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Interaction of the MET Receptor Tyrosine Kinase Gene and Air Pollution Exposure in Autism Spectrum Disorder

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Abstract

Background—Independent studies report association of autism spectrum disorder with air pollution exposure and a functional promoter variant (rs1858830) in the MET receptor tyrosine kinase (*MET*) gene. Toxicologic data find altered brain Met expression in mice after prenatal exposure to a model air pollutant. Our objective was to investigate whether air pollution exposure and *MET* rs1858830 genotype interact to alter ASD risk.

Methods—We studied 252 cases of autism spectrum disorder and 156 typically developing controls the Childhood Autism Risk from Genetics and the Environment Study. Air pollution exposure was assigned for local traffic-related sources and regional sources (particulate matter, nitrogen dioxide and ozone). *MET* genotype was determined by direct re-sequencing.

Results—Subjects with both *MET* rs1858830 CC genotype and high air pollutant exposures were at increased risk of autism spectrum disorder compared with subjects who had both the CG/GG

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genotypes and lower pollutant exposures. A statistical test of multiplicative interaction identified a statistically significant effect between NO₂ and *MET* CC genotype (p=0.03)

Conclusions—*MET* rs1858830 CC genotype and air pollutant exposure may interact to increase autism spectrum disorder risk.

Autism and autism spectrum disorders are complex neurodevelopmental disorders characterized by deficits in social interaction, communication, and behavioral flexibility. The complex phenotypic presentation of these disorders suggests that multiple genetic and environmental factors contribute to risk, and gene-environment interactions are widely believed to underlie autism spectrum disorders. Few studies have addressed joint risk from specific genetic susceptibility in combination with a specific environmental exposure or class of exposures.¹ In previous independent studies, we have identified (1) increased autism spectrum disorder risk among children exposed to high levels of local near-roadway traffic-related air pollution and regional particulate matter near the time of birth^{2,3}; (2) increased autism spectrum disorder risk among children with the C allele of the *MET* gene promoter variant rs1858830,^{4,5} which is associated with decreased expression of MET protein in brain⁶ and immune system⁷; and (3) decreased MET protein expression in brain and altered behavior in offspring of mouse dams exposed during pregnancy to the polycyclic aromatic hydrocarbon benzo(a)pyrene (a component of traffic-related air pollution and particulate matter).⁸ Based on these independent autism spectrum disorder associations and the biological link between benzo(a)pyrene and *MET*, we hypothesized that a gene-environment interaction contributes to autism spectrum disorder risk.

In children, as in animals, prenatal polycyclic aromatic hydrocarbon exposure has been associated with intelligence (IQ) deficits at age 5 years as well as with increased anxiety, depression, and inattention at age 6–7.^{8–10} In this study we investigated the relationship of air pollution exposure and genotype at the *MET* rs1858830 locus with autism spectrum disorder.

Methods

Description of Sample

The Childhood Autism Risks From Genetics and the Environment Study is a population-based, case-control study of preschool children from California. Participants were born in California and lived with at least one English- or Spanish-speaking biologic parent in one of the study catchment areas related to specific regional centers in California. Subjects were 24 to 60 months of age at the time of recruitment; additional details on study design are provided elsewhere.¹¹ For this analysis, cases met criteria for autism or autism spectrum disorder based on the Autism Diagnostic Observation Schedules and the Autism Diagnostic Interview-Revised. Typically developing controls were children who received a score of less than 15 on the Social Communication Questionnaire and also showed no evidence of other types of developmental delay (composite scores greater than 70 on Mullen Scales of Early Learning and Vineland Adaptive Behavior Scales). We assigned air pollution exposure to 669 study participants based on their residential histories and available exposure databases (as described below).³ For 63 percent of participants, parents agreed to give blood and

consented to share biospecimens with researchers outside of the original study team. This analysis includes 251 cases with a confirmed diagnosis of autism or autism spectrum disorder and 156 controls with typical development.

In parental interviews we collected data on demographic characteristics, medical conditions and environmental exposures, including residential history.¹¹ Residential histories recorded dates and address locations where the mother lived, beginning at conception through the most recent place of residence, as well as any other place of residence where the child lived. These dates and addresses were used to develop air pollution exposure metrics.³ Prenatal and birth addresses were used to develop a weighted average of pollution exposure. In this analysis, we focus on air pollution exposure during the prenatal period.

Air Pollution Exposure Assignment

We assigned modeled estimates of traffic-related air pollution exposure to study participants using the CALINE4 line-source air-quality dispersion model.¹² Included in the model is information on roadway geometry, link-based traffic volumes, period-specific meteorological conditions (wind speed and direction, atmospheric stability, and mixing heights), and vehicle emission rates.³ CALINE4 pollutant concentration estimates are indicators of the traffic-related air pollutant mixture rather than of a specific pollutant. We estimated residential exposure derived from freeways, non-freeways, and all roads located within 5 km of the home.

We also used regional air quality data to assign exposure for particulate matter less than 2.5 and less than 10 microns in diameter (PM_{2.5} and PM₁₀), nitrogen dioxide, and ozone using data from the US Environmental Protection Agency Air Quality System (www.epa.gov/ttn/airs/airsaqs) supplemented for Southern California by the University of Southern California's Children's Health Study data for 1997–2009.³ When no Federal Reference/Equivalent Method data for particulate matter were available for a given monitoring station in the Air Quality System, Children's Health Study continuous particulate matter data were used. The monthly air quality data from monitoring stations located within 50 km of each residence were used for spatial interpolation of ambient concentrations. The spatial interpolations were based on inverse distance-squared weighting of data from up to four closest stations located within 50 km of each participant residence; however, if one or more stations were located within 5 km of a residence, then only data from the stations within 5 km were used for the interpolation.

Genotyping Methods

Blood was collected from participants as part of the study protocol, with genomic DNA extracted from peripheral blood leukocytes using standard methods (Puregene kit; Gentra Inc). As the rs1858830 SNP falls within a highly GC-rich region, indirect genotyping methods fail when using genomic DNA. A 652-bp fragment containing the rs1858830 SNP was amplified from 15 ng genomic DNA with primers 5'-GATTCCTCTGGGTGGTG-3' (Forward) and 5'-CAAGCCCCATTCTAGTTTCG-3' (Reverse). Polymerase chain reaction (PCR) analysis was performed with the KOD Xtreme Hot Start Polymerase kit (EMD Millipore), which is designed to amplify regions with high

GC content. Cycling conditions were: 95°C for 5 min followed by 35 cycles of 95°C for 30s, 68°C for 30s and 72 °C for 1 min. Specific amplification of the 652-bp product was confirmed by agarose gel electrophoresis. Each PCR product was subjected to direct re-sequencing using an ABI 3730xl using Big Dye Terminator chemistry. Genotype at the *MET* rs1858830 locus was determined from the sequencing result using Sequencher software (Gene Codes, Ann Arbor, MI, USA).

Statistical Analysis

Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for air pollution exposure and *MET* genotype. We examined each pollutant separately, categorizing children as “high exposure” if the pregnancy average exposure for traffic related air pollution, PM_{2.5} or PM₁₀, nitrogen dioxide, or ozone was in the top 25% of the exposure distribution. Participants in the other 75% served as “low exposure” in our analyses. These categorizations are consistent with findings identified in our previous work.³ We also explored more and less extreme exposure-cut points (eTable 1). Because previous research demonstrated an increased risk of autism spectrum disorder due to over-transmission of the C allele and because functional studies suggest the *MET* CC genotype is associated with decreased *MET* expression, we compared the CC genotype to the CG and GG genotypes in our analyses.⁵ Analyses were adjusted for potential confounders, including child’s sex and ethnicity, maximum education level in the home, maternal age, home ownership and prenatal smoking.

Results

Genotyped subjects were similar to ungenotyped subjects in autism spectrum disorder status and air pollution exposure (eTable 1). Genotyped subjects were less likely to have a mother who smoked during pregnancy and less likely to have high nitrogen dioxide exposure compared with ungenotyped subjects. *MET* rs1858830 genotype frequencies did not vary across cases and controls ($\chi^2=1.40$, 2df). We did not find an increased risk of autism spectrum disorder for the *MET* CC genotype compared with CG/GG genotypes (crude OR= 0.9 [95% CI= 0.6–1.4]). Autism spectrum disorder was associated with exposure to the top quartile of traffic-related air pollution (1.7 [1.0–2.7]), particulate matter less than 10 microns in diameter (2.5 [1.6–4.3]), particulate matter less than 2.5 microns in diameter (1.9 [1.2–3.1]), and nitrogen dioxide (1.7 [1.1–2.7]).

We then parameterized our model based on both *MET* genotype and air pollution exposure. Synergistic effects were observed between *MET* CC genotype and local traffic-related air pollution, regional PM₁₀, and regional nitrogen dioxide exposure; adjusted ORs were, respectively, 2.9 (1.0–10.6), 3.2 (1.3–9.1), and 3.6 (1.3–13), comparing the high-risk genotype and highly exposed children to those with low exposure and without the risk genotype (Table). Statistical tests of multiplicative interaction identified a statistically significant effect between NO₂ and *MET* CC genotype (p=0.03) and borderline significant effects between local traffic-related air pollution and *MET* CC genotype (p=0.09). Analyses exploring alternative cut-points found the persistence of joint effects of traffic-related air pollution and *MET* CC genotype using either lower or higher cut points for defining high

exposure (eTable 2). Joint effects of *MET* CC genotype with PM₁₀ or nitrogen dioxide are additionally present at higher cutpoints.

Discussion

Examination of joint pollution and gene effects suggest that subjects with both the *MET* rs1858830 CC genotype and high air pollutant exposure were at increased risk of autism spectrum disorder compared with subjects who had both the CG/GG genotypes and lower air pollutant exposure. Given that the *MET* CC genotype had no impact in the 75% of the population with lower air pollutant exposures, these data suggest a gene-environment interaction for autism spectrum disorder based on *MET* genotype and air pollution exposure. These results require independent replication and a more detailed understanding of the underlying biology. However, these data add to the literature supporting a role for gene-environment interactions in autism spectrum disorder etiology. They also point to the contribution of common alleles for which gene-only analyses show inconsistent evidence of a link to autism spectrum disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Crude and Adjusted^a Associations of Air Pollution Exposure, *MET* rs1858830 Genotype, and Autism Spectrum Disorder Risk (n=407)

Table

Exposure Quartile	<i>MET</i> Genotype	No. Autism Cases (n=251)	No. Controls (n=156)	Crude OR (95%CI)	aOR (95% CI)
Traffic-Related Air Pollution					
Top ^b	CC	18	4	2.9 (1.1–10.4)	2.9 (1.0–10.6)
	CG/GG	38	34	1.3 (0.78–2.3)	1.3 (0.73–2.2)
Bottom 3	CC	55	27	0.72 (0.43–1.2)	0.80 (0.47–1.4)
	CG/GG	140	91	1.00	1.00
PM _{2.5}					
Top ^c	CC	23	8	2.0 (0.88–4.9)	2.1 (0.92–5.4)
	CG/GG	33	30	1.7 (0.99–2.0)	1.7 (0.96–3.1)
Bottom 3	CC	55	22	0.75 (0.43–1.3)	0.82 (0.46–1.5)
	CG/GG	140	96	1.00	1.00
PM ₁₀					
Top ^d	CC	24	6	2.9 (1.2–8.0)	3.2 (1.3–9.1)
	CG/GG	32	32	2.2 (1.3–4.0)	2.1 (1.2–3.9)
Bottom 3	CC	58	19	0.72 (0.41–1.3)	0.76 (0.43–1.4)
	CG/GG	137	99	1.00	1.00
Nitrogen Dioxide					
Top ^e	CC	21	4	3.4 (1.2–11.8)	3.6 (1.3–12.7)
	CG/GG	35	34	1.3 (0.74–2.2)	1.2 (0.71–2.1)
Bottom 3	CC	53	27	0.66 (0.38–1.1)	0.72 (0.41–1.3)
	CG/GG	142	91	1.00	1.00
Ozone					
Top ^f	CC	16	12	0.86 (0.39–1.9)	0.95 (0.42–2.2)
	CG/GG	40	26	1.3 (0.75–2.3)	1.2 (0.67–2.2)
Bottom 3	CC	47	23	0.99 (0.57–1.7)	1.0 (0.59–1.9)

Exposure Quartile	MET Genotype	No. Autism Cases (n=251)	No. Controls (n=156)	Crude OR (95%CI)	aOR (95% CI)
	CG/GG	178	95	1.00	1.00

^a Models adjusted for child's sex, child's ethnicity (Hispanic vs. White, Black/Asian/Other vs. White), maximum education of parents (parent with highest of four levels: college degree or higher vs. some high school, high school degree, or some college education), maternal age (>35 years vs. ≤35 years), prenatal smoking (self-report of ever- vs. never-smoked while pregnant), and home ownership (owner vs. renter).

^b 30.2 parts per billion.

^c 16.0 microgram/meters cubed.

^d 29.2 microgram/meters cubed.

^e 17.5 parts per billion.

^f 41.8 parts per billion.