one of the following cognitive evaluations (n = 64); Wechsler Intelligence Scale for Children—3rd Edition [WISC-III, Wechsler, 1991], Wechsler Intelligence Scale for Children—4th Edition [WISC-IV, Wechsler, 2003], Wechsler Adult Intelligence Scale—3rd Edition [WAIS-III, Wechsler, 1997], Wechsler Preschool and Primary Scale of Intelligence—3rd Edition [WPPSI-III, Wechsler, 2002], Wechsler Abbreviated Scale of Intelligence [WASI, Wechsler, 1999], Stanford-Binet—4th Edition [Thorndike et al., 1986], and Leiter International Scale of Intelligence-Revised [Leiter-R, Roid and Miller, 1997]. In addition, the parent/caregiver of the patients (n = 62) were administered the Vineland Adaptive Behavior Scales, Interview Edition [VABS, Sparrow et al., 1984] to assess adaptive functioning.

The assessment of the medical and genetic conditions consisted of a clinical history, a review of medical and psychological records including neuroimaging and previous genetic testing, and a medical and neurological examination previously described in Riddle et al. [1998]. Cytogenetic testing was not routinely completed, but was often included in the medical records. FMR1 DNA testing was performed on all patients. DNA testing was performed on DNA isolated from peripheral blood leukocytes using both Southern Blot and PCR analysis. Details of the methods are previously described in Tassone et al.

**TABLE I. Description of Participants**

<table>
<thead>
<tr>
<th>Measures</th>
<th>FXS alone (n = 33)</th>
<th>FXS + ASD (n = 57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSIQ score*</td>
<td>61.00±14.87</td>
<td>52.22±11.54</td>
</tr>
<tr>
<td>Vineland score**</td>
<td>52.90±14.79</td>
<td>41.79±14.79</td>
</tr>
</tbody>
</table>

*n = 28, †n = 36, ‡n = 24, *p < 0.05, **p < 0.01.
RESULTS

Autism and PDDNOS

Of the 90 males, 57 (63.3%) met the criteria for ASD according to the ADOS-G and the DSM-IV criteria for Autistic Disorder. Twenty-nine (32%) subjects had autism and 28 (31%) had PDDNOS. The remaining 33 (37%) did not meet criteria for ASD. Those with autism and PDDNOS clinically are on a continuum and since many of the individuals scored at or just below the cut-off for autism; they were combined in our analysis. Often in studies of autism, these two groups are combined into the ASDs category [Harris et al., 2008]. These percentages are quite consistent with our overall numbers for FXS + ASD. In addition, there were no significant differences between the FXS and PDDNOS (FXS + PDDNOS) and FXS and autism (FXS + autism) groups in IQ (P = 0.92), VABS score (P = 0.06), and age (P = 0.83). Combining these groups enhanced statistical power for comparison across those with ASD and those without.

If we compare the presence of secondary medical diagnoses between the FXS and autism group (FXS + autism) and FXS and PDDNOS (FXS + PDDNOS) group we saw that in the FXS + PDDNOS group, 11 (39.3%) had secondary medical diagnoses and in the FXS + autism group, 11 (37.9%) had secondary medical diagnoses. Systematic comparisons between the two groups, FXS + PDDNOS and FXS + autism, showed no significant difference (Chi square = 0.011, df = 1, P = 0.916). The presence of secondary medical diagnoses does not differentiate the FXS + PDDNOS from the FXS + autism group. Therefore, we combined the group into FXS + ASD for further analysis (Table II).

CNS Associated Medical Disorders

Twenty-eight (31.1%) of the 90 subjects were found to have an associated medical disorder that

### TABLE II. Medical/Genetic Results of the Two Groups (FXS Alone and FXS + ASD)

<table>
<thead>
<tr>
<th></th>
<th>FXS alone (n = 33)</th>
<th>FXS + ASD (n = 57)</th>
<th>Chi-square test (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Percentage</td>
<td>n</td>
</tr>
<tr>
<td>Full mutation</td>
<td>19</td>
<td>57.6</td>
<td>37</td>
</tr>
<tr>
<td>Mosaic</td>
<td>14</td>
<td>42.4</td>
<td>20</td>
</tr>
<tr>
<td>No medical problems</td>
<td>27</td>
<td>81.8</td>
<td>55</td>
</tr>
<tr>
<td>Seizures</td>
<td>4</td>
<td>12.1</td>
<td>16</td>
</tr>
<tr>
<td>MRI abnormalities</td>
<td>1</td>
<td>3.0</td>
<td>2</td>
</tr>
<tr>
<td>Genetic abnormalities</td>
<td>1</td>
<td>3.1</td>
<td>4</td>
</tr>
<tr>
<td>Total medical problems</td>
<td>6</td>
<td>18.2</td>
<td>22</td>
</tr>
</tbody>
</table>
affects the CNS after a detailed medical history, clinical exam, and further testing of a subgroup. Twenty of the subjects had seizures; three had MRI abnormalities, that is, white matter disease, cystic abnormalities, and optic atrophy with microcephaly; and five had genetic abnormalities, four with Prader–Willi like phenotype (PWP) and one with XYY. Age effects were examined between participants with a secondary medical diagnoses and participants without a secondary medical diagnoses using Mann–Whitney U-test, and there was no significant group difference ($Z = -1.24$, $P = 0.21$).

In the FXS alone group, 6 (18.2%) subjects had secondary medical diagnoses and in the FXS + ASD group, 22 (38.6%) had secondary medical diagnoses (Table II). Systematic comparisons between the two groups, FXS + ASD and FXS alone, showed a significant difference (Chi squared $= 4.064$, df $= 1$, $P = 0.04$). These results indicate that the group with FXS + ASD had approximately twice as many medical problems that affect the CNS than the group with FXS alone (Table II). Age effects were also examined in relation to secondary medical diagnoses within the FXS alone group and the FXS + ASD group using Mann–Whitney U-test. There were no significant group differences in age between individuals with a secondary medical diagnoses and individuals without a secondary medical diagnoses in the FXS alone group ($Z = -0.841$, $P = 0.40$) or in the FXS + ASD group ($Z = -0.730$, $P = 0.47$).

Considering seizures only, the FXS alone group had 4 (12.1%) subjects who had seizures and the FXS + ASD group had 16 (28.1%) subjects who had seizures; although more than twice as many had seizures in the FXS + ASD group, this difference was not significant ($P = 0.15$, Table II).

**DISCUSSION**

Autism occurs in approximately 30% of young males with FXS, but why autism occurs in only this subgroup of patients with FXS is not known. This study demonstrates a higher rate of secondary medical problems in those with FXS + ASD compared with FXS alone. In addition, we found a significantly lower IQ in those with FXS + ASD compared to FXS alone. These results suggest that additional brain dysfunction related to secondary medical diagnoses that affect the CNS can increase the risk for autism for those with FXS. This could be associated simply to more general damage to the brain which could lower IQ or to a secondary medical diagnosis that specifically relates to gene dysregulation, that is, PWP. These results are consistent with the findings of several studies showing lower IQ in those with FXS with autism or ASD compared to those with FXS alone [Rogers et al., 2001; Bailey et al., 2001a; Kaufmann et al., 2004; Loesch et al., 2007]. A number of genetic disorders can lead to autism without FXS including sex chromosome abnormalities, 15q duplication, PKU, and a marker X chromosome [Cohen et al., 2005; Schaefer and Lutz, 2006]. Genetic disorders can be documented in 15–40% of patients with autism with a careful assessment including cytogenetic studies with FISH testing and metabolic studies [Reddy, 2005; Schaefer and Lutz, 2006].

**Seizures**

The most prevalent secondary medical diagnosis in FXS is seizures, which was first reported by Lubs in [1969]. Other studies have described seizures in FXS and the rate varies between 10% and 40% [Musumeci et al., 1999; Sabaratnam et al., 2001; Berry-Kravis, 2002]. Rates of seizures are increased in the general population with developmental disability, thus FXS is not unique in this regard. We found seizures by medical history in 22.2% of the subjects overall, including 12.1% in FXS alone and 28.1% in FXS + ASD. It is also possible that occult EEG abnormalities occurred more frequently in those with FXS + ASD, but that was not evaluated here. Seizures can occur in 18–29% in those with autism without FXS [Olsson et al., 1988; Rapin and Katzman, 1998]. The underlying neuronal abnormalities leading to neuronal excitability and susceptibility to seizures may be associated with the development of autism in those with FXS. In some cases, prolonged seizures may cause CNS tissue damage which may predispose to autism. The electrical abnormalities associated with seizures may also disrupt connectivity in the brain, which may also predispose individuals with FXS and seizures to autism [Belmonte and Bourgeron, 2006]. If further studies confirm the association between seizures and autism, this would suggest that treating seizure activity in young children with FXS may decrease their risk of having an ASD. In addition, some genes associated with autism may also predispose to seizures such as the ARX gene [Nowicki et al., 2008]. Recently, a mutation in the gene encoding the contactin-associated protein-like 2 (CASPR2) was identified as being associated with childhood onset epilepsy and autism in a sample of Old Order Amish children [Strauss et al., 2006].

**Genetic Disorders**

The next most prevalent secondary medical problem, in our sample of males with FXS, was genetic disorders. Prader–Willi syndrome (PWS) is a genetic disorder with a 15q deletion and a phenotype that includes severe hyperphagia, significant obesity, hypogonadism, and growth hormone deficiency, demonstrating hypothalamic dysfunction. The Prader–Willi phenotype (PWP) in FXS is not due to a 15q deletion, but consists of
obesity, hyperphagia, hypotonia, and often hypogonadism [de Vries et al., 1993; de Vries and Niermeijer, 1994; Nowicki et al., 2007]. The PWP was seen in four patients described here, all with ASD, and we consider this an additional genetic abnormality because a decrease in the expression of cytoplasmic interacting FMR1 protein (CYFIP) has been seen in boys with FXS and PWP [Nowicki et al., 2007]. The gene for CYFIP is located at 15q [Chai et al., 2003]. Patients with PWP do not have short stature, as seen in PWS, but hyperphagia and obesity are present and approximately 50% have small genitilia or delayed puberty as reported in Nowicki et al. [2007].

We found a low rate of secondary cytogenetic abnormalities, with just one patient with XXY syndrome; although cytogenetic studies were not routinely carried out in our patients. The association of sex chromosome abnormalities with FXS has been reported in the past, primarily FXS with XXY [Chudley, 1990; Kupke et al., 1991; Milunsky et al., 1993]. Two cases of 46,XY and 47,XYY mosaicism in association with FXS have been reported [Bodurtha et al., 1993; Milunsky et al., 1993]. Although there have been some reports of autism in males with XYY syndrome [Nicolson et al., 1998; Geerts et al., 2003], ASD was not seen in our case of XYY + FXS.

This study suggests that secondary medical diagnoses that affect the CNS should be examined in patients with FXS and autism or ASD. These abnormalities may occur silently and include differences in gene expression, such as CYFIP expression in patients with FXS and the PWP. Another example of gene expression changes in autism is the low expression of MeCP2 in the brains of individuals diagnosed with autism but without Rett Syndrome or fragile X. Although the MeCP2 protein regulates the FMR1 gene expression, we do not know the expression of MeCP2 in the brains of individuals with FXS, either with or without autism.

Our findings are consistent with the hypothesis that children with FXS and ASD are more likely to have a secondary medical problem compared to those with FXS alone. It is important to note that there may have been a sample bias since it is probable that more of the FXS + ASD participants who were more severe in presentation to their doctors might have been more likely to be referred for evaluation of the secondary medical diagnoses, that is, EEG studies. In other words, it is probable that not every participant was equally screened for a secondary medical diagnosis. Our research suggests that clinicians should be careful to rule out seizures or EEG abnormalities in individuals with FXS + ASD. Treatment with an anticonvulsant in those with seizures may help subsequent social development in those with autism with or without FXS. Although additional genetic problems such as Down syndrome or the Prader–Willi phenotype are recognizable by their physical phenotype, some may involve occult expression abnormalities of genes such as MeCP2, CYFIP, or other yet unidentified genes. The search for additional gene dysregulation associated with autism in FXS may also be pertinent to individuals with autism of unknown etiology.

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