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# Brief Report: Duplication of Chromosome 15q11–13 in Two Individuals with Autistic Disorder[1]

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Autism is a behavioral syndrome and, like most behavioral syndromes, it is etiologically heterogeneous. The importance of hereditary factors in the etiology of this disorder has been demonstrated through twin and family studies (reviewed in Folstein & Piven, 1991). In a small portion of cases cytogenetic abnormalities have been reported in association with autism; however, other than the fragile site at Xq27.3 no specific cytogenetic abnormality has been consistently identified (Cohen et al., 1991; Piven, Gayle, Landa, Wzorek, & Folstein, 1991). Several reports in the cytogenetics literature have noted duplications in chromosome 15q11-13 in individuals thought to be autistic (Schinzel, 1981, Wisniewski, Hassold, Heffelfinger, & Higgins, 1979), however, detailed behavioral descriptions and standardized diagnostic criteria for autism are not included in these reports. Schinzel (1981) reported one case of a male with a duplication of chromosome 15 (pter—q12 or q13) mental retardation and "features of autism." Wisniewski et al. (1979) reviewed 19 cases reported in the literature with similar cytogenetic abnormalities (i.e., duplication of the proximal

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portion of the long arm of chromosome 15) and noted that five (three male and two female) were described as autistic. In the same paper, Wisniewski et al. (1979) presented the results of their direct examination of five individuals with a duplication of the proximal portion of chromosome 15. The behavioral symptoms of one of the five subjects presented, a female, were suggestive of autism. Schinzel (1981) has also noted that mild dysmorphic features, hyperactivity, decreased attention, aggression, seizures, and poor motor coordination have been reported in association with duplication of chromosome 15 (pter—q12 or q13). Gillberg et al. (1991) reported the clinical descriptions of six males with autism and an associated partial tetrasomy of chromosome 15 (pter—q13). Two subjects had moderate, three had severe, and one had profound mental retardation. Three were hyperactive. A number of physical abnormalities were noted in about half of the cases including epicanthal folds; hypertelorism; high-arched palate; large, protruding ears; kyphosis; hypotonia, and muscular rigidity.

In this paper, we describe two children with DSM-III-R (American Psychiatric Association, 1987) autistic disorder and duplication of chromosome 15q11-q13, confirmed using molecular cytogenetic techniques. These results are consistent with previous studies by Wisniewski et al. (1979), Schinzel (1981), and Gillberg et al. (1991) who reported the presence of a duplication of chromosome 15 (pter—q13) in autistic individuals. We suggest that this region may have etiologic significance in some cases of autism.

## CLINICAL REPORT

### *Case 1*

L.N. is the only child of nonconsanguinous parents. At age 10 she was referred to the Outpatient Child Psychiatry Clinic at the University of Iowa for treatment of increasing aggression. Family history reveals L.N.'s father had learning difficulties as well as academic and behavioral problems in school. He had a psychiatric hospitalization as a teenager for unknown reasons. As an adult, he has had significant problems with the law including several jail terms for various misdemeanors. The paternal family history is also remarkable for two paternal uncles who have had problems with alcohol and substance abuse. Both paternal uncles have had several incarcerations for unknown charges. The maternal family history is remarkable for L.N.'s mother having a history of alcohol abuse although she has been abstinent for the past 8 years. Maternal grandmother has a diagnosis of cerebral palsy.

L.N. had a prenatal history of maternal hyperemesis gravidarum during the first trimester. There was also a first trimester history of maternal alcohol and tobacco use of unknown amount. Delivery was induced as a result of being post-dates. The neonatal course was unremarkable.

L.N. walked at 12 months. Single words were observed at 18 months; full sentences were noted at 3 years of age. On examination, her speech was monotonous with an occasional high pitched whine. There was a history of repetitive speech with immediate and delayed echolalia, pronoun reversal, idiosyncratic word use, and frequent irrelevant remarks. Solitary and repetitive play activities were preferred. L.N. was uninterested in peers and was indiscriminantly affectionate towards people. There was no evidence of specific attachments to family members. Eye contact was diminished and limited facial expressiveness was noted in social situations. L.N. had motor stereotypies, including frequent hand flapping. Tantrums occurred frequently when routines were altered or if there were minor changes in the environment. She had a restricted range of interests and a preoccupation with the color purple.

Physical examination was unremarkable; including no evidence of the stigmata of fetal alcohol syndrome. Routine laboratory testing was normal. Psychological assessment revealed a composite IQ score of 53 on the Stanford-Binet 4th edition. EEG was abnormal with a sharp and slow wave focus in the right frontal lobe. Magnetic resonance brain scan demonstrated multiple, small foci of increased T2 signal intensity in the right inferior temporal lobe at the junction of the gray and white matter. Diagnosis was DSM-III-R Autistic Disorder and Moderate Mental Retardation. L.N. has subsequently developed tonic-clonic seizures and is being treated with Phenytoin.

### *Case 2*

J.R. is the second child of healthy nonconsanguinous parents. At age 6 she was referred to the University of Iowa Child Psychiatry Inpatient Unit for increasing aggression and hyperactivity. Family history revealed a maternal cousin with a history of attention deficit disorder and learning difficulties. There was no history of psychiatric disorder in the patient's parents or her one older female sibling. There was no history of pregnancy or delivery complications. As an infant, difficulties with sucking and swallowing were reported which resulted in failure to gain weight and several hospitalizations. J.R. first walked at 24 months.

On examination, speech consisted predominantly of frequent immediate and delayed echolalic verbalizations, varying from single words to sim-

ple, full sentences. Occasional spontaneous use of meaningful single words (e.g., "cookie," "brush," "swing") was noted as well as occasional two- to three-word sentences. J.R. had marked social deficits. She generally had no interest in seeking out staff or peers for reciprocal social interactions. At times she became upset and would cling indiscriminantly to a nearby adult. There was no evidence of specific attachments to family, staff, or peers. Play consisted of repetitive manipulation of objects or parts of objects with no pretend or imaginative play. Stereotyped hand movements and upper body rocking were frequently observed. With changes in activities, temper tantrums often occurred which consisted of repetitive and aggressive behavior (e.g. slapping staff repeatedly on the back). J.R. insisted on adhering to certain routines. For example, she often insisted that a story or song be included as part of a bedtime routine; if not, a tantrum would result.

Physical examination revealed mildly dysmorphic features including anteverted nostrils, bilateral epicanthal folds, clinodactyly of the fifth digits bilaterally, broad great toes, and a high-arched palate. Because of her marked inattention and aggressive behavior, a standardized IQ test could not be reliably administered. Routine laboratory testing was unremarkable. EEG was normal and a CT scan of the head revealed slightly enlarged lateral and third ventricles. J.R. exhibited some improvement of aggressive behavior after behavioral modification and Imipramine treatments were employed. Diagnosis was DSM-III-R Autistic Disorder and Probable Severe Mental Retardation.

## CYTOGENETIC STUDIES

### *Case 1*

G-banded chromosome analysis from a peripheral blood lymphocyte revealed the patient to have 46,XX/46,XX,dup(15) (q11.2—q13) karyotype (i.e., a duplication within chromosome 15 of a small portion of the proximal long arm). A focused analysis of chromosome 15 showed duplication of the 15q11-13 region in five out of 10 cells. Fluorescence in situ hybridization (FISH) was performed using ONCOR's Prader-Willi region A probe (D15S11) to determine the feasibility of detecting the duplication. The FISH technique involves taking a known piece of DNA (the probe), that can be made to fluoresce, and observing whether it binds (hybridizes) to complementary DNA from the subject. The presence of a complementary stretch of DNA in the subject is indicated by the appearance of a fluorescent spot on the chromosome. Preliminary results indicated a size

difference in signals between the two homologies in 14 out of 26 cells suggesting the existence of mosaicism. That is, the fluorescent signal was much larger than expected in some cells (14/26), indicating the presence of a larger than expected stretch of DNA (i.e., due to the duplicated portion of the chromosome) and confirming that the duplication was in the proximal portion (q11-13) of the long arm of chromosome 15. Duplication of the q11-13 region of chromosome 15 on a portion of cells, together with the normal presence of this segment of chromosome 15 on a homologous chromosome (i.e., the 23 autosomal chromosomes are each present in pairs in each cell) resulted in a partial trisomy or three copies of this chromosomal material in over half of the cells observed. Parents were chromosomally normal.

### *Case 2*

G-banded chromosome analysis from a peripheral blood sample revealed the patient to have 47,XX,+mar in all cells. This patient had an extra, small piece of chromosomal material — the "marker" chromosome (i.e., 47 chromosomes were detected). The marker was found to represent isodicentric (15) (pter — q13). That is, the marker was the result of a fusion of the pieces of chromosome 15 made from the short arm, centromere, and proximal portion of the long arm. Confirmation of this interpretation was done after C-, NOR-, and DAPI staining. FISH studies were performed with three different centromeric specific probes. Two of the probes specific for the centromeres of chromosomes 13 and 21 as well as for chromosomes 14 and 22, revealed no hybridization (i.e., binding) to the marker chromosome. However, pTRA-20 hybridized to the centromere, indicating that the marker chromosome was derived from chromosome 15. This was confirmed with a chromosome 15 specific library, which also hybridized to the marker chromosome. Thus the patient appeared to be tetrasomic (i.e., had four copies) for the region q11 to q13. Chromosome studies of both parents revealed no abnormalities.

## DISCUSSION

The identification of a consistent cytogenetic abnormality is often an important preliminary step in elucidating the genetic etiology of medical syndromes. In autism, a disorder in which hereditary factors have been shown to have a role, only the fragile site at Xq27.3 has been consistently associated with autism and autistic features in some individuals (Cohen et

al., 1991; Piven et al., 1991). In this paper, we present two individuals with duplication 15q11-13, autistic disorder, and mental retardation. The fact that we report only two cases raises the possibility that this is merely a chance association. However, the narrowly defined parameters of this cytogenetic abnormality (from q11 to q13) and the previous reports of other possible (Schinzel, 1981; Wisniewski et al., 1979) and definite (Gillberg et al., 1991) cases of autism with similar duplications, suggest that this area of chromosome 15 may be a useful locus for future linkage studies in autism.

Further support for the possible importance of this region in behavioral and mental retardation syndromes comes from investigations of Prader-Willi syndrome (PWS) and Angelman syndrome (AS). These two phenotypically distinct mental retardation syndromes have been shown to involve deletions of chromosome 15 at q11-13 and imprinting (Ledbetter et al., 1981, 1982). In PWS, this deletion is transmitted paternally, whereas in individuals with AS it is transmitted maternally. This region of chromosome 15 also contains the gene encoding the beta-3 subunit of the gamma-amino butyric acid (GABA) receptor (Wagstaff et al., 1991). Although no studies have reported an association between GABA and autism, GABA is the major inhibitory neurotransmitter in the mammalian brain and it may have a role in the pathogenesis of autism (Elliott & Ciranello, 1987).

Several additional issues require comment. First, in both cases presented in this paper the chromosomal abnormalities involved duplication of chromosomal material. Although less common than deletions, partial duplications of chromosomes have been associated with specific medical conditions and most likely represent an increased gene dosage secondary to the duplicated genetic material (Lupski et al., 1992). Second, L.N. was noted to be considerably less intellectually and behaviorally impaired than J.R. These milder abnormalities may be the result of mosaicism noted in L.N. (i.e., only half of the cells contained the abnormal duplication) or, the fact that the abnormality detected in L.N. was a partial trisomy, whereas in J.R. it was a partial tetrasomy. Robinson et al. (1993) in a study of five nonautistic, mentally handicapped individuals with inv dup (15) found evidence to suggest that the dosage of the q11-13 region was a major factor in determining clinical severity. In that study, cases with three copies of this region (i.e., partial trisomy) were more mildly affected than those with four copies (i.e., partial tetrasomy).

Finally, molecular cytogenetic techniques such as fluorescence in situ hybridization and chromosomal painting have resulted in increasingly more precise and definitive identification of subtle chromosomal rearrangements. Molecular abnormalities underlying medical syndromes are often detected following initial reports of cytogenetic defects, highlighting the importance

of detecting these clinical–pathological associations. Clinicians and researchers should make use of these techniques in individuals with developmental neuropsychiatric disorders as a way to gain preliminary clues for future molecular genetic studies.

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