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Longitudinal changes in PON1 enzymatic activities in Mexican–American mothers and children with different genotypes and haplotypes

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ABSTRACT

The paraoxonase 1 (PON1) enzyme prevents low-density lipoprotein oxidation and also detoxifies the oxon derivatives of certain neurotoxic organophosphate (OP) pesticides. PON1 activity in infants is low compared to adults, rendering them with lower metabolic and antioxidant capacities. We made a longitudinal comparison of the role of genetic variability on control of PON1 phenotypes in Mexican–American mothers and their children at the time of delivery ($n = 388$ and 338, respectively) and again 7 years later ($n = 280$ and 281, respectively) using generalized estimating equations models. At age 7, children's mean PON1 activities were still lower than those of mothers. This difference was larger in children with genotypes associated with low PON1 activities (*PON1*$_{-108TT}$, *PON1*$_{192QQ}$, and *PON1*$_{-909CC}$). In mothers, PON1 activities were elevated at delivery and during pregnancy compared to 7 years later when they were not pregnant ($p<0.001$). In non-pregnant mothers, *PON1* polymorphisms and haplotypes accounted for almost 2-fold more variation of arylesterase (AREase) and chlorpyrifos-oxonase (CPOase) activity than in mothers at delivery. In both mothers and children, the five *PON1* polymorphisms (*192*, *55*, *−108*, *−909*, *−162*) explained a noticeably larger proportion of variance of paraoxonase activity (62–78%) than AREase activity (12.3–26.6%). Genetic control of PON1 enzymatic activity varies in children compared to adults and is also affected by pregnancy status. In addition to known *PON1* polymorphisms, unidentified environmental, genetic, or epigenetic factors may also influence variability of PON1 expression and therefore susceptibility to OPs and oxidative stress.

Introduction

Paraoxonase 1 (PON1) is a high-density-lipoprotein (HDL)-associated enzyme that plays a role in both organophosphate (OP) sensitivity and oxidative stress (Azarsiz et al., 2003). PON1 can metabolize the toxic oxon derivatives of several OP pesticides, which are known to be acutely neurotoxic (Costa et al., 2005a). There is growing evidence that PON1 may play a role in diseases related to oxidative stress including diabetes and heart disease (Li et al., 2003, 2005; Bhattacharyya et al., 2008). *In vitro* and *in vivo* studies have demonstrated that PON1 has antioxidant properties, preventing LDL and HDL oxidation (Aviram and Rosenblat, 2004) and protecting against atherosclerosis (Tward et al., 2002; Rosenblat et al., 2006). Current studies suggest lipophilic lactones are the primary substrate for PON1 (Draganov et al., 2005; Khersonsky and Tawfik, 2005), and it is through this mechanism that PON1 is involved in lipid peroxidation. Although PON1 was named for its esterase activity towards OPs, the endogenous function of this enzyme is more likely its lipolactonase activity (Draganov et al., 2005). In humans, there is a wide variability of PON1 enzymatic activities among adults (Deakin and James, 2004). Individuals with low PON1 activity may be more susceptible to pesticide exposures and oxidative stress since their metabolic capacity and antioxidant defenses are lower compared to those with average or high PON1 activities. Thus, understanding the determinants of PON1 variability, including genetics and age, and how they confer susceptibility to disease or exposures may have broad public health significance.

Several common polymorphisms in the coding and promoter regions of the *PON1* gene influence substrate-specific PON1 enzyme activities (Ferre et al., 2003; Costa et al., 2005b). The single nucleotide polymorphism (SNP) at codon 192 leads to an amino acid substitution from the active-site residue glutamine (Q) to arginine (R) and the catalytic efficiency of the *PON1*$_{192}$ R alloform towards the oxon derivatives of OP pesticides parathion and chlorpyrifos is greater than that of the *PON1*$_{192}$ Q alloform in *in vitro* substrate-specific assays. Animal experiments in transgenic mice expressing human *PON1*$_{192}$ Q and R alloforms have demonstrated that indeed mice expressing the R alloform are more resistant to chlorpyrifos-oxon (CPO) exposure than mice expressing the Q alloform (Cole 2005). For paraoxon, however, the catalytic efficiency even in the faster R alloform is too slow to provide any protection from *in vivo* exposures (Li et al., 2000). Recent studies found that the *PON1*$_{192}$ genotype explains a large portion of the variability of *in vitro* PON1 activity towards paraoxon (POase activity); it accounts for 59% of the variability among Caucasian and

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African–American adults (Bhattacharyya et al., 2008) and 48% of the variability in a Mexican–American population (Rainwater et al., 2009). Several promoter polymorphisms are also known to influence PON1 expression including $PON1_{-108}$, $PON1_{-162}$, and $PON1_{-909}$ (positions are relative to the translation start site). The $PON1_{-108}$ SNP exerts the most noticeable effect on PON1 quantity, as measured by arylesterase activity, accounting for 22.4% of the variability. The $PON1_{-108CC}$ genotype is associated with 2-fold higher PON1 levels compared to the $PON1_{-108TT}$ genotype (Brophy et al., 2001; Deakin et al., 2003). The association of the SNPs at positions −162 and −909 with AREase activity likely is due in part to their strong linkage disequilibrium (LD) with the $PON1_{-108}$ SNP (Brophy et al., 2001; Holland et al., 2006). Similarly, the coding SNP $PON1_{L55M}$ is also associated with AREase activity; however, most of this effect is attributable to LD with $PON1_{-108}$ (Brophy et al., 2001). While several studies have described the important genetic contribution of these PON1 SNPs on phenotypic variation in multiple populations, few have characterized how the relative influence of genetic control may change through different stages of childhood and by pregnancy status. Furthermore, although genetic polymorphisms account for a large portion of PON1 variability, it is not sufficient in epidemiological studies to consider *PON1* genotypes alone (Richter and Furlong, 1999). PON1 phenotypes range broadly even between individuals with the same *PON1* genotypes because enzyme quantity also varies within these groups (Furlong et al., 2006; Holland et al., 2006). Therefore, studies that measure PON1 activities are more informative than studies that rely solely on *PON1* genotype data.

Children are particularly vulnerable to environmental exposures because they practice behaviors that can lead to increased exposure and often have lower metabolic capacities than adults (Landrigan et al., 2004; Neri et al., 2006; Wigle et al., 2007). For example, children's susceptibility to the toxic metabolites of OPs and oxidative stress may be heightened as several studies have demonstrated that PON1 activity is lower in newborns compared to adults (Chen et al., 2003; Holland et al., 2006). Early hypotheses suggested that PON1 developmental expression reaches mature levels at or near age 2 (Cole et al., 2003); however, we recently followed a large cohort of Mexican–American children from birth to age 7 and found that their PON1 activities continue to increase past age 2 until at least age 7 (Huen et al., 2009a). This age-dependent increase of PON1 enzymatic activity was modified by genetic polymorphisms. For example, children with $PON1_{192}$ R alleles and $PON1_{-108}$ C alleles experienced a steeper rise in activity as they got older compared to children with $PON1_{192}$ Q alleles and $PON1_{-108}$ T alleles. These findings suggest that the window of susceptibility to both oxidative stress and OP exposure may be much longer than previously believed and children with certain genotypes may be particularly vulnerable.

Initially, we reported PON1 activity in a subset of 130 mother–child pairs from the Center for Health Assessment in Children and Mothers of Salinas Valley (CHAMACOS) cohort and determined the effects of genotypes and haplotypes on PON1 phenotype and status (Furlong et al., 2006; Holland et al., 2006). In the present study, we performed a longitudinal comparison of the role of genetic control on PON1 enzymatic activities in the entire CHAMACOS birth cohort of mothers and their children at the time of birth and also 7 years later. We also compared PON1 activities between mothers and children at both time points and determined differences in PON1 activities in mothers during pregnancy, at delivery, and 7 years later when they were not pregnant.

Materials and methods

Study subjects. CHAMACOS is a longitudinal birth cohort study of the effects of pesticide and other environmental exposures on neurodevelopment, growth, and respiratory disease in children from primarily Mexican–American families (Eskenazi et al., 2003). The Salinas Valley, which is located in Monterey County, CA, is intensively farmed with approximately 200,000 kg of OPs applied annually (DPR, 2007). Six hundred and one pregnant women were enrolled in 1999–2000, and 531 were followed through the birth of a live infant. Mothers were primarily young ($M = 25.6 \pm 5.3$ years), married, low-income, Mexican-born, and Spanish-speaking. Many were farm workers themselves (44%) and/or lived with farm workers at the time of enrollment (84%). Ethnicity of children and their mothers was based on mothers' self-report. In this analysis, we included only women and children who were of Hispanic origin, the majority of which were Mexican (>90%), to avoid potential confounding by ethnicity. Study protocols were approved by the University of California, Berkeley Committee for Protection of Human Subjects. Written informed consent was obtained from all mothers, and verbal assent was obtained from the children at 7 years of age.

Blood collection and processing. Blood specimens were collected from mothers during pregnancy at the time of their glucose tolerance test (approximately 26-week gestation) and also at the hospital shortly before or after delivery. They were collected from children at the time of delivery (umbilical cord blood) and when the children were approximately 7 years old (mean ± SD: 7.05 ± 0.15 years). Heparinized whole blood was collected in BD vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ), centrifuged, divided into plasma, buffy coats and red blood cells, and stored at −80 °C. Serum and blood clots were collected in vacutainers containing no anticoagulant. DNA was isolated from clots as described previously (Holland et al., 2006).

Determination of PON1 genotypes. DNA isolated from clots was available for genotyping for 431 mothers and 434 children. The coding polymorphisms, $PON1_{192}$ and $PON1_{55}$, and the promoter polymorphism, $PON1_{-162}$, were genotyped using the TaqMan real-time PCR method. Primers for the nucleotide sequence flanking the SNP and probes specific for the SNPs were custom-designed by Applied Biosystems, Inc. (Foster City, CA). The promoter SNPs, $PON1_{-909}$ and $PON1_{-108}$, were genotyped using a fluorogenic allele-specific genotyping assay (Amplifluor). The $PON1_{-108}$ assay required a two-part nested PCR strategy, where the region surrounding the SNP was pre-amplified using non-allelic flanking primers. The amplicon was then diluted and used as the template for the Amplifluor assay. Quality assurance procedures for genotyping all five *PON1* SNPs included assessment of randomly distributed blank samples in each plate and duplicates of randomly selected samples with independently isolated DNA from the same subjects. Repeated analysis (4% of samples) in several runs showed a high degree (>99%) of concordance. All discrepancies were resolved with additional genotyping.

Determination of PON1 enzymatic activities. PON1 enzyme activity was measured in heparinized plasma samples from 275 pregnant mothers (26-week gestation), 388 mothers and 338 newborns at delivery (312 complete mother–child pairs), and 300 mothers and 281 children at age 7 (246 complete mother–child pairs). In this article, the 26-week gestation time point is referred to as pregnancy, measurements made in mothers at the time of delivery are referred to as delivery, and measurements made in umbilical cord blood are referred to as birth or newborns. At the 7-year collection, 21 mothers were pregnant and were excluded from the analysis. PON1 enzyme measurements were obtained from 481 mothers in total; of those, 228 had measurements at two time points, and 127 had measurements at all 3 time points. Enzyme measurements were available from 428 children of whom 191 had measurements at both time points. All samples that were previously assayed for PON1 activity in 130 mother–child pairs (Holland et al., 2006) were completely re-assayed simultaneously with all remaining samples in the CHAMACOS cohort. We found high correlations ($r \sim 0.51$–0.79, $p < 0.0005$) with previous