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# An Investigation of Porphyrinuria in Australian Children with Autism

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Two recent studies, from France (Nataf et al., 2006) and the United States (Geier & Geier, 2007), identified atypical urinary porphyrin profiles in children with an autism spectrum disorder (ASD). These profiles serve as an indirect measure of environmental toxicity generally, and mercury (Hg) toxicity specifically, with the latter being a variable proposed as a causal mechanism of ASD (Bernard et al., 2001; Mutter et al., 2005). To examine whether this phenomenon occurred in a sample of Australian children with ASD, an analysis of urinary porphyrin profiles was conducted. A consistent trend in abnormal porphyrin levels was evidenced when data was compared with those previously reported in the literature. The results are suggestive of environmental toxic exposure impairing heme synthesis. Three independent studies from three continents have now demonstrated that porphyrinuria is concomitant with ASD, and that Hg may be a likely xenobiotic to produce porphyrin profiles of this nature.

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Autism is a neurodevelopmental disorder presenting in childhood that affects up to 1 in 150 children in the United States (Centers for Disease Control, 2006) and 1 in 160 in Australia (Wray & Williams, 2007). Autism is characterized by severe impairments in socialization, communication, and behavior (American Psychiatric Association, 1994). The prevalence of autism is increasing at epidemic rates (Yazbak, 2003) that cannot be accounted for by changing diagnostic criteria or improved diagnostic systems (Blaxill et al., 2003; Croen et al., 2002).

Mercury (Hg) toxicity has been proposed as a causal mechanism whereby a small subset of children are uniquely sensitive to Hg and, in such individuals, exposure triggers a cascade of events leading to autism (Bernard et al., 2001;

Mutter et al., 2005; Kern & Jones, 2006). Urinary porphyrins provide a convenient and non-invasive measure of xenobiotic exposure generally (Brewster, 1988) and of Hg specifically (Woods et al., 2005; Heyer et al., 2006).

Excess urinary porphyrin excretion (porphyrinuria) results from the inhibition of enzymatic steps in conditions including genetic deficiencies in heme production enzymes, hepatitis, renal disease, and erythroid disease (Gross et al., 2000), as well as by heavy metal inhibition (Bowers et al., 1992; Woods, 1996). The causal relationship between Hg and porphyrinuria has been demonstrated both in rats (Pingree et al., 2001) and in humans (Woods et al., 1993).

The steps in the heme pathway most vulnerable to heavy metal inhibition are those that involve uroporphyrin decarboxylase (Woods & Kardish, 1983) and coproporphyrinogen oxidase (Woods et al., 2005). The result of these inhibitions is specific elevations of urinary coproporphyrin and pentacarboxyporphyrin levels. Although nonmetal agents targeting the heme pathway also elevate urinary porphyrin levels (Daniell et al., 1997), precoproporphyrin (also known as keto-isocoproporphyrin) is produced by *in vivo* conversion of pentacarboxyporphyrin in the presence of heavy metal, providing a specific porphyrin marker for Hg exposure (Woods et al., 2005; Heyer et al., 2006).

Two previous studies reported porphyrinuria among autistic subjects consistent with elevated body burden of Hg (Nataf et al., 2006; Geier & Geier, 2007). The pattern is one of generalized porphyrinuria with marked elevation of coproporphyrin and of precoproporphyrin and of the ratio of coproporphyrin/uroporphyrin. This study aimed to examine this phenomenon among a group of Australian autistic children.

## METHODS

### Subjects

Urinary porphyrin profiles were obtained from 41 consecutive patients with an ASD presenting to the first author's psychology clinic from October 2006 through March 2008. Each patient was previously diagnosed with an ASD, by a health professional, based upon accepted international

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standards (American Psychiatric Association, 1994). Table 1 provides a summary of patient sample characteristics. Subjects were excluded if they had ever undergone any specific therapy to remove Hg (chelation) or if the child had confirmed fragile X, Prader–Willi/Angelman, or Rett syndrome.

### Measures

All patients were tested by the ISO-certified Laboratoire Philippe Auguste for urinary porphyrins using high-pressure liquid chromatography with fluorometric detection, including uroporphyrin (UP), heptacarboxyporphyrin (7cxP), hexacarboxyporphyrin (6cxP), pentacarboxyporphyrin (5cxP), precoproporphyrin (PrCP), and coproporphyrin (CP: types I and III).

### Statistical Analysis

Raw data (ASD and control) from the Nataf et al. (2006) study and normal control data from the Minder and Schneider-Yin (1996) study were obtained. Consequently, mean porphyrin

levels (standardized to creatinine levels) and the CP/UP ratio was calculated for the Australian sample and compared to these data sets. Porphyrin levels and ratios for the Geier and Geier study (2007) were ascertained from their published paper, as the raw data were not available. A one-way between-groups analysis of variance (ANOVA) was conducted to compare ASD and control group CP/UP ratios. This ratio is an important measure, as coproporphyrin is postulated to be directly affected by Hg, with uroporphyrin to a lesser extent. Tukey HSD post hoc comparisons determined differences between pairs of means.

### RESULTS

Table 1 shows the mean differences between the ASD patients and the non-ASD controls. Elevations on all porphyrins for the ASD groups in comparison to both control and laboratory reference range are evident, with the greatest discrepancies evident for precopro- and coproporphyrin.

A statistically significant difference was found in the CP/UP ratio between the present study ASD data, Nataf et al. (2006)

**TABLE 1**  
Patient Characteristics and Porphyrin Levels of Children With and Without Autism

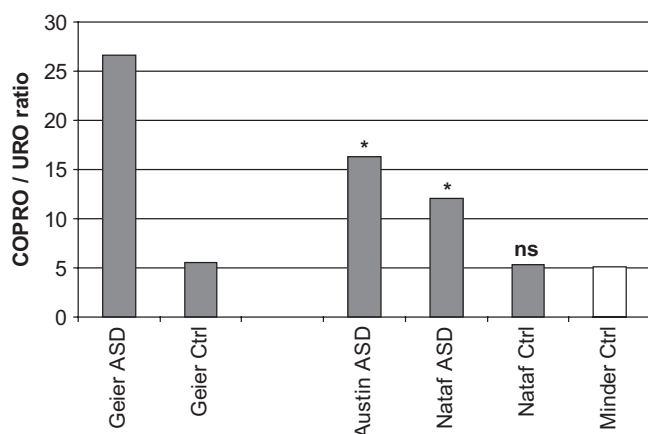
|                        | Austin and<br>Shandley | Geier and Geier (2007)     |                          | Nataf et al. (2006) |               | Minder and<br>Schneider-Yin<br>(1996) | Laboratory<br>reference<br>range <sup>a</sup> |
|------------------------|------------------------|----------------------------|--------------------------|---------------------|---------------|---------------------------------------|-----------------------------------------------|
|                        | ASD                    | ASD                        | Control                  | ASD                 | Control       | Control                               | Control                                       |
| Sample characteristics |                        |                            |                          |                     |               |                                       |                                               |
| <i>n</i>               | 41                     | 37                         | 9                        | 106                 | 12            | 111                                   | —                                             |
| Gender<br>(M:F)        | 30:11                  | —                          | —                        | 79:27               | 7:5           | —                                     | —                                             |
| Mean age               | 6.0                    | —                          | —                        | 6.4                 | 10.3          | 7.0                                   | —                                             |
| Age range              | 1–16                   | 3–22                       | 3–20                     | 2–15                | 4–16          | 2–15                                  | —                                             |
| Porphyrins             |                        |                            |                          |                     |               |                                       |                                               |
| UP                     | 20.09 (10.36)          | 28.51 (38.37)              | 16.89 (21.60)            | 16.25 (9.63)        | 12.86 (5.11)  | 19.20 (14.44)                         | 8–20                                          |
| 7cxP                   | 5.06 (3.12)            | 3.70 (7.16)                | 2.89 (3.18)              | 2.94 (1.86)         | 2.32 (0.92)   | —                                     | 2.5–4.5                                       |
| 6cxP                   | 1.03 (.97)             | 1.97 (4.56)                | 0.44 (0.53)              | 0.93 (0.79)         | 0.56 (0.28)   | —                                     | 0.5–1.5                                       |
| 5cxP                   | 5.19 (2.33)            | 1.57 (1.04)                | 1.00 (0.50)              | 4.02 (2.46)         | 1.99 (1.23)   | —                                     | 2–4                                           |
| PrCP                   | 20.34 (10.69)          | —                          | —                        | 18.13 (13.07)       | 7.95 (5.41)   | —                                     | 5–9                                           |
| CP                     | 289.61 (175.75)        | 299.70 (1910)              | 162.20 (43.20)           | 185.80 (159.14)     | 71.25 (49.45) | 134.42 (117.54)                       | 100–200                                       |
| Porphyrin ratios       |                        |                            |                          |                     |               |                                       |                                               |
| PrCP/UP                | 1.17 (0.72)            | 1.67 <sup>b</sup> (2.80)   | 0.36 <sup>c</sup> (0.09) | 1.20 (0.72)         | 0.60 (0.38)   | —                                     | 0.2–0.5                                       |
| CP/UP                  | 16.31 (9.37)           | 26.66 <sup>b</sup> (49.19) | 5.56 <sup>c</sup> (1.48) | 12.11 (8.99)        | 5.35 (3.56)   | 5.12 (5.50)                           | 5–9                                           |

*Note.* Data are given as means with SD in parentheses. Porphyrin levels are expressed as nmol/g CRT. Abbreviations: CRT = creatinine; UP = uroporphyrins I and III; 7cxP = heptacarboxy porphyrin; 6cxP = hexacarboxy porphyrin; 5cxP = pentacarboxy porphyrin; PrCP = precoproporphyrin; CP = coproporphyrins I & III.

<sup>a</sup>The laboratory reference range was obtained from Laboratoire Philippe Auguste, Paris, France.

<sup>b</sup>*n* = 11.

<sup>c</sup>*n* = 5.



**FIG. 1.** Coproporphyrin (CP)/uroporphyrin (UP) ratio means. Asterisk denotes significant difference to the Minder and Schneider-Yin (1996) control group at  $\alpha < .05$ ; ns = not significant. Geier and Geier (2007) means not included in statistical comparisons as raw data were unavailable.

ASD and control data, and Minder and Schneider-Yin (1996) control data. Post hoc comparisons indicated that the CP/UP ratio for the two ASD groups (Austin and Shandley, and Nataf et al., 2006) differed significantly from both control groups (Nataf et al., 2006, and Minder & Schneider-Yin, 1996). The two ASD groups did not differ significantly from each other, and likewise, the two control groups did not differ significantly. Figure 1 graphically represents the differences in CP/UP ratio across groups.

## DISCUSSION

The present study provides further support from an independent cohort that porphyrinuria is a concomitant biological occurrence in ASD. Furthermore, this study provides further evidence suggestive of an environmental toxicant variable, consistent with Hg, contributing to the maintenance, and possibly development, of ASD. Given the consistency of the emerging research, health authorities worldwide need to move without delay to further elucidate the specific nature of the toxic insult. Research needs to focus on the form, source, dose, and timing of the toxic exposure and, importantly, appropriate treatment for those already exposed.

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