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Polymorphisms of pesticide-metabolizing genes in children living in intensive farming communities

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HIGHLIGHTS

- 10 polymorphic gene variants of pesticide-metabolizing enzymes were analyzed.
- Allelic frequency, linkage disequilibrium and haplotype analysis were studied.
- SNPs and CNVs frequencies were broadly consistent with European populations.
- Adverse genotype combinations conferring a greater genetic risk were suggested.
- These combinations can be used in future studies to predict adverse health effects.

ARTICLE INFO

ABSTRACT

Polymorphisms in genes encoding xenobiotic-metabolizing enzymes (XME) are important parameters accounting for the wide inter-individual variability to environmental exposures. Paraoxonase-1 (PON1), butyrylcholinesterase (BChE) and Cytochrome-P450 constitute major classes of XME involved in the detoxification of pesticide chemicals, in particular organophosphates. This study explored the allelic frequency, linkage disequilibrium and haplotype analysis of ten common polymorphic variants of seven key genes involved in organophosphate metabolism (BCHE-K, BCHE-A, PON1 Q192R, PON1 L55M, PON1 −108C/T, CYP2C19 G681A, CYP2D6 G1846A, CYP3AP1 −44G/A, GSTM1*0 and GSTT1*0) in a children population living near an intensive agriculture area in Spain. It was hypothesized that individuals with unfavorable combinations of gene variants will be more susceptible to adverse effects from organophosphate exposure. Genomic DNA from 496 healthy children was isolated and amplified by PCR. Hydrolysis probes were used for the detection of eight specific SNPs and two copy number variants (CNVs) by using TaqMan® Assay-based real-time PCR. Frequencies of SNPs and CNVs in the target genes were in Hardy–Weinberg equilibrium and broadly consistent with European populations. Linkage disequilibrium was found between the three PON1 genetic polymorphisms studied and between BCHE-K and BCHE-A. The adverse genotype combination (unusual BCHE variants, PON1 55MM/−108TT and null genotype for both GSTM1 and GSTT1) potentially conferring a greater genetic risk from exposure to organophosphates was observed in 0.2% of our study population. This information allows broadening our knowledge about differential susceptibility toward environmental toxicants and may be helpful for further research to understand the inter-individual toxicokinetic variability in response to organophosphate pesticides exposure.

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1. Introduction

Genetic variations in genes encoding xenobiotic-metabolizing enzymes (XME) largely account for the variability in the response of individuals to environmental chemicals. These variations have been associated with differences in toxicokinetic of xenobiotics and may modify the bioeffective dose at critical target organs, eventually resulting in toxicity from environmental and occupational chemical exposures. Thus, genetic differences in these enzymes may entail an increased risk of (or susceptibility to) environmentally and occupationally related diseases (Tanaka, 1999).

Long-term low-dose exposure to pesticides has been associated with an increased risk of developing a number of chronic diseases, including cancers at different sites, neurodegenerative disorders, neuropsychological deficits and congenital malformations (González-Alzaga et al., 2014; Dardiotis et al., 2013; Mostafalou and Abdollahi, 2013; Parrón et al., 2011, 2014), as has been observed in our study area. However, epidemiological studies have provided conflicting results owing to the late onset of effects and the possible contribution of other causal factors. One potential effect modifier in these studies is genetic variability of pesticide metabolism genes, which at the same level of exposure may allow individuals to metabolize these compounds at different rates (Howard et al., 2010), rendering a distinct metabolite profile. These genetically-based metabolic differences may determine whether or not clinical symptoms (or even intoxication) appear and also predispose individuals to the aforementioned chronic diseases after long-term pesticide exposure.

Although all the pathways for pesticide detoxification are not fully understood, animal studies have indicated the involvement of three main systems in organophosphates (OPs) metabolism: the cytochrome P450 enzymes (CYP2D6, CYP2C19, CYP3A), glutathione S-transferases (GSTM1, GSTT1), and the esterases paraoxonase-1 (PON1), butyrylcholinesterase (BChE) and carboxylesterases (CEs) (Araoud, 2011; Liu et al., 2006). Thus, inheritance of the unfavorable versions of the different polymorphic genes may render an individual more or less susceptible to the adverse effects of pesticides (Bolognesi, 2003) and hence they are strong candidate susceptibility factors for pesticides and diseases. Interestingly, these XME can also metabolize chemicals other than pesticides, such as pharmaceuticals (CYP450s) and reactive species (PON1, GSTs).

Although the individual allelic distribution of the major genes encoding for XME is well established in various populations, information on the degree of linkage disequilibrium between polymorphic variants (single nucleotide polymorphisms –SNPs– and copy number variants –CNVs–) and haplotype distribution is limited. In this study, a healthy Spanish children population living in intensive agriculture areas where pesticides are largely used was screened for genetic polymorphisms in the genes coding for the most important OPs pesticide-metabolizing enzymes (BCHE, PON1, CYP450 and GSTs). In the study area (Southeastern Spain), the amount of insecticides–acaricides–nematocides used accounts for 66.8% of the total pesticide consumption in such area, with OPs representing about 30% of the total amount (tons) of insecticides–acaricides–nematocides sold (Gómez-Martín et al., 2015). The frequencies of SNPs and CNVs as well as the prevalence of haplotypes and combined polymorphisms were explored in order to identify those individuals most at risk for potential adverse health effects from chronic exposure to OPs pesticides.

2. Materials and methods

2.1. Study population

Participant's recruitment took place in public schools from Almeria, Granada and Huelva, three provinces of Andalusia (South

Spain) with large areas devoted to intensive agriculture under plastic greenhouses where horticultural crops are grown: 26,264 ha in the province of Almería and 2814 ha in Granada coastline. The coast of the province of Huelva is another target area of plastic greenhouses (1213 ha) where strawberries crops are mostly grown. These areas were considered as of high pesticide use in previous studies (Parrón et al., 2011, 2014). Sixteen schools were randomly selected from the 155 public schools existing in the study area (Adra, Almeria, El Ejido, Berja and Vicar in Almeria province; Almonte, Huelva and Moguer in Huelva province; and Carchuna, Granada and Torrenueva in Granada province). The random selection allows participants to be representative of the general population of the study area from which they were drawn, thus supporting the generalizability of the results obtained. Children aged 3–11 years registered in these sixteen schools were invited to take part in the study in December 2009. Spanish-speaking children, with at least one parent also Spanish-speaking, living in towns from the selected study area were considered eligible for the study. A total of 496 children met the inclusion criteria and had informed and signed consent by parents or guardians to participate in the study. Authorizations from Ministries of Health and Education of Andalusia were obtained to get access to these schools. The study protocol was approved by the Biomedical Research Ethics Committee of Granada Province.

2.2. Sample collection and DNA extraction

Buccal mucosa cells collection and DNA extraction procedures were carried out according to the method detailed in Freeman et al. (2003) with some modifications. Cotton swabs on wooden sticks were used in schools to remove cells by scraping the inside of the mouth. These swabs were then placed in Slagboom buffer (100 mM NaCl, 10 mM Tris–HCl pH 8.0, 10 mM EDTA pH 8, 0.5% wt/vol SDS) with Proteinase K (0.2 mg/mL). After three weeks storage at room temperature, DNA was extracted. Proteins were removed using ammonium acetate (2M final concentration), and DNA was precipitated with isopropyl alcohol. The DNA was re-suspended in 500 µl of Tris-EDTA (TE) buffer and stored at −20 °C.

DNA purity and concentration were assessed by UV absorbance at 260 and 280 nm using Infinite® 200 NanoQuant (Tecan, Switzerland), and samples were considered to be sufficiently pure if the ratio of absorbance at 260 and 280 nm was 1.8 ± 0.31 (Santella, 2006). The mean DNA concentration found was 67 ± 91 µg/mL. A total of 10 or 20 ng of dried-down genomic DNA was used per reaction for SNP and CNV assays, respectively.

2.3. Genotyping

Hydrolysis probes were used for the detection of eight specific SNPs and two copy number variants (CNVs) by using TaqMan® Assay-based real-time PCR. Specific TaqMan® Predesigned SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) were used: C___2411904_20 (BCHE D70G, dbSNP: rs1799807), C__27479669_20 (BCHE A539T, rs1803274) and C__11708905_10 (PON1 −108C/T, rs705379). TaqMan® Drug Metabolism Genotyping Assays (Applied Biosystems, Foster City, CA, USA) were also used: C___2259750_20 (PON1 L55M, rs854560), C___2548962_20 (PON1 Q192R, rs662), C__25986767_70 (CYP2C19 G681A, rs4244285), C__27102431_D0 (CYP2D6 G1846A, rs3892097). The protocols specifically recommended by the manufacturer were followed.

Genotyping of *CYP3AP1* −44G/A (rs2177180) polymorphism was performed using Custom Taqman® SNP Genotyping assays (Assay-by-Design) (Applied Biosystems, Warrington, Cheshire, UK), which consist of a mix of unlabeled polymerase chain reaction