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Letter to the Editor

DO KNOWN MUTATIONS IN NEUROLIGIN GENES (NLGN3 AND NLGN4) CAUSE AUTISM?

Classical autism is an early onset neurodevelopmental disorder that belongs to a group of heterogeneous conditions known as autism spectrum disorders (ASD) which include classical autism, Asperger syndrome, and pervasive development disorder-not otherwise specified (PDD-NOS) (Lord, Rutter & Le Couteur, 1994). Classical autism is approximately four times more common in males than in females (Volkmar, Szatmari & Sparrow, 1993). The sex ratio is greater for Asperger syndrome, with a male to female ratio of eight to one (Volkmar *et al*., 1993). Although this sex ratio discrepancy is unexplained, it suggests the involvement of X chromosome genes and possibly genomic imprinting and/or skewness of X chromosome inactivation in females with autism (Talebizadeh Bittel, Veatch, Kibiryeva, & Butler).

Recently, Jamain *et al*. (2003) reported mutations in two X linked neuroligin genes, NLGN3 and NLGN4, in male individuals with ASD. The neuroligins are encoded by a family of five genes producing cell-adhesion molecules essential for the formation of functional neural synapses (Jamain *et al*., 2003). These authors hypothesized that the identified neuroligin mutations may abolish formation, stabilization or recognition of specific synapses essential for the communication processes which is implicated in patients with autism. They screened for mutations in NLGN3 (localized at Xq13), NLGN4 (Xp22.3) and NLGN4Y (Yq11.2) in 158 subjects (140 males and 18 females), including 36 pairs of affected siblings and 122 trios with an affected ASD individual. The NLGN3 mutation was a C→T transition in exon 6 which changed a highly conserved arginine residue to cysteine (R451C) in a Swedish family with two affected brothers, one with typical autism and the other with Asperger syndrome. The mother was heterozygous for this substitution but otherwise healthy. The NLGN3 mutation was not found in 200 unaffected controls (100 females and 100 males). In another Swedish family, the authors reported a 1-bp insertion (1186insT) in exon 5 of the NLGN4 gene in two affected sons (autism and Asperger) resulting in a frameshift mutation causing a premature termination. It was concluded that the mutation was *de novo* and originated in the mother. This mutation was not seen in 350 unrelated and unaffected controls (250 females and 100 males). Similarly, members of a large French family were analyzed for NLGN4 gene mutations including males with non specific X-linked mental retardation with or without autism or pervasive developmental disorder (Laumonnier *et al*., 2004). They found a 2-bp deletion in exon 5 in six affected males in which DNA was available (one with autism, one with pervasive developmental disorder, four with non specific mental retardation). This deletion was not observed in 200 healthy male controls. Therefore, both published reports found different mutations but in the same coding region (exon 5) of the NLGN4 gene.

To determine whether reported mutations in NLGN3 or NLGN4 occurs in both males and females with ASD in the American population, we screened 67 autistic subjects (64 with classical autism and 3 with Asperger syndrome). Forty-one females and 26 males (63 white, 3 black, and 1 other) were analyzed with an age range of 2–37 years. Our subject group included a higher percentage of females than expected in the general population of individuals with ASD due to recruitment of females with autism for X chromosome inactivation studies. Thirty-seven individuals with ASD were recruited from the Autism Center at the University of Missouri-Columbia Hospitals and Clinics (Talebizadeh *et al*., 2002) and 30 from the Autism Genetics Research Exchange (AGRE) (Los Angeles, CA). The diagnosis was established with the use of the Autism Diagnostic Interview-Revised (ADI-R) (Lord, Rutter, & Le Couteur, 1994) and by clinical genetic evaluation. Chromosome analysis and fragile X testing were reportedly normal. Genomic DNA was previously extracted from peripheral blood and PCR amplification performed using specific primers designed to produce a DNA fragment containing the mutation for both neuroligin genes (exon 6 in NLGN3 and exon 5 in NLGN4) reported by Jamain *et al*.

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(2003) and Laumonnier *et al.* (2004). The PCR fragment was then sequenced using a capillary electrophoresis ABI 310 sequencer. The reported mutations in the two neuroligin genes were not detected in our group of individuals with ASD (American males and females), suggesting that these specific gene mutations are not common or found in a low frequency in the autism population. However, mutations in other exons may be present in these genes but not detectable in our study design. In summary, the reports by Jamain *et al.* (2003) and Laumonnier *et al.* (2004) do emphasize that X chromosome genes should be studied to shed light on the etiology of heterogeneous neurodevelopmental conditions, including ASD.

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