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An *OGG1* polymorphism is associated with mitochondrial DNA content in pesticide-exposed fruit growers

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ABSTRACT

Exposure to pesticides has the capacity to cause mitochondrial dysfunction. An increase mitochondrial DNA (mtDNA) content has also been suggested to relate with DNA damaging agent. In mitochondria, the manganese superoxide dismutase (MnSOD) is a scavenger of reactive oxygen species, and the 8-oxoguanine DNA glycosylase (OGG1) is the major DNA glycosylase for the repair of 8-oxoG lesions. However, the alteration of mtDNA content elicited by pesticide exposure in people with genetic variations in *MnSOD* or *OGG1* has not been investigated. In this study, the mitochondrial to nuclear DNA ratio was quantified in the peripheral blood of 120 fruit growers who experienced pesticide exposure and 106 unexposed controls by real-time quantitative polymerase chain reaction (real-time qPCR). Questionnaires were administered to obtain demographic data and occupational history. The *MnSOD* and *OGG1* genotypes were identified by the PCR based restriction fragment length polymorphism analysis. After adjusting for confounding effects, multiple regression model revealed that subjects experiencing high or low pesticide exposure had a greater mtDNA content than that of controls. The *OGG1* Ser-Ser genotype was also associated with an increased mtDNA content. No association between *MnSOD* genotype and mtDNA content was revealed. Thus, subjects experiencing pesticide exposure had greater mtDNA content and the *OGG1* genotype may modulate mtDNA content in pesticide-exposed fruit growers.

1. Introduction

Pesticides have a very important role in agriculture, and their potential health hazards have been the focus of substantial public interest. Defects in mitochondria have been identified in various cancers (Carew and Huang, 2002) and some degenerative disorders (Orth and Schapira, 2001), and environmental exposure to pesticides has the capacity to cause mitochondrial dysfunction (Yamano and Morita, 1995). Mitochondrial DNA (mtDNA) damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. This may be due to a high level of oxygen radicals localized at the mitochondrial inner membrane, the lack of efficient mtDNA repair mechanisms, and/or the absence of DNA-binding proteins such as histones (Yakes and Van Houten, 1997). Importantly, changes in mtDNA content have also been suggested to be a response to endogenous and exogenous oxidative stress in human cells (Lee et al., 1998).

Manganese superoxide dismutase (superoxide dismutase 2, SOD2/MnSOD) is a reactive oxygen species (ROS) scavenger in mitochondria (Robinson, 1998). It is synthesized in the cytosol and posttranscriptionally modified for transport into the mitochondrion (Shimoda-Matsubayashi et al., 1996). Within the mitochondria, MnSOD catalyzes the dismutation of superoxide radicals, thereby producing hydrogen peroxide and oxygen. A decrease in MnSOD levels may lead to an imbalance among production and consumption of ROS and free radicals as well as possibly affect to mtDNA. One single nucleotide polymorphism at position −9 of human *MnSOD* (rs4880) has been identified: GTT (valine) to GCT (alanine) (Rosenblum et al., 1996). This polymorphism can alter the

Abbreviations: 8-oxoG, 8-oxoguanine; BER, base excision repair; Ct, cycle number; GLM, general linear model; MnSOD, manganese superoxide dismutase; mtDNA, mitochondrial DNA; OGG1, 8-oxoG glycosylase; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; ROS, reactive oxygen species.

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secondary structure of the protein from a β-pleated sheet to an α-helical conformation (Shimoda-Matsubayashi et al., 1996), and this might affect its cellular localization along with its transport into the mitochondria, leavening mitochondria with an incomplete defense against superoxide radicals.

8-Oxoguanine (8-oxoG) is one of the primary base lesions formed upon oxidative attack against mtDNA in mammalian cells (Bohr et al., 2002). Because 8-oxoG can mispair with adenine, leading to a G:C → A:T transversion, this lesion is highly mutagenic. 8-oxoG is formed in mammalian cells either as a byproduct of normal metabolism or due to oxidative stress generated by exogenous sources, such as pesticides and their metabolites. The 8-oxoG glycosylase (OGG1) protein is the major DNA glycosylase involved in the repair of 8-oxoG lesions (de Souza-Pinto et al., 2001; Kang et al., 1995). A C/G polymorphism at nucleotide position 1245 in exon 7 of the *hOGG1* gene (rs1052133) results in an amino-acid substitution from serine to cysteine at codon 326 (Kim et al., 2003). The repair activity of OGG1 was found to be greater for 326Ser than 326Cys by a functional complementation activity assay of an *Escherichia coli* mutant defective in 8-oxoG repair (Kohno et al., 1998).

In the present study, mtDNA content in the peripheral blood of pesticide-exposed subjects and unexposed controls was compared; and the association of polymorphisms in the *MnSOD* and *OGG1* genes in pesticide-exposed fruit growers with mtDNA content was investigated.

2. Materials and methods

2.1. Study population

The present investigation was a cross-sectional study performed in Tungshin, which is a town located in central Taiwan. The agricultural population of Tungshin is approximately 25,000 people, based on recent population statistics (Taichung County Government, 2009). Traditionally, local farmer associations provide the farmer insurance, finance support, marketing services, and educational training for their members, which consist of commercial and hobby farmers. Citrus, pears, peaches, grapes, persimmons, carambola, and plums constitute more than 95% of the total crop area consisting of 6000 ha. In these farms, pesticides are regularly applied all year round. Airblast sprayers are predominantly used for the application of pesticides. Fruit growing is typically a family business in Tungshin; therefore, exposure is not only limited to the fruit grower, but also to all family members, such as the farmers' wives and children, who often participate in orchard work. During harvesting, hired seasonal workers may also be exposed to crop pesticide residues.

A total of 120 subjects with regular pesticide exposure and 106 unexposed controls who agreed to participate in the present study underwent detailed questionnaire and health examinations. Hired seasonal workers were not included in the current study. An attempt has also been made to minimize possible biases from ethnicity and lifestyle by selecting control subjects originating from the same geographic area and having the same ethnicity of that of the pesticide-exposed subjects. Control occupations included housewives, teachers, clerks, non-farm laborers, skilled workers, small-business persons, and professionals. Among these individuals, none had received any therapeutic irradiation or were taking any medications. All participants were provided with a written description of the study. Those who were unable to read the description had it read to them. Voluntary written consent was obtained from all participants. The study design was approved by the institutional review board of the Chung Shan Medical University, Taichung, Taiwan.

2.2. Epidemiologic information

Information pertaining to personal characteristics was collected from the study subjects using interviewer-administered questionnaires. The structured questionnaire contained questions that covered demographic characteristics and lifestyle, including habits of cigarette smoking, alcohol consumption, and detailed occupational and medical histories. The subject's smoking history included the number of cigarettes smoked daily and the duration of the subject's smoking habit. A variable termed "pack-years" was coined as an indicator of cumulative smoking dose and was defined as the number of packs of cigarettes smoked daily multiplied by the number of years of active smoking. Most Taiwanese farmers have been alerted to the risk of alcohol induced liver damage and have a good understanding that drinking alcohol makes pesticide poisoning worse (Eddleston et al., 2009). In general, alcohol drinking during the period of pesticide application is an unallowable behavior. A concern in the current study was that if pesticide exposed subjects with this condition were included, their prevalence of alcohol drinking would be lower than that of the controls. If there was a substantial difference in mtDNA content between pesticide exposed and control groups, a false association of altered mtDNA content and decreased alcohol drinking might be presented. Therefore, subjects who drink alcohol were removed from the data analysis.

2.3. Assessment of pesticide exposure

Exposure to pesticides consisted of diluting, mixing, loading, and spraying of pesticides as well as maintaining and cleaning the equipment; these tasks were mostly performed by the orchard owner. Other tasks performed in the orchards were bending of branches, thinning of fruit, and pruning. During harvesting, tasks included sorting and transporting fruit, which often required extra labor. For the study, information on past pesticide use by name, amount, area of pesticide application, numbers of treatments per season, years of agrochemical exposure, and use of personal protection equipment was obtained via interviewer-administered questionnaires. Types of work in the orchards were also obtained. The mean orchard size was 1.28 ha (range, 0.06–4.85 ha). The pesticides used by the fruit growers during the preceding 6 months before the medical examination consisted of almost 40 different compounds (Table 1), including organophosphates, carbamates, pyrethroid, insecticides, fungicides, and growth regulators; whereas the application of organochlorines was negligible. On average, each exposed person had applied pesticide about three times a month, with an average cumulative spraying duration of about 7 h/month (range, 2–28 h/month).

Unfortunately, the doses of pesticide exposure could not be calculated for the study subjects due to the lack of environmental monitoring data. Thus, fruit growers were categorized as having low or high pesticide exposure by a modification of the criteria developed by Scarpato et al. (1996): (a) for each subject spraying pesticides, the number of hectares treated was determined and pesticide exposure was calculated by multiplying the average number of treatments × the number of hectares sprayed; (b) the median value of the distribution obtained in (a) was determined, and fruit growers with exposure values less than or greater than the median were assigned to the low or high exposure class, respectively; and (c) subjects who did not directly handle pesticides (e.g., only involved in cutting or harvesting fruits) were considered to have low exposure.

2.4. Copy number of mtDNA

Venous blood was collected into heparinized tubes from all subjects and was separated into plasma, buffy coat, lymphocytes, and red blood cells. These samples were processed the same day and stored at −70 °C. Real-time quantitative polymerase chain reactions (real-time qPCRs) were used to determine the mtDNA copy number in human lymphocytes according to the method developed by Liu et al. (2003) with some modifications. The *ND1* gene (mtDNA) and *β-actin* gene (nuclear DNA) were quantified by real-time qPCR. To evaluate mtDNA copy number, the mitochondrial to nuclear DNA ratio was calculated by dividing the quantity of the *ND1* gene by the corresponding *β-actin* quantity. Primers and the PCR program for the *ND1* gene amplification were also different between our study and the study of Liu et al. The *ND1* gene (264-bp) was amplified using the following primers: forward 5′-GGA GTA ATC CAG GTC GGT-3′ and reverse 5′-TGG GTA CAA TGA GGA GTA GG-3′. The *β-actin* gene (195-bp) was amplified using the following primers: forward 5′-TGG CAT TGC CGA CAG GAT-3′ and reverse 5′-GCT CAG GAG GAG CAA TGA TCT-3′. A total of 20 ng of lymphocyte DNA was used in the real-time qPCR for the determination of the threshold cycle number (Ct) of the nuclear and mitochondrial genes, respectively. Lymphocytes DNA was added to 10 μM of primers and 12.5 μL of 2× SYBR Green PCR Master Mix kit in a final volume of 25 μL. The 2× SYBR Green PCR Master Mix kit supplied by Applied Biosystems (Foster City, CA, USA) contained 250 units AmpliTaq gold DNA polymerase, 100 units AmpEase UNG, 400 μM of dNTP mix with dUTP, 25 mM $MgCl_2$, and 10× SYBR Green PCR buffer. Real-time qPCR was performed using an ABI Prism 7700 Sequence Detector (Applied Biosystems). The PCR program consisted of a 13 min initial denaturation step at 95 °C followed by 40 cycles of 15 s at 95 °C, 15 s at 60 °C, and 45 s at 72 °C. The raw data were processed using the software accompanying the ABI PRISM 7700 Sequencing Detection System (Applied Biosystems). The Ct values for *β-actin* and *ND1* were concurrently determined in each real-time qPCR run and analyzed using ABI Prism SDS Software v9.1 (Applied Biosystems). The efficiency of qPCR was analyzed and standard regression analyses were performed during each run with different amounts of two standard DNA fragments. One was a 264-bp DNA fragment of *ND1* gene, which was amplified from 1.2×10^{-5} to 1.2×10^{1} ng of lymphocytes DNA. The other was a 195-bp DNA fragment of *β-actin* gene amplified from 1.2×10^{-4} to 1.2×10^{2} ng. The experiments were performed in duplicate for each sample. The Ct values were accepted in each qPCR run when correlation efficient was greater than 0.98 and the efficiency was between 1.95 and 2.00. The mean coefficient of variation was 1.35%.

2.5. Genotyping of polymorphic MnSOD and OGG1 genes

Genotyping was performed on genomic DNA extracted from peripheral blood lymphocytes. The determination of the *MnSOD* Ala-9Val (rs4880) genotypes was performed using methods described by Ambrosone et al. (1999). Briefly, for *MnSOD* gene analysis, restriction fragment length polymorphism (RFLP) was detected by differences in *Ngo*MIV site cleavage following PCR amplification. Primers used for the amplification of the *MnSOD* gene were 5′-ACC AGC AGG CAG CTG GCG CCG G-3′ and 5′-GCG TTG ATG TGA GGT TCC AG-3′. A total of 0.5 μL of DNA was added to a