Genes Controlling Affiliative Behavior as Candidate Genes for Autism

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Background: Autism spectrum disorders (ASD) are neurodevelopmental disorders of complex etiology, with a recognized substantial contribution of heterogeneous genetic factors; one of the core features of ASD is a lack of affiliative behaviors.

Methods: On the basis of the existing literature, in this study we examined the hypothesis of allelic associations between genetic variants in six genes involved in control of maternal and affiliative behaviors (*OXT, OXTR, PRL, PRLR, DβH*, and *FOSB*). One hundred and seventy-seven probands with ASD from 151 families ($n = 527$) were assessed with a set of related instruments capturing multiple facets of ASD. Multivariate and univariate phenotypes were constructed from these assessments and subjected to genetic linkage and association analyses with PBAT and FBAT software.

Results: The resulting pattern of findings, in general, confirmed the hypotheses of the significance of the genes involved in the development of affiliative behaviors in the manifestation of ASD (p values ranging from .000005 to .05); statistically speaking, the strongest results were obtained for allelic associations with the *PRL, PRLR,* and *OXTR* genes.

Conclusions: These preliminary data provide additional support for the hypothesis that the allelic variants of genes necessary for the development of species-typical affiliative behaviors are associated with ASD. Independent replication of these findings is needed and studies of other genes associated with affiliative behaviors are indicated.

Key Words: Affiliative behaviors, allelic association studies, autism spectrum disorders, *DβH*, *FOSB*, *OXT*, *OXTR*, *PRL*, *PRLR*

There is convincing evidence that autism spectrum disorders (ASD), a family of disorders characterized by impairments in social, linguistic, and motor functioning, are highly heritable (1). Yet, specific genetic mechanisms underlying this heritability have not yet been discovered. In addition, a substantial number of environmental factors are known to lead, at least in their extremes, to autism-like symptoms (2). Correspondingly, the dominant hypothesis regarding the etiological factors underlying ASD is oligogenic inheritance with possible epistatic interactions among common risk-predisposing genetic variants and detrimental environments (3).

As of today, only approximately 10% of all ASD cases have been attributed to known etiologies (4,5). Recognition of the complexity of the genetic mechanisms of autism explains why its biology remains elusive and molecular mechanisms underlying this biology mostly speculative or unknown. The field is exploring multiple lines of inquiry, one of which is to investigate a componential nature of the disorder (6) and formulate hypotheses targeting its specific facets (i.e., social, linguistic, and motor impairments). One such hypothesis is aimed at social impairment in ASD and links animal-based investigations of species-typical social behaviors (e.g., affiliative behaviors, pair bonding, social processing) to studies of ASD (7–9). Capitalizing on this line of thinking, we designed a study to test a subset of the genes implicated in aspects of affiliative behavior (7).

Oxytocin (OT) is a neurohypophysial peptide that has emerged in studies of rodents (7), nonhuman primates (10), and humans (11,12) as an important mediator of affiliative behavior. There are also specific hypotheses (supported by varying degrees of evidence) linking oxytocin and ASD, specifically, that autistic children tend to be characterized by lower levels of plasma OT (13,14) and that infusion of OT is related to the reduction of repetitive behaviors in patients with ASD (15). For example, it has been hypothesized that administration of OT during labor can generate excess OT in the fetal brain. Such excesses might lead to downregulation of OT receptors and, subsequently, to imbalance of the OT system and unavailability of OT for further signal transduction cascades (16). A general dysregulation of the OT system has been conjectured in humans with autism (9). Although there is indirect support from animal studies for this hypothesis (9), experimental studies with humans are difficult to conduct, and descriptive studies with humans have produced contradictory results (17–19). Also of note is that maternal OT has been related to switching γ-aminobutyric acid (GABA) signaling in the fetal brain during delivery (20). Because GABA signaling is seen as one of the disrupted process in ASD (21), it is possible that oxytocin is indirectly associated with this deficiency. Thus, there is suggestive evidence connecting OT and ASD, but the underlying mechanics of this connection are not known. Oxytocin exerts its functions in the brain through OT ligands and receptors and the corresponding genes. Linkage and association studies of ASD have also provided evidence regarding the potential role of oxytocin in the etiology of autism. Specifically, a combined analysis of Autism Genetic Resource Exchange (AGRE) and a sample of Finnish families of probands with autism (22) implicated region 3p24–26, harboring the oxytocin receptor gene, as a susceptibility region for autism. In this study, the signal was stronger in the Finnish sample. Another whole-genome scan also pointed to this region as the second

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Received September 13, 2007; revised November 8, 2007; accepted November 9, 2007.

0006-3223/08/$34.00
doi:10.1016/j.biopsych.2007.11.015

best locus (23). Association studies with Chinese Han (24) and US Caucasian families (25) of probands with autism implicated the oxytocin receptor gene as a candidate gene for autism (24). Correspondingly, in this study, we considered both the OT (*OXT*, OMIM 167050: GenBank accession NM_000915; chromosome 20p13) and the OT receptor (*OXTR*, OMIM 167055 GenBank accession NM_000916; chromosome 3p26) genes as possible candidate genes for ASD.

Similar to OT, prolactin (PRL) is a pituitary hormonal peptide. Prolactin has been implemented in parturition, stimulation of mammary gland development during pregnancy, stimulation of milk synthesis, and regulation of lactation, making it an important agent in regulating affiliative behaviors (7). The literature contains some suggestive evidence regarding the role of PRL in the manifestation of autism. Specifically, there are studies reporting an elevation of serum PRL in autism (26), especially in probands with seizures (27), and responsiveness of the PRL system to the administration of m-chlorophenylpiperazine (m-CPP) to adults with autism (28). Similar to studies of OT in autism, the literature is suggestive but not causative and not without complexities. Specifically, 50% of the patients in a study indicating an elevated serum PRL were receiving neuroleptic treatment, and although the drugs were stopped for the purposes of the study, an insignificant amount of the medication could have remained in the blood and resulted in an elevated amount of PRL (26). It is also interesting to note that the administration of m-CPP resulted not only in significantly increased PRL response when compared with control subjects but also in an increase in repetitive behaviors (28); yet, the nature of the association between increased PRL and increased repetitive behaviors is not clear. In the current study, we investigated both the PRL ligand (*PRL*, OMIM 176760; GenBank accession NM_000948; chromosome 6p22) and the PRL receptor (*PRLR*, OMIM 176761; GenBank accession NM_000949; chromosome 5p13) as ASD candidate genes. Various genome-wide screens point to the regions harboring these genes as of interest (for 6p [29] and for 5p [29–31]); however, neither region maintained significance in a meta-analytic reevaluation (32).

Another biological agent on which the literature on autism and affiliative behavior converge is the enzyme dopamine beta hydroxylase (DβH), involved in the catalyses of dopamine to norepinephrine. The DβH is coded by a single gene. There are several animal models in which loss of this gene is strongly associated with deficits in affiliative behaviors (for a review, see [7]). Studies in humans have reported decreased serum DβH in probands with autism and their parents (33,34). In addition, some evidence suggests that maternal variation in DβH is associated with the manifestation of autism in their children (34,35). The gene coding for the DβH enzyme (*DβH*, OMIM 609312; GenBank accession NM_000787) is located on chromosome 9q, which has been indicated as a region of interest in at least one genome-wide screen for autism (29).

The last candidate gene considered in this study was *FOSB* (OMIM 164772; GenBank accession NM_0006732; chromosome 19q), part of the Fos gene family, whose proteins can form transcription factor complexes. Through their transcription functions, these proteins are involved with proliferation, differentiation, and transformation. The *FOSB* gene has homologs in many species. Mice lacking *fosb* show marked deficits in affiliative behavior, including a failure to retrieve and nurse pups (7). This gene has never been investigated as a candidate gene in autism, although four independent genome-wide scans identified 19q as a region of interest (29,31,36,37).

Methods and Materials

Participants

To test our hypotheses on the relevance of one or more of six genes involved in control of affiliative behaviors (*OXT, OXTR, PRL, PRLR, DβH*, and *FOSB*) to ASD, we genotyped DNA from a sample of probands with autism and their families available through the Yale Child Study Center. The sample consisted of 527 participants (322 male, and 205 female), of which 177 were classified as probands (Table 1). These participants formed 151 nuclear families. Forty-one percent of the families had only one child (n = 62), 39% had two children (n = 57), 17% had three children (n = 25), and only five families had four–five children (3 and 2, respectively). Most of the proband children (154, or 87%) were male; the gender distribution among parents or non-proband siblings was approximately equal. The majority of the sample was Caucasian (93%). Additional relevant characteristics of this sample are shown in Table 1. The study was approved by the Yale institutional review board.

Phenotyping

Probands and their relatives were evaluated with a number of clinical instruments used for ASD diagnosis (Autism Diagnostic Interview, ADI [38], and Autism Diagnostic Interview–Revised, ADI-R [39]; Autism Diagnostic Observational Schedule, ADOS [40], and Autism Diagnostic Observational Schedule–Generic, ADOS-G [41]; and Vineland Adaptive Behavior Scales [42]), from which a number of phenotypes were developed. Specifically, the ADI and ADI-R generated five indicators, "Social Interactions," "Communication," "Restricted/Repetitive Behaviors," "Onset," and "ADI-Based Diagnosis." The ADOS and ADOS-G also resulted in four quantitative indicators, "Social Skills," "Communication Skills," "Stereotyped Behaviors," and "Imaginative Skills," and the "ADOS-Based Diagnosis" qualitative variable. The administration of the Vineland resulted in four subscores, "Communication," "Daily Living Skills," "Socialization," and "Motor Skills," and the "Vineland Composite Score." In addition, "Clinical Diagnosis" (any type of ASD, yes/no) was used in our analyses;

Table 1. Descriptive Statistics on the Sample of Families of the Autism Spectrum Disorder Probands

Total Number of People Genotyped	472
Male (%)	62.3
Female (%)	37.7
Number of Probands/Family	
1	129
2	21
3	2
Average Age of the Probands	10.97
Average Full-Scale IQ of the Probands	96.65
Average Vineland Composite Score of the Probands	55.38
Probands' Diagnoses	
Autism	84
Asperger's Disorder	46
Pervasive Developmental Disorder-Not Otherwise Specified	40
Childhood Disintegrative Disorder	7

The IQ was assessed by a variety of means, including the Wechsler Intelligence Scale for Children (WISC), Weschler Preschool and Primary Scale of Intelligence (WPPSI), the Differential Ability Scales (DAS), the Mullen, the Kaufman Assessment Battery for Children (K-ABC), and the Leiter Test of Intelligence (Leiter-R), depending on the proband's age and level of functioning and the availability of particular versions of these tests at a given time of testing.