



Recent progress in the genetics and epigenetics of paraoxonase: why it is relevant to children's environmental health

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Purpose of review

Children are more susceptible to exposures *in utero* and during early childhood that may result in developmental problems and chronic diseases. Novel discoveries in the field of molecular epidemiology that can help explain susceptibility to exposures and disease will be demonstrated using the multifunctional enzyme paraoxonase 1 (PON1) as an example.

Recent findings

The broad PON1 variability in humans, partly due to differences in genetics and age, can confer differential susceptibility because this enzyme can detoxify organophosphate pesticides and has antioxidant properties. Epigenetics plays a significant role in the mediation of the effects of environmental exposure on human health and is hypothesized to be a major contributing factor to the early-life origins of adult disease. Studies highlighted in this review demonstrate the relationship of *PON1* polymorphisms with microRNA binding in addition to a link between DNA methylation in the transcriptional regulatory region with changes in PON1 enzyme levels. Other important methodologies such as ancestry informative markers and lactonase activity can enhance studies involving PON1.

Summary

This PON1 model demonstrates that integrating genetic and epigenetic factors, as well as other novel methodologies, can improve our understanding of important susceptibility factors linked to pediatric disease.

Keywords

differential susceptibility, enzymatic activity, obesity, oxidative stress, single nucleotide polymorphisms

INTRODUCTION

Paraoxonase 1 (PON1) is an enzyme involved in oxidant defense by hydrolyzing oxidized lipids [1] and also plays a key role in the detoxification of some organophosphate pesticides [2]. Thus, individuals with low PON1 levels and activities may be more susceptible to organophosphate exposures and oxidative stress, which occurs when there is an excess of damaging reactive oxygen species. PON1 genetic variants and lower enzyme levels have been linked to adverse health outcomes including oxidative stress-related conditions such as cardiovascular disease and obesity [3–7]. Therefore, it is of considerable clinical interest to characterize the protective role of endogenous antioxidant enzymes against the development of obesity and metabolic syndrome (MetS) in children. Previous reviews of PON1 research have shown that age and genetics are

key factors associated with PON1 variability and, thus, susceptibility. Here, we highlight novel developments in PON1 research including epigenetic mechanisms such as DNA methylation and non-coding RNAs, as well as the effects of genetic admixture, especially important for studies of minority populations, and the relationship of genetic and epigenetic markers with obesity and metabolic disease.

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KEY POINTS

- The broad variability of PON1 levels and activities in humans can confer differential susceptibility because this multifunctional enzyme can detoxify organophosphate pesticides and has antioxidant properties.
- Genetics and age are strong determinants of PON1 variability and, therefore, susceptibility to oxidative stress and organophosphate exposure.
- Epigenetic modifications such as DNA methylation and miRNAs may provide additional insights into the mechanisms of PON variability.
- In addition to commonly used measurements for PON1 status, as well as AREase and POase activity, future studies of PON1 function may be enhanced by incorporation of lactonase activity as a reliable indicator of PON1 phenotype.
- In order to use PON1 as a model to predict risk of obesity and other health outcomes, it should integrate enzyme activities, genetic and epigenetic factors, and take into account genetic ancestry as a potential confounding factor.

PARAOXONASE SUBSTRATE-SPECIFIC ACTIVITIES, SINGLE NUCLEOTIDE POLYMORPHISMS, AND STATUS

Human PON1 enzyme is a 43kDa protein composed of 354 amino acids. It is referred to as a multifunctional enzyme because it can metabolize a number of substrates including some oxon forms of organophosphate pesticides (this is where the name paraoxonase originates from) and aryl esters, among others. Spectrophotometric methods have been established for the most commonly used substrates for the measurement of PON1 molecular phenotype. These include phenyl acetate and paraoxon, which measure arylesterase (AREase) and paraoxonase (POase) activity, respectively. AREase activity is considered an indirect measure of PON1 protein level and has been highly correlated with western blot and ELISA experiments [8,9]. POase activity reflects both quantity and catalytic efficiency of the enzyme.

More recently, several studies have demonstrated that the native substrates for PON1 are likely lactones [10] and researchers are now beginning to incorporate the use of lactonase substrates such as dihydrocoumarin [11¹¹] and 5-thiobutyl butyrolactone (TBBL) in PON1 experiments [11¹¹,12¹²,13]. For instance, Ferre *et al.* [12¹²] found that TBBLase, but neither POase nor PON1 concentrations, determined by ELISA, was significantly associated with obesity status in prepubertal and adolescent

children (age 9–15 years) as well as several obesity markers such as BMI, body fat percentage, and triglyceride levels (Table 1). Obese children with MetS had even lower levels of TBBLase than obese children who did not have MetS [12¹²]. As lactones are the endogenous target of PON1, lactonase activity may be an important and relevant marker of PON1 molecular phenotype particularly for studies related to oxidative stress rather than organophosphate pesticide exposure.

The polymorphic *PON1* gene is a member of the *PON* family cluster of genes including *PON1*, *PON2*, and *PON3*, which all reside adjacent to each other on chromosome 7. *PON1* specifically has been mapped along the chromosome 7q21.3–22.1 region [17,18] and contains nine exons. Over 200 gene variants have been identified in the *PON1* gene; however, only a few promoter single nucleotide polymorphisms (SNPs) significantly influence protein levels as measured by AREase activity [19–23]. In particular, rs705379 (*PON1*₋₁₀₈), is the strongest predictor of AREase activity. Furthermore, the *PON1*_{-108CC} genotype is associated with two-fold higher PON1 enzyme levels compared with the *PON1*_{-108TT} genotype [24,25]. The SNP at the position 192 (rs662) in the coding region impacts the catalytic efficiency of the enzyme in detoxifying organophosphate pesticides with the *PON1*_{192QQ} genotype coding for the enzyme with the lowest efficiency [26]. A number of studies have examined the association of numerous *PON1* SNPs with PON1 molecular phenotype but the majority of SNPs do not explain a substantial amount of additional variation of enzyme quantity or activity in comparison to the commonly studied *PON1*₋₁₀₈ and *PON1*₁₉₂ SNPs [22,27]. One recent study identified a few rare *PON1* variants and some transacting variants located in the *FTO* and *SERPINA12* genes that displayed a modest association with AREase activity independent of common *PON1* SNPs [28]. However, these associations were not as strong as those with previously identified *PON1* SNPs.

Although most studies of PON1 focus on just a small set of the most common *PON1* SNPs, one recent study characterized 16 *PON1* genetic variants and their relationship with several different substrate-specific activities, including lactonase (using dihydrocoumarin substrate), POase, AREase, and diazoxonase (DZOase) activities in prepubertal children (ages 4–16 years) [11¹¹]. The relationships of these SNPs with the different substrate-specific PON1 activities were highly dependent on their location in the gene. For instance, promoter region SNPs were highly associated with AREase, lactonase, and DZOase, whereas 3' untranslated SNPs were more strongly associated with POase and less

Table 1. Summary of studies related to paraoxonase, obesity and metabolic syndrome in children

Authors	Study population	Age (years)	PON1 activities measured	PON1 associations with obesity and MetS	SNPs
Andersen <i>et al.</i> [14]	141 children (88 pesticide exposed and 53 unexposed)	6–11	POase	NS	rs662 (<i>PON1</i> _{192QQ}) (+obesity) (+waist circumference)
Huen <i>et al.</i> [15 ^{***}]	373 children	2 and 5	AREase	2 yrs – AREase (+obesity)	2 yrs –rs662 (<i>PON1</i> _{192QQ}) (+obesity) 5 yrs –rs662 (<i>PON1</i> _{192QQ}) (+waist circumference)
Ferre <i>et al.</i> [12 ^{***}]	110 obese children and adolescents	9–15	POase Lactonase	POase (–obesity) Lactonase (–obesity)(–MetS)	NS
Krzystek-Korpacka <i>et al.</i> [16 [*]]	156 children and adolescents (47 normal weight, 27 overweight and 82 obese).	14 ± 2	AREase	AREase (–waist circumference)	NS
Ruperez <i>et al.</i> [11 ^{***}]	189 normal weight and 179 obese prepubertal children	4–13	POase Lactonase AREase DZOase	NS	rs854566 (–obesity)

AREase, arylesterase; DZOase, diazoxonase; MetS, metabolic syndrome; NS, no significant relationship; POase, paraoxonase SNPs, single nucleotide polymorphisms. Negative or positive association with obesity and other health outcomes is indicated by (–) or (+) symbols.

associated with other substrate-specific activities. Interestingly, Ruperez *et al.* [11^{***}] also identified an intronic SNP, rs854566, which was inversely associated with obesity and also significantly associated with all four substrate-specific activities measured. None of the PON1 activities differed between obese and nonobese children; however, lactonase activity was correlated with markers involved in lipid metabolism like high density lipoprotein and ApoA1 [11^{***}].

Several studies have demonstrated that PON1 status, which includes measures of both *PON1*₁₉₂ genotype, which affects catalytic efficiency toward some substrates, and protein levels (AREase assay), may be a more comprehensive descriptor of PON1 molecular phenotype and a more accurate predictor of disease [17,29,30].

OTHER GENETIC EFFECTS: ADMIXTURE AND ANCESTRY INFORMATIVE MARKERS

In genetic association studies of admixed populations, heterogeneity of genetic background can lead to spurious associations if ancestry is related to both a candidate gene and the disease outcome of interest (Fig. 1a); this is also referred to as genetic confounding due to population stratification [31]. Structured association methods enable us to adjust for potential genetic confounding. This is done by genotyping a number of ancestry informative markers, genetic variants known to vary widely between different ethnic groups, and using the known frequencies among reference populations

to estimate proportion of ancestry in individuals. The estimated parameters can then be included in statistical models as covariates. Figure 1b and c show examples of proportional ancestry distribution estimated by the genotyping of over 100 ancestry informative markers for two different studies of Latino populations, Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS) [15^{***}] (Mexicans) and Genes-environments and Admixture in Latino Americans (GALAI) [32]. Ancestral distributions range quite broadly within populations in both studies and between populations for GALAI.

Both functional *PON1* genetic variants *PON1*₁₉₂ and *PON1*_{–108}, among others, vary substantially among ethnic groups. For instance, the frequency of the Q allele for the *PON1*₁₉₂ SNP is 0.73 for Caucasians [33], 0.37 for African–Americans [33], and 0.48 for Mexicans [21,34]. Furthermore, many of the oxidative stress-related health outcomes ranging from birth weight to obesity and cardiovascular disease differ in prevalence by ethnic groups, making genetic ancestry an important factor to consider in PON1 studies, especially in admixed populations. We recently examined associations of PON1, obesity, and genetic ancestry in young Mexican–American children [15^{***}]. Although a trend of higher African ancestry with higher BMI z-scores and odds of obesity was observed, these relationships did not reach statistical significance after adjusting for multiple testing. We also identified a strong increased odds of obesity in children with the *PON1*_{192QQ} genotype at ages 2 and 5 and found

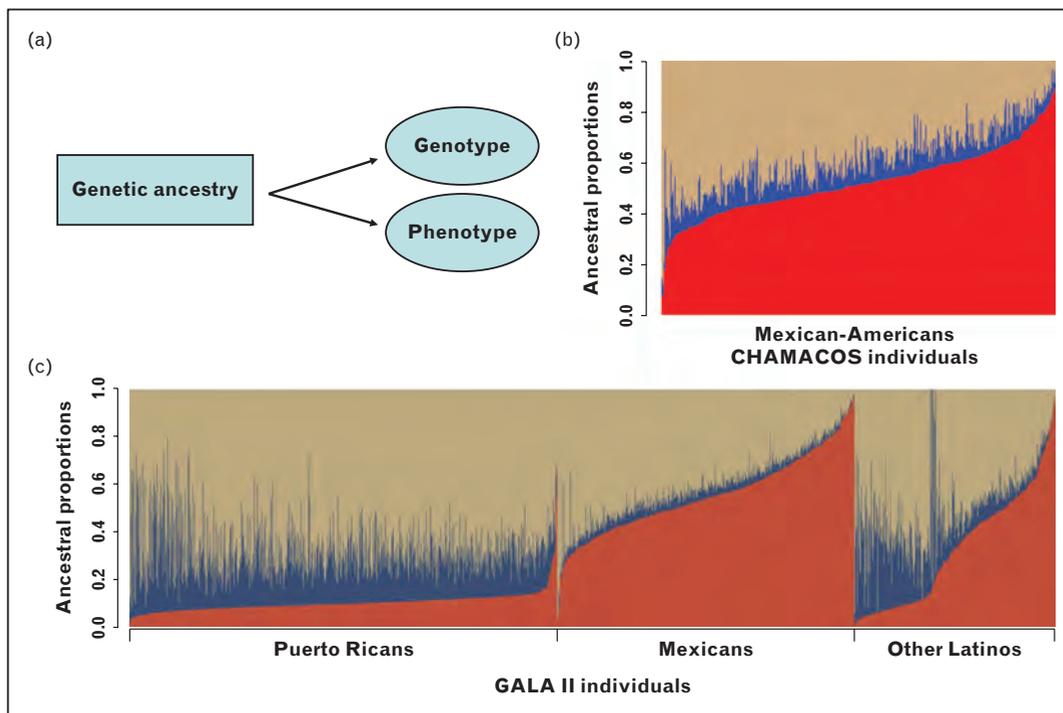


FIGURE 1. (a) shows that if differences in ancestry are associated both with genotype and the outcome of interest, genetic ancestry can act as a source of genetic confounding. Bar plot of genetic ancestry estimates are generated by STRUCTURE software (Stanford University, Stanford, CA). Estimates were expressed as proportion of European, African and Native American ancestry in (b) Mexican–American Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS) children ($n=375$) and (c) GALAII participants of Hispanic origin ($n=6021$). Each vertical bar represents the ancestral distribution in one participant. For each participant, the proportions of Native American (red), African (blue), and European (tan) ancestry are displayed. Figure 1b is reproduced (with minor modifications) with permission from [15[■]] and Figure 1c is reproduced with permission from [32]. PON1, paraoxonase 1; SNPs, single nucleotide polymorphisms.

similar trends in relation to waist circumference at age 5. After controlling for genetic ancestry, this relationship with *PON1*₁₉₂ genotype remained, although beta coefficients changed moderately (9–15%), demonstrating suggestive evidence of genetic confounding by population stratification.

Presence of genetic confounding may explain some of the inconsistencies between reported studies as it can introduce bias, yet to our knowledge, few other studies have attempted to adjust for population stratification in *PON1* genetic association studies [11[■],35].

PARAOXONASE EPIGENETICS

PON1 genetics does not completely explain the broad variability of PON1 molecular phenotype and other factors should be considered. There is a growing recognition that epigenetics may play an important role in key biological processes and mechanisms of disease development [36–38]. Epigenetic mechanisms regulate gene expression without changes in DNA sequence and include DNA methylation, histone modifications, and noncoding

RNAs [39–41]. Here, we focus on DNA methylation and microRNA (miRNA) because, in contrast to chromatin modification assays that are mainly qualitative and require large sample volumes, the state-of-the-art methodologies available for these marks are more amenable to human population studies. Furthermore, a 2011 review of PON1 research describing potential regulators of PON1 expression highlighted epigenetics as an unexplored field [42].

DNA methylation is the most extensively investigated epigenetic mechanism and refers to the potential of a cytosine (C) base to be methylated at its fifth carbon if followed by a guanine (G) base in the DNA code, called a CpG site. The human genome contains about 30 million CpG sites. CpG sites are distributed throughout several regions of the genes referred to as CpG islands, shores, shelves, and gene bodies. CpG islands are stretches of DNA with a high frequency of CpG dinucleotides that often occur in proximity to gene promoter regions [43]. It was previously believed that the majority of functional changes occurred in CpG islands, but new research has shown that DNA methylation changes along CpG shores (regions within 2 kb of

islands) and within the gene body may also have functional effects on gene expression [44,45]. The amount and patterns of DNA methylation are established during the prenatal period and may vary by tissue and cell type [46,47]. Gain or loss of DNA methylation, referred to as hypermethylation and hypomethylation can lead to gene silencing or over-expression, respectively [48].

The *PON1* promoter has one CpG island comprising 19 CpG sites with a second island located near exon 7 (eight CpG sites). There are a total of 287 CpG sites in *PON1* including 66, 48, and 146 CpG sites within shores, shelves, and other regions referred to as the open seas, respectively (Fig. 2). Data on *PON1* methylation are scarce. Only one study has examined associations between *PON1* methylation at several CpG sites with molecular phenotype [49]. De la Iglesia *et al.* [49] reported an inverse association between methylation levels of several promoter region CpG sites and AREase activity in 47 adults with MetS in an energy-restricted dietary weight-loss intervention. This relationship was strongest for CpG sites that were closest in proximity to the *PON1* transcription start site. Additionally, a parallel decline in AREase activity was seen with decreases in several obesity-related parameters such as BMI, fat mass, blood pressure, and triglyceride levels. Another small study of 24 adults also reported an association between methylation at two *PON1* promoter CpG sites with body weight and waist circumference, providing further evidence that *PON1* DNA methylation may influence obesity risk [50].

Another class of epigenetic marks includes miRNAs, which are small (~23 nucleotide) noncoding RNAs that regulate gene expression by pairing with protein-coding mRNAs and directing their posttranscriptional repression. To date, approximately 2500 miRNAs have been identified in humans; however, the majority of their target-

binding sites are not yet known [51]. Putative-binding sites within coding sequences can be identified by sequence complementarity to candidate miRNAs [52]. MiRNAs regulate protein expression by the cleavage of homologous mRNA or by specific inhibition of translation. It is estimated that 10–30% of human protein-coding genes are targets of miRNA binding [53], and aberrant expression of miRNA has been implicated in numerous diseases [54,55]. MiRNAs are an excellent epigenetic biomarker to study for the following reasons: they are ubiquitously expressed in tissues and body fluids including blood, urine, and saliva; as miRNAs are released into the bloodstream from target tissues (i.e., brain, liver) circulating miRNAs may reflect profiles of target tissue [56]; and they are highly stable and resistant to RNase activity as well as effects of pH and temperature in stored specimens over time [55].

In-silico analyses using miRanda software (Memorial Sloan Kettering Cancer Center, New York, NY), and considering conservation and good support vector regression (SVR) scores, have yielded 25 putative miRNA binding sites in the *PON1* 3' untranslated region (Fig. 2). One recent study showed that a *PON1* SNP located in a different miRNA binding site (miR-616) was associated both with changes in *PON1* expression and increased risk of ischemic stroke and carotid atherosclerosis [57]. Unlike the other putative miRNAs that may target *PON1*, this is the only miRNA that has been functionally validated and shown by reporter assay to bind *PON1*. Furthermore, Liu *et al.* [57] were able to show that the *PON1* SNP, rs3735590, affected the binding affinity of miR-616 to *PON1* and resulted in differences in *PON1* expression. These data demonstrate the molecular mechanisms through which the interplay of genetics and epigenetics influence *PON1* expression and demonstrate further the clinical significance of *PON1* variability. Although the complex interactions between DNA methylation, miRNAs, and genetics are not yet

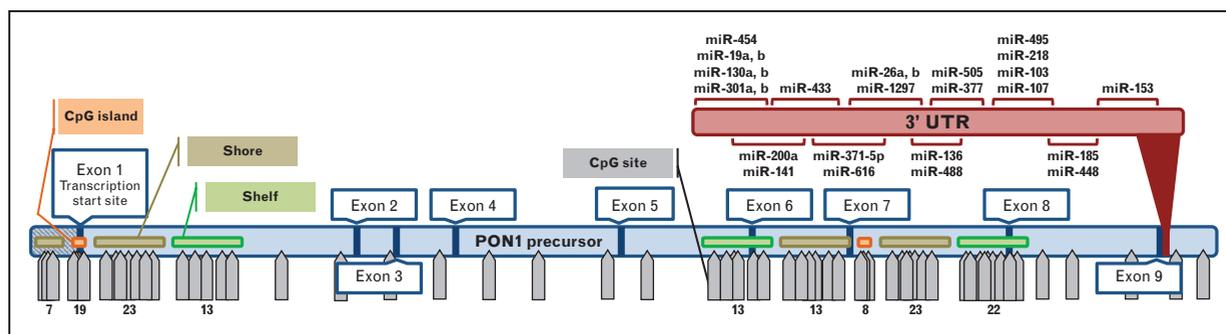


FIGURE 2. Map of *PON1* CpG sites putative miRNA binding sites. *PON1* contains two CpG islands, one in the promoter region and one in exon 7. It has 278 CpG sites spread across the CpG islands, shores, shelves, and open seas. In-silico analyses using the most recent version of miRbase (version 21) has identified 25 putative miRNA binding sites in *PON1*. However, thus far, none of these sites has been functionally validated with *PON1*. miRNA, microRNA; *PON1*, paraoxonase 1.

well understood, recent data suggest that miRNA gene expression can also be regulated by aberrant DNA methylation of miRNA genes [58–61].

PARAOXONASE, OBESITY, AND METABOLIC SYNDROME

Few studies have examined the associations of PON1 substrate-specific activities or genotypes with obesity or MetS in children; however, this field of research has grown substantially over the past 2 years (Table 1). AREase activity, a marker of PON1 enzyme quantity, was inversely associated with obesity status [62] and waist circumference [16[■]] in two studies of children and adolescents. Yet one study of older children in Spain, which measured PON1 concentration by ELISA [12[■]], and our study in 2-year-old Mexican–American children [15[■]] found positive associations of PON1 quantity with obesity. Interestingly, at age 5, we found that AREase activity was inversely associated with obesity status in *PON1*_{192QQ} but not *PON1*_{192RR} children, indicating the associations with obesity can change with age and by genotype [15[■]]. Lactonase (TBBLase) and PON1-specific activity (POase divided by PON1 concentration) were also inversely associated with several obesity parameters (BMI, % body fat) in children [12[■]]. The only other study to examine lactonase activity using dihydrocoumarin did not observe associations of lactonase or other substrate-specific activities with obesity in prepubertal children, but did report a relationship of lactonase activity with lipid profiles [11[■]].

Two studies, including ours, found higher BMI z-scores, waist circumference, and odds of obesity with the *PON1*_{192Q} allele in young children [15[■]] as well as school-age children [14]. However, some studies did not observe the same relationship [11[■], 12[■]]. Furthermore, Ruperez *et al.* [11[■]] identified a different intronic *PON1* SNP that was significantly associated with several substrate-specific PON1 activities (DZOase, AREase, and lactonase) as well as obesity. Ruperez *et al.* [11[■]] and Ferre *et al.* [12[■]] also examined associations with haplotypes, which incorporate combinations of multiple SNPs on a single chromosome [63]. Nevertheless, based on our study [22] and recent publications that also examined *PON1* haplotypes [11[■], 12[■]], there is no apparent advantage in using haplotypes in the analysis of PON1 effects.

PERSPECTIVES

Although findings have been inconsistent, a number of studies have identified potential relationships between *PON1* genetic variants and substrate-

specific activity with obesity and MetS. Several factors may help to explain differences observed between studies. First, most studies included participants with large age ranges (more than 2 years) but did not adjust for age in their analyses with substrate-specific activities even though it has been shown that PON1 levels and activities increase with age through at least age 7 [64,65]. Additionally, it is possible that the relationship of PON1 with obesity may differ by age. Another important difference between studies was the ethnic composition of the populations included, which may be particularly relevant because allele frequencies of many common *PON1* SNPs vary so widely between ethnic groups. For future studies, it will be important to adjust for potential confounding by population stratification in PON1 studies related to obesity, particularly in admixed populations. Finally, it should be pointed out that no studies of children's obesity have looked at the relationship of PON1 epigenetics with obesity despite solid preliminary evidence that DNA methylation and miRNAs may affect PON1 variability. Liu *et al.* [57[■]] demonstrated that the complex interactions between genetics and epigenetics are an important molecular mechanism that affects PON1 expression and important oxidative stress-related health outcomes in adults. Overall, incorporation of novel emerging methodologies and analyses, such as epigenetics, lactonase activity, and control for genetic confounding, into PON1 research may help to further characterize molecular mechanisms affecting PON1 variability and susceptibility to organophosphate exposure and oxidative stress-related health conditions.

CONCLUSION

PON1 serves an important example of a susceptibility factor, whose characterization can help us to understand the cause of multiple diseases. Here we have highlighted several methodologies that may broaden the scope of PON1 research and help to explain some of the molecular mechanisms linking PON1 to disease. More generally, many of these concepts can be applied to other susceptibility markers involved in metabolism, inflammation, or other important biological pathways. Identifying the factors that modulate gene expression, accounting for differences in study populations, and trying to assess the complex interplay of different molecular markers (genetics, epigenetics, enzyme levels, etc.) may increase our understanding of these susceptibility markers. This is essential for elucidation of the cause of complex diseases in children and reliable interpretation of data used for personalized medicine.

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Conflicts of interest

None.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Li HL, Liu DP, Liang CC. Paraoxonase gene polymorphisms, oxidative stress, and diseases. *J Mol Med* 2003; 81:766–779.
2. Costa L, Cole T, Jansen K, Furlong CE. Paraoxonase (PON1) and Organophosphate Toxicity. In: Mackness B, editor. *The paraoxonases: their role in disease development and xenobiotic metabolism*. Springer; 2008. pp. 209–220.
3. Harley KG, Huen K, Schall RA, et al. Association of organophosphate pesticide exposure and paraoxonase with birth outcome in Mexican-American women. *PLoS One* 2011; 6:e23923.
4. Bhattacharyya T, Nicholls SJ, Topol EJ, et al. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA* 2008; 299:1265–1276.
5. Ferretti G, Bacchetti T, Masciangelo S, et al. HDL-paraoxonase and membrane lipid peroxidation: a comparison between healthy and obese subjects. *Obesity (Silver Spring)* 2010; 18:1079–1084.
6. Leduc V, Poirier J. Polymorphisms at the paraoxonase 1 L55M and Q192R loci affect the pathophysiology of Alzheimer's disease: emphasis on the cholinergic system and beta-amyloid levels. *Neurodegener Dis* 2008; 5(3–4):225–227.
7. Ryckman KK, Morken NH, White MJ, et al. Maternal and fetal genetic associations of PTGER3 and PON1 with preterm birth. *PLoS One* 2010; 5:e9040.
8. Connelly PW, Maguire GF, Picardo CM, et al. Development of an immunoblot assay with infrared fluorescence to quantify paraoxonase 1 in serum and plasma. *J Lipid Res* 2008; 49:245–250.
9. Kujiraoka T, Oka T, Ishihara M, et al. A sandwich enzyme-linked immunosorbent assay for human serum paraoxonase concentration. *J Lipid Res* 2000; 41:1358–1363.
10. Draganov DI, Teiber JF, Speelman A, et al. Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. *J Lipid Res* 2005; 46:1239–1247.
11. Ruperez AI, Lopez-Guarnido O, Gil F, et al. Paraoxonase 1 activities and genetic variation in childhood obesity. *Br J Nutr* 2013; 110:1639–1647. This study reported a novel *PON1* SNP (rs854566) that was negatively associated with obesity in prepubertal children.
12. Ferre N, Feliu A, Garcia-Heredia A, et al. Impaired paraoxonase-1 status in obese children. Relationships with insulin resistance and metabolic syndrome. *Clin Biochem* 2013; 46:1830–1836. This study suggested that PON1 may play a role in the metabolic alteration observed in obese children leading to cardiovascular diseases and diabetes later in life.
13. Gugliucci A, Numaguchi M, Caccavello R, Kimura S. Paraoxonase 1 lactonase activity and distribution in the HDL subclasses in the cord blood. *Redox Rep* 2014; 19:124–132.
14. Andersen HR, Wohlfahrt-Veje C, Dalgard C, et al. Paraoxonase 1 polymorphism and prenatal pesticide exposure associated with adverse cardiovascular risk profiles at school age. *PLoS One* 2012; 7:e36830.
15. Huen K, Harley K, Beckman K, et al. Associations of PON1 and genetic ancestry with obesity in early childhood. *PLoS One* 2013; 8:e62565. This study reported models of PON1 adjusted for genetic ancestry and found moderate evidence of genetic confounding by population stratification.
16. Krzystek-Korpacka M, Patryn E, Hotowy K, et al. Paraoxonase (PON)-1 activity in overweight and obese children and adolescents: association with obesity-related inflammation and oxidative stress. *Adv Clin Exp Med* 2013; 22:229–236. This study reported a decrease in PON1 AREase activity associated with central rather than general obesity in overweight and obese children and adolescents.
17. Costa LG, Richter RJ, Li WF, et al. Paraoxonase (PON 1) as a biomarker of susceptibility for organophosphate toxicity. *Biomarkers* 2003; 8:1–12.
18. Primo-Parmo SL, Sorenson RC, Teiber J, et al. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. *Genomics* 1996; 33:498–507.
19. Eskenazi B, Huen K, Marks A, et al. PON1 and neurodevelopment in children from the CHAMACOS study exposed to organophosphate pesticides in utero. *Environ Health Perspect* 2010; 118:1775–1781.
20. Eskenazi B, Kogut K, Huen K, et al. Organophosphate pesticide exposure, PON1, and neurodevelopment in school-age children from the CHAMACOS study. *Environ Res* 2014; 134C:149–157.
21. Holland N, Furlong C, Bastaki M, et al. Paraoxonase polymorphisms, haplotypes, and enzyme activity in Latino mothers and newborns. *Environ Health Perspect* 2006; 114:985–991.
22. Huen K, Barcellos L, Beckman K, et al. Effects of PON polymorphisms and haplotypes on molecular phenotype in Mexican-American mothers and children. *Environ Mol Mutagen* 2011; 52:105–116.
23. Huen K, Harley K, Bradman A, et al. Longitudinal changes in PON1 enzymatic activities in Mexican-American mothers and children with different genotypes and haplotypes. *Toxicol Appl Pharmacol* 2010; 244:181–189.
24. Deakin S, Leviev I, Brulhart-Meynet MC, et al. Paraoxonase-1 promoter haplotypes and serum paraoxonase: a predominant role for polymorphic position -107, implicating the Sp1 transcription factor. *Biochem J* 2003; 372:643–649.
25. Brophy VH, Jampsa RL, Clendenning JB, et al. Effects of 5' regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am J Hum Genet* 2001; 68:1428–1436.
26. Humbert R, Adler DA, Distechi CM, et al. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat Genet* 1993; 3:73–76.
27. Camps J, Marsillach J, Joven J. The paraoxonases: role in human diseases and methodological difficulties in measurement. *Crit Rev Clin Lab Sci* 2009; 46:83–106.
28. Kim DS, Burt AA, Crosslin DR, et al. Novel common and rare genetic determinants of paraoxonase activity: FTO, SERPINA12, and ITGAL. *J Lipid Res* 2013; 54:552–560.
29. Brophy VH, Jarvik GP, Richter RJ, et al. Analysis of paraoxonase (PON1) L55M status requires both genotype and phenotype. *Pharmacogenetics* 2000; 10:453–460.
30. Jarvik GP, Rozek LS, Brophy VH, et al. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1(192) or PON1(55) genotype. *Arterioscler Thromb Vasc Biol* 2000; 20:2441–2447.
31. Ziv E, Burchard EG. Human population structure and genetic association studies. *Pharmacogenomics* 2003; 4:431–441.
32. Drake KA, Torgerson DG, Gignoux CR, et al. A genome-wide association study of bronchodilator response in Latinos implicates rare variants. *J Allergy Clin Immunol* 2014; 133:370–378.
33. Chen J, Kumar M, Chan W, et al. Increased influence of genetic variation on PON1 activity in neonates. *Environ Health Perspect* 2003; 111:1403–1409.
34. Rojas-Garcia AE, Solis-Heredia MJ, Pina-Guzman B, et al. Genetic polymorphisms and activity of PON1 in a Mexican population. *Toxicol Appl Pharmacol* 2005; 205:282–289.
35. Lee YL, Teitelbaum S, Wolff MS, et al. Comparing genetic ancestry and self-reported race/ethnicity in a multiethnic population in New York City. *J Genet* 2010; 89:417–423.
36. Robertson KD. DNA methylation and human disease. *Nat Rev Genet* 2005; 6:597–610.
37. Tammen SA, Friso S, Choi SW. Epigenetics: the link between nature and nurture. *Mol Aspects Med* 2013; 34:753–764.
38. Waterland RA. Is epigenetics an important link between early life events and adult disease? *Horm Res* 2009; 71 (Suppl 1):13–16.
39. Foley DL, Craig JM, Morley R, et al. Prospects for epigenetic epidemiology. *Am J Epidemiol* 2009; 169:389–400.
40. Ho SM, Tang WY. Techniques used in studies of epigenome dysregulation due to aberrant DNA methylation: an emphasis on fetal-based adult diseases. *Reprod Toxicol* 2007; 23:267–282.
41. Pennisi E. Behind the scenes of gene expression. *Science* 2001; 293:1064–1067.
42. Schrader C, Rimbach G. Determinants of paraoxonase 1 status: genes, drugs and nutrition. *Curr Med Chem* 2011; 18:5624–5643.
43. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. *J Mol Biol* 1987; 196:261–282.
44. Ball MP, Li JB, Gao Y, et al. Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nat Biotechnol* 2009; 27:361–368.
45. Irizarry RA, Ladd-Acosta C, Wen B, et al. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat Genet* 2009; 41:178–186.

46. Bibikova M, Chudin E, Wu B, *et al.* Human embryonic stem cells have a unique epigenetic signature. *Genome Res* 2006; 16:1075–1083.
47. Lister R, Pelizzola M, Downen RH, *et al.* Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* 2009; 462:315–322.
48. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev* 2002; 16:6–21.
49. de la Iglesia RMM, Sánchez-Muniz FJ, Zulet MA, Martínez JA. Arylesterase activity is associated with antioxidant intake and paraoxonase-1 (PON1) gene methylation in metabolic syndrome patients following an energy restricted diet. *EXCLI Journal* 2014; 13:416–426.
- This is the first study that reported methylation levels of some CpG sites of the *PON1* gene and their association with AREase activity.
50. Gomez-Uriz AM, Goyenechea E, Campion J, *et al.* Epigenetic patterns of two gene promoters (TNF-alpha and PON) in stroke considering obesity condition and dietary intake. *J Physiol Biochem* 2014; 70:603–614.
51. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res* 2014; 42 (Database issue):D68–D73.
52. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; 136:215–233.
53. Kusenda B, Mraz M, Mayer J, *et al.* MicroRNA biogenesis, functionality and cancer relevance. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2006; 150:205–215.
54. Rowles J, Wong M, Powers R, *et al.* FTO, RNA epigenetics and epilepsy. *Epigenetics* 2012; 7:1094–1097.
55. Tijssen AJ, Pinto YM, Creemers EE. Circulating microRNAs as diagnostic biomarkers for cardiovascular diseases. *Am J Physiol Heart Circ Physiol* 2012; 303:H1085–H1095.
56. Zen K, Zhang CY. Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. *Med Res Rev* 2012; 32:326–348.
57. Liu ME, Liao YC, Lin RT, *et al.* A functional polymorphism of PON1 interferes with microRNA binding to increase the risk of ischemic stroke and carotid atherosclerosis. *Atherosclerosis* 2013; 228:161–167.
- This is the first study that validated a human microRNA that modulates *PON1* gene expression.
58. Lussier YA, Stadler WM, Chen JL. Advantages of genomic complexity: bioinformatics opportunities in microRNA cancer signatures. *J Am Med Inform Assoc* 2012; 19:156–160.
59. Lujambio A, Calin GA, Villanueva A, *et al.* A microRNA DNA methylation signature for human cancer metastasis. *Proc Natl Acad Sci U S A* 2008; 105:13556–13561.
60. Morita S, Takahashi RU, Yamashita R, *et al.* Genome-wide analysis of DNA methylation and expression of microRNAs in breast cancer cells. *Int J Mol Sci* 2012; 13:8259–8272.
61. Suzuki H, Maruyama R, Yamamoto E, *et al.* DNA methylation and microRNA dysregulation in cancer. *Mol Oncol* 2012; 6:567–578.
62. Koncsos P, Seres I, Harangi M, *et al.* Human paraoxonase-1 activity in childhood obesity and its relation to leptin and adiponectin levels. *Pediatr Res* 2010; 67:309–313.
63. Stephens JC, Schneider JA, Tanguay DA, *et al.* Haplotype variation and linkage disequilibrium in 313 human genes. *Science* 2001; 293:489–493.
64. Gonzalez V, Huen K, Venkat S, *et al.* Cholinesterase and paraoxonase (PON1) enzyme activities in Mexican-American mothers and children from an agricultural community. *J Expo Sci Environ Epidemiol* 2012; 22:641–648.
65. Huen K, Harley K, Brooks J, *et al.* Developmental changes in PON1 enzyme activity in young children and effects of PON1 polymorphisms. *Environ Health Perspect* 2009; 117:1632–1638.