Altered Vascular Phenotype in Autism

Correlation With Oxidative Stress

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Background: Autism is a neurologic disorder characterized by impaired communication and social interaction. Results of previous studies showed biochemical evidence for abnormal platelet reactivity and altered blood flow in children with autism.

Objective: To evaluate the vascular phenotype in children with autism.

Design and Main Outcome Measures: Urinary levels of isoprostane F_2\alpha-VI, a marker of lipid peroxidation; 2,3-dinor-thromboxane B_2, which reflects platelet activation; and 6-keto-prostaglandin F_1\alpha, a marker of endothelium activation, were measured by means of gas chromatography-mass spectrometry in subjects with autism and healthy control subjects.

Setting and Subjects: Children with a clinical diagnosis of autism attending the Pfeiffer Treatment Center.

Results: Compared with controls, children with autism had significantly higher urinary levels of isoprostane F_2\alpha-VI, 2,3-dinor-thromboxane B_2, and 6-keto-prostaglandin F_1\alpha. Lipid peroxidation levels directly correlated with both vascular biomarker ratios.

Conclusion: Besides enhanced oxidative stress, platelet and vascular endothelium activation also could contribute to the development and clinical manifestations of autism.

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stable metabolite of the endothelium cyclooxygenase-derived prostacyclin. Moreover, the relative contribution of oxidative stress in inducing persistent abnormalities in platelet and endothelial function remains to be determined.

Here, we report the first observation that oxidative stress is increased and directly correlated with the rate of systemic Tx2 and prostacyclin biosynthesis in a group of subjects with autism compared with findings in controls.

### METHODS

#### STUDY PARTICIPANTS

The participants in the study were 26 children with autism who had not undergone any treatment and 12 healthy control children. The test subjects were outpatients at the Pfeiffer Treatment Center who had a diagnosis of autism on the basis of the criteria for autistic disorder as defined in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. The same criteria applied to all control subjects, each of whom (1) was a healthy, well-adjusted child who was not receiving any kind of therapy; (2) had no relatives with a diagnosis of autism spectrum disorder and had good behavior and academic standing; (3) had no mental disorder, including autism spectrum disorder; and (4) received no medication for at least 1 month and stopped receiving any multivitamin 2 weeks before sample acquisition. The study was approved by the institutional review board of the Pfeiffer Treatment Center, and their parents attested to the absence of these potential confounders in the prior month.

#### BIOCHEMICAL ANALYSES

Data were always analyzed in a blinded fashion. Urinary isoprostane F2α-VI (iPF2α-VI), 2,3-dinor-TxB2, and 6-keto-PGF1α were measured in urine spot samples by using standardized gas chromatography–mass spectrometry-assays, as previously described. Briefly, each sample was first spiked with the corresponding deuterated internal standard, extracted by means of a solid phase extraction column, purified by means of a thinline chromatography step, and finally assayed by means of negative ion chemical ionization gas chromatography–mass spectrometry.16,17 A urine aliquot (0.1 mL) was used to measure creatinine concentration with a commercially available, standardized colorimetric assay (Sigma-Aldrich Co, St Louis, Mo). Results were always normalized for urinary creatinine concentration, as previously described.17

#### STATISTICAL ANALYSIS

Data are presented as mean ± SEM. Statistical analysis was performed by using nonparametric 1-way analysis of variance (Kruskall-Wallis test) and Dunn posttest comparison. Correlations between variables were examined by using linear regression analysis. Only P values lower than .05 were regarded as significant.

As shown in the Table, we recruited 38 individuals: 26 subjects with autism and 12 control subjects. Although they were well matched for sex (autistic, 22 [85%] boys; controls, 10 [80%] boys), subjects with autism were younger than the controls (mean, 4.6 vs 6.7 years) (Table). Next, we compared the iPF2α-VI contents in the urine of children with autism with that of the controls. The urinary levels of iPF2α-VI were significantly higher in children with autism (5.2 ± 0.5 ng/mg creatinine) than in the controls (3.1 ± 0.3 ng/mg creatinine, P<.01) (Table). The data were also significant when children with autism were compared with the controls of the same sex (boys, 4.9 ± 0.5 vs 2.9 ± 0.4 ng/mg creatinine; girls, 6.6 ± 0.8 vs 3.5 ± 0.8 ng/mg creatinine; P<.05 for both). No significant difference in urinary creatinine levels was observed between the 2 groups of subjects (Table). No effect of age was observed on iPF2α-VI levels either in the subjects with autism or control subjects (r2=0.05, P=.18) (Figure 1).

Figure 2 (upper panel) illustrates the urinary levels of 2,3-dinor TxB2 in the 2 groups investigated. Similar to the findings for iPF2α-VI, 2,3-dinor TxB2 content in the urine of subjects with autism was significantly higher than that in control subjects (2.9 ± 0.2 vs 1.8 ± 0.1 ng/mg creatinine, P<.01).

Next, we investigated the endogenous biosynthesis of prostacyclin by assaying the urinary levels of its stable metabolite 6-keto-PGF1α. As shown in Figure 2 (lower panel), subjects with autism had a much higher urinary

### Table. Demographics of the Study Population

<table>
<thead>
<tr>
<th>Population</th>
<th>Age, y</th>
<th>Sex, M/F</th>
<th>Regression, Yes/No</th>
<th>Isoprostane F2α-VI, ng/mg Creatinine</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autistic (n = 26)</td>
<td>4.6 ± 0.2</td>
<td>22/4</td>
<td>14/12</td>
<td>5.2 ± 0.5†</td>
<td>15.3 ± 1.7</td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>6.7 ± 0.4‡</td>
<td>10/2</td>
<td>NA</td>
<td>3.1 ± 0.3</td>
<td>14.1 ± 2.6</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SEM and number of subjects.
†P<.01.
‡P<.05.

Abbreviation: NA, not applicable.

SI conversion factor: To convert creatinine to micromoles per liter, multiply by 88.4.
excretion of 6-keto-PGF₁α than did controls (2.01±0.2 vs 1.07±0.3 ng/mg creatinine, \( P = .005 \)). Levels of 2,3-dinor-TXB₂ directly correlated with 6-keto-PGF₁α in subjects with autism (\( r^2 = 0.22, P = .01 \)), suggesting a common mechanism of formation. Finally, we observed a linear relationship between those 2 metabolites and urinary iP₃-VI levels (2,3-dinor-TXB₂: \( r^2 = 0.30, P = .003 \); and 6-keto-PGF₁α: \( r^2 = 0.33, P = .002 \)) (Figure 3).

The major finding of this study is that autism is characterized by increased in vivo oxidative stress, which associates and directly correlates with biochemical signatures of platelet and vascular endothelial activation.

Autism is a complex neurologic disorder; its pathogenesis involves an interaction among multiple genetic, epigenetic, and environmental factors.\(^1\text{-}^5\) Oxidative imbalance is a feature of the autistic syndrome, and several lines of evidence support the hypothesis that oxidative stress also may play a functional role in this disease. Thus, autism is characterized by a lower antioxidant defense system, higher free radical production, and improvement of behavioral symptoms after antioxidant administration.\(^6\text{-}^9,18\text{-}^20\)

Consistent with a previous report,\(^10\) our results indicated that children with autism showed higher rates of in vivo lipid peroxidation than did controls. More important, in our study, this increase was independent of the age of the subjects investigated. A subgroup of children with autism is characterized by regression, a potential confounding factor. However, we found no difference between regressed vs early-onset subgroups along the variables we measured. Moreover, we observed that girls in the autism group and in the control group did not show a significant difference in isoprostane levels when compared with levels in boys. However, this con-

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**Figure 1.** Correlation between urinary levels of isoprostane F₂α-VI (iPF₂α-VI) in the subjects investigated and their age (\( r^2 = 0.05, P = .18 \)).

**Figure 2.** A, Urinary levels of 2,3-dinor-thromboxane B₂ (2,3-dinor-TXB₂) in subjects with autism and control subjects. B, Urinary levels of 6-keto-prostaglandin F₁α (6-keto-PGF₁α) in subjects with autism and control subjects. Asterisk indicates \( P < .01 \); horizontal lines, means.

**Figure 3.** Correlation between urinary levels of isoprostane F₂α-VI (iPF₂α-VI) with 2,3-dinor-thromboxane B₂ (2,3-dinor-TXB₂) (\( P = .003 \)) (A) iPF₂α-VI with 6-keto-prostaglandin F₁α (6-keto-PGF₁α) (\( P = .002 \)) in subjects with autism (B).
Conclusion may be secondary to the relatively small size of the girls in our study. Finally, by contrast with a previous study, isoprostane levels in the subjects with autism in our study did not show a bimodal distribution. This difference could be due to different inclusion/exclusion criteria adopted in recruiting the subjects. Thus, by contrast with that study, the subjects with autism in our study were not taking any medication. Another possible reason is the fact that while Ming et al measured 8-iso-prostaglandin F2α, a class III F2-isoprostane, we assayed iPF2α-VI, a member of class VI. Thus, previous reports have shown that in vivo, for mechanisms not completely understood, there could be a preferential formation of one isomer vs the other of this large family. Measurement of iPF2α-VI has been characterized as a reliable and specific method of investigating lipid peroxidation in vivo and reflects a status of enhanced oxidative stress, regardless of the underlying pathophysiological triggers. Interestingly, iPF2α-VI belongs to a class of lipids, the F2-isoprostanes that are also characterized by biological activities. Thus, they modulate the vascular phenotype of the oxidative stress response by inducing, among other phenomena, platelet activation and vasoconstriction. For this reason, we investigated the relationship between oxidative stress and the vascular phenotype in subjects with autism. This study represents the first observation, to our knowledge, that the rates of TxA2 and prostacyclin biosynthesis, markers of platelet and endothelial activation, respectively, are not only significantly increased in autism but also are closely correlated with the rate of oxidative stress. Because F2-isoprostanes promote platelet aggregation and vasoconstriction, the correlation of these variables in our study allows some speculation about a direct effect of these bioactive lipids on the vascular phenotype in autism. However, our data do not establish a causal relationship among the variables we measured. Abnormal markers of platelet activation and vasoconstriction in autism may result from many factors, including other biologically active species resulting from free radicals. In general, abnormalities of the vascular phenotype can be reflected clinically by an abnormal blood flow. In this regard, it is interesting that results of multiple neuroimaging studies demonstrate brain hypoperfusion in autism. Further elucidation of the relationship of oxidative stress and vascular homeostasis to the pathogenesis of autism, including the possible influence of F2-isoprostane on tissue perfusion, could lead to improvements in therapy.

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