OBSTETRICS

Fetal DNA methylation of autism spectrum disorders candidate genes: association with spontaneous preterm birth

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OBJECTIVE: Autism spectrum disorder (ASD) is associated with preterm birth (PTB), although the reason underlying this relationship is still unclear. Our objective was to examine DNA methylation patterns of 4 ASD candidate genes in human fetal membranes from spontaneous PTB and uncomplicated term birth.

STUDY DESIGN: A literature search for genes that have been implicated in ASD yielded 14 candidate genes (*OXTR*, *SHANK3*, *BCL2*, *RORA*, *EN2*, *RELN*, *MECP2, AUTS2, NLGN3, NRXN1, SLC6A4, UBE3A, GABA, AFF2*) that were epigenetically modified in relation to ASD. DNA methylation in fetal leukocyte DNA in 4 of these genes (*OXTR*, *SHANK3*, *BCL2*, and *RORA*) was associated with PTB in a previous study. This study evaluated DNA methylation, transcription (reverse transcription polymerase chain reaction), and translation patterns (immunostaining and Western blot) in fetal membrane from term labor ($n = 14$), term not in labor (TNIL; $n = 29$), and spontaneous preterm birth (PTB; $n = 27$). Statistical analysis was performed with analysis of variance; a probability value of $< .05$ was significant.

RESULTS: Higher methylation of the *OXTR* promoter was seen in fetal membranes from PTB, compared with term labor or TNIL. No other gene showed any methylation differences among groups. Expression of *OXTR* was not different among groups, but the 70 kDa OXTR protein was seen only in PTB, and immunostaining was more intense in PTB amniocytes than term labor or TNIL.

CONCLUSION: Among the 4 genes that were studied, fetal membranes from PTB demonstrate differences in *OXTR* methylation and regulation and expression, which suggest that epigenetic alteration of this gene in fetal membrane may likely be indicating an in utero programing of this gene and serve as a surrogate in a subset of PTB. The usefulness of *OXTR* hypermethylation as a surrogate for a link to ASD should be further evaluated in longitudinal and in vitro studies.

Key words: epigenetics, hydroxymethylation, *OXTR*, programing

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Autism spectrum disorder (ASD) is a complex group of heterogeneous neurodevelopmental disorders that can cause significant social and behavioral challenges. According to the Centers for Disease Control and Prevention,[1] ASD affects an estimated 1 in 88 individuals in the United States with a male-to-female ratio of 4:1.[2] The prevalence of ASD has increased over the past 2 decades, almost doubling in number.[3] Despite decades of research, the cause of ASD remains unclear, although several epidemiologic, genetic, epigenetic, and environmental factors have been proposed. It is thought currently that the mechanism underlying the cause of ASD is most likely polygenic and/or potentially epistatic and has environmental and genetic interactions that increase the risk of ASD.[4,5] A number of perinatal risk factors such as behavior (cigarette, alcohol, and drug abuse), stress, metabolic syndrome, poor nutritional status, environmental contaminants, advanced maternal age, and the use of antidepressant medications during pregnancy that can cause epigenetic alterations (eg, DNA methylation) in ASD-associated genes have been reported.[6,7] In addition to the aforementioned risk factors, several epidemiologic studies have linked preterm birth (PTB; <37 weeks of gestation) to the development of ASD.[8]

Coincidently, the number of infants born preterm has also increased during the past 2 decades, which creates

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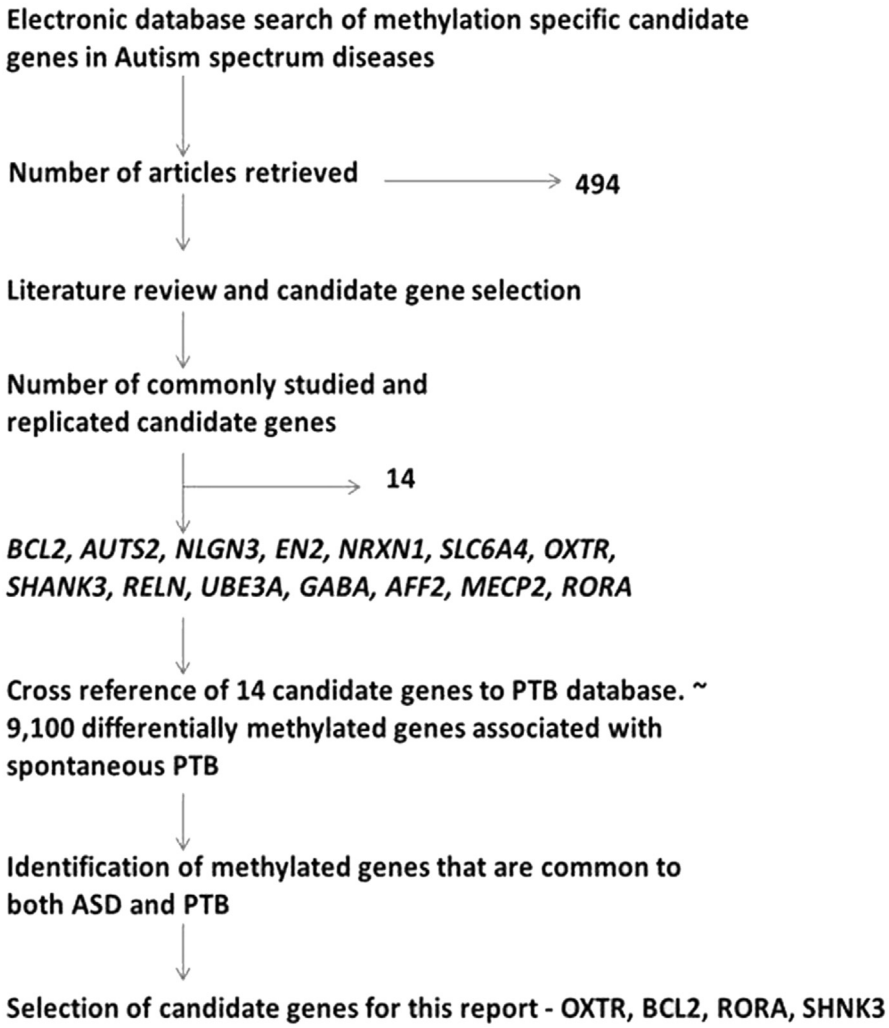
FIGURE 1
Schema used for selection of candidate genes for this study



ASD, autism spectrum disorder; *BCL2*, B-cell lymphoma 2; *OXTR*, oxytocin receptor; *PTB*, preterm birth; *RORA*, RAR-related orphan receptor alpha; SHANK3, SH3 and multiple ankyrin repeat domains.

Behnia. In utero programing of OXTR by methylation in preterm birth. Am J Obstet Gynecol 2015.

significant emotional devastation and strain on both the individual and the family. PTB continues to be a significant public health concern as a leading cause of infant morbidity and death. Spontaneous preterm labor comprises 70% of PTB and is a syndrome that is attributable to multiple pathologic processes.[9] Multiple mechanisms have been implicated in PTB such as cervical disease,[10] uterine over-distention,[11] decidual senescence,[12] senescence of fetal membranes,[13,14] and decline in progesterone action.[15] Because risk factors for PTB and ASD are similar, it is unclear whether these risk factors increase risk for both conditions independently or whether ASD results from prematurity (or low birthweight). We have recently reported fetal epigenetic (DNA methylation) differences that associate with gestational age in those with PTB.[16-18] However, no study has yet evaluated the potential molecular link between PTB and ASD.

Epigenetic alterations, a potential risk factor for both ASD and PTB, refer to functionally relevant modifications to the genome that influences gene expression without involving a change in nucleotide sequence.[19] Epigenetic modifications include DNA methylation, hydroxymethylation, and modifications of histone proteins that are complexed with DNA to form the chromatin.[19] It has been reported that environmentally induced epigenetic changes in ASD-risk genes during the postnatal period cause oxidative status change in the brain. Therefore, altered redox status has been reported as a pathophysiologic condition of ASD.[20] In animal studies, administration of valproate, a histone deacetylase inhibitor that influences epigenetic patterns during early fetal development, results in autistic-like behavior.[21] Adverse maternal environment (deficit in the maternal 5-HT_{1a}) has also been reported to induce DNA methylation changes in genes that are involved in synapse formation and function in offspring.[22] Based on these observations, we hypothesize that the risk factors for PTB and the hostile environment that results in PTB may also affect the expression of ASD-susceptibility genes in fetal tissues, which is an indicator of in utero programing, potentially increasing ASD risk during the early stages of life. The primary objective of our study was to evaluate DNA methylation of genes that are implicated in both ASD and PTB and to characterize DNA methylation, gene expression, and protein levels of these genes in fetal membrane tissue from PTB compared with normal term birth. If methylation-specific epigenetic changes in ASD susceptible genes are identifiable in fetal membranes from PTB, it may serve as a biomarker for screening neonates from high-risk pregnancies (PTB) for the development of ASD. These findings are also expected to generate hypotheses for future studies of fetal programing that is associated with PTB that may predispose to ASD.

To achieve this goal, an extensive literature review was conducted on genes that are linked to ASD with the use of an electronic database (PubMed). Our search was limited to genes for which methylation levels associate with ASD. A catalogue of genes was generated, and this list was compared with genes for which methylation levels associated with PTB in our previously published study.[16] Methylated genes that associate with both ASD and PTB were selected (Figure 1) and included oxytocin receptor (*OXTR*),