Mauritius Research Council

Interaction between PON1 GIN192Arg Polymorphism and type 2 Diabetes in Agricultural Workers Exposed to Herbicides.

Final Report

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Mauritius Research Council

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Interaction between *PON1* Gln192Arg polymorphism and type 2 diabetes in agricultural workers exposed to herbicides.

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ABSTRACT:

Background: Type 2 diabetes (T2D) is a metabolic disorder of multifactorial aetiology where age, genetic factors and environment play a role. Human serum paraoxonase (PON1) is an enzyme located on high-density lipoprotein (HDL) that is involved in preventing peroxidation of low-density lipoprotein. PON1 is also involved in the detoxification of organophosphate (OP) pesticides or herbicides

A previous cross-sectional study was undertaken in 2005 in 73 agricultural workers exposed to insecticides to study prevalence of diabetes and check for a relationship between the *PON1 Gln192Arg* polymorphism and T2D (Peermamode, 2005). We found an increased frequency of heterozygotes, and a lower frequency of *PON1 192Gln/Gln* homozygotes among individuals with T2D compared to normoglycaemic individuals, p=0.05.

The present study was carried out to confirm association between the same polymorphism and T2D in agricultural workers exposed to herbicides which are more widely used in the sugar industry in Mauritius.

Methods: After our project was granted clearance by the Ethics Committee of the University of Mauritius, we recruited from February to March 2008, 181 male agricultural workers exposed to herbicides at MDA and FUEL sugar estates. After they provided informed consent, we collected studied their glucose metabolism status and lipid profile. DNA was extracted and PON1*Gln192Arg* genotyping was performed using PCR-RFLP technique. Information was sought on exposure to herbicides, use of protective equipment, medical history, lifestyle and family history of type 2 diabetes. They benefited from a clinical examination, anthropometric and blood pressure measurements. A p-value ≤ 0.05 was considered to be significant.

Results: As in 2005, overall prevalence of T2D (19%) was comparable to men of the same age. Our recent study confirmed association between the *PON1 Gln192Arg* polymorphism and T2D. Diabetic sprayermen exposed to herbicides were more often homozygous for the *Gln192* allele (50%, n=30) than non-diabetic sprayermen (24.4%, n=122), OR=3.12, Cornfield 95% CI: 1.3<OR<7.14, p=0.01. Important risk factors for T2D appeared to be age, homozygosity for the *Gln192* allele and hypertension. Hypertension itself was however 3 times more likely in labourers who were homozygous for the *Arg192* allele.

Discussion and Conclusion: We found an interaction between exposure to pesticides or herbicides and the *PON1 Gln192Arg* polymorphism on T2D, the risk allele appeared to be the *Gln192* allele in exposure to herbicides, and the *Arg192* allele in exposure to insecticides. We also found unexpected pleiotropic effects for the polymorphism on T2D and hypertension in labourers exposed to herbicides. Further studies are warranted to confirm the impact of that polymorphism in exposed populations.

KEYWORDS:

PON1 Gln192Arg polymorphism, type 2 diabetes, hypertension, occupational exposure to herbicides.

I-INTRODUCTION

Mauritius has had for a long time an agricultural economy, relying heavily on sugar production. Even though the economy is more diversified nowadays, the use of pesticides/herbicides in sugarcane plantations, vegetables and fruit plantations is quite common.

Type 2 diabetes (T2D) is a metabolic disorder of multifactorial aetiology where age, genetic factors and environment, which includes toxic pollutants (e.g. herbicides), play a role. In Mauritius T2D has achieved epidemic proportions. According to the latest report on the Non Communicable Disease (NCD) survey carried out in 2004, prevalence of diabetes among adults above 30 years of age in the Mauritian Population was 19.3 % (Ministry of Health & Quality of Life, 2006).

Association between exposure to organophosphates and type 2 diabetes:

Pesticides and herbicides represent a large and important class of environmental chemicals that are often associated with adverse health effects. Among pesticides, insecticides are of most concern with regard to potential adverse effects on the nervous system.

A major class of insecticides and herbicides is represented by the organophosphates (OP), triesters of phosphoric acid that exert their toxicity primarily by inhibiting the enzyme acetylcholinesterase (AChE), (Costa, 2001). Genetic variations in AChE and in biotransformation enzymes that are involved in bioactivation and detoxification processes of OPs may render an individual more or less susceptible to adverse effects of those OPs.

Persistent Organic Pollutants (POPs) such as polychlorinated dibenzo-p-dioxins are used as pesticides and these are known to accumulate in the body through the food chain (Fisher, 1999). Several prospective cohort studies including those mentioned below have shown that individuals occupationally or accidentally exposed to POPs had increased risk of diabetes.

- Beard et al. (2003) compared mortality of 1,999 outdoor staff, working as part of an insecticide application program during 1935–1996, with that of the Australian population. That study reported increased deaths due to diabetes and also a higher prevalence of diabetes among survivors in the occupationally exposed group.
- The Ranch Hand study (Calvert et al., 1999) and the National Institute for Occupational Safety and Health study (Longnecker & Michalek, 2000) of U.S. chemical workers, reported an elevated incidence of diabetes in individuals who had high levels of serum dioxin relative to others examined in that study. Moreover the U.S. Department of Veterans Affairs (DVA) added type 2 diabetes to the list of presumptive diseases associated with exposure to dioxin-containing Agent Orange in Vietnam. Several dioxin exposure studies demonstrated that there is limited/suggestive evidence of an association between exposure to the herbicides used in Vietnam or the contaminant dioxin and T2D (Department of Veterans Affairs, 2001).
- Results of cross-sectional studies investigating association between serum concentrations of POPs and diabetes prevalence in 2,016 adult participants, in the National Health and Nutrition

Examination Survey 1999–2002 (conducted by the Centre for Disease Control and Prevention in the USA), showed striking dose-response relations between serum concentrations of six selected POPs and prevalence of diabetes (Lee et al., 2006).

Moreover Saldana et al. (2007) showed that pregnant women who reported being occupationally exposed to pesticides during the first trimester had a two-fold increased risk of developing gestational diabetes mellitus but those with residential/incidental exposures had no risk.

Influence of paraoxonase (PON1) on detoxification of organophosphate compounds and on prevention of lipid peroxidation.

Paraoxonase (PON1) is a liver and plasma enzyme that hydrolyzes the active metabolites of several organophosphorus (OP) insecticides. Age is an important factor in PON1 activity and susceptibility to OP toxicity. PON synthesis begins before 28 weeks gestation and increases until 12 months of age, by which adult levels are reached. However, under the age of two, enzyme activity is lower than in adults (Ecobichon, 1972). In a study on developmental progression of PON1 and other biotransformation enzymes involved in OP detoxification, foetuses and infants had increased risk of neurotoxicity from OP exposure (Atterberry et al., 1997).

The PON1 mediated hydrolysis of OPs, especially chlorpyrifos and thion, is the most important route for their detoxification (Mutch et al., 1992), and is a major factor determining their toxicity to vertebrates including man (Mackness, 1989). Other organophosphate (OP) substrates of PON1 include chlorpyrifos oxon (CPO) and diazoxon (DZO), the active metabolites of chlorpyrifos and diazinon, respectively, as well as the nerve agents sarin and soman (Sorensen et al, 1999).

PON1 is thus the main means of protection of the nervous system against neurotoxicity of OPs. This was proved by animal studies: IV injection of rabbit PON1 (which has a seven-fold higher plasma activity), increased serum PON1 activity in rats and mice, providing increased protection against toxicity from dermal, oral and IV exposure to chlorpyrifos, with the greatest protection to brain and diaphragm tissues (Costa et al., 1990; Li et al., 1995). Other studies have shown that PON1 knock out mice (PON1 -/-) had increased sensitivity to OPs as compared to wild type mice (PON1 +/+) while hemizygotes had intermediate sensitivity (Shih et al, 1998; Li et al., 2000).

Human serum paraoxonase-1 (PON1) was also described as an enzyme that was almost exclusively associated with high-density lipoprotein (HDL) (Blatter et al., 1993), protecting against peroxidation of LDL cholesterol, thereby preventing the development of atherosclerosis (Mackness et al., 1993).

Influence of PON1 Gln192Arg or Q192R polymorphisms on detoxification of OPs

PON1 gene displays several polymorphisms that influence both its level of expression and its catalytic activity, thereby determining the rates at which a given individual will detoxify a specific insecticide (Costa et al, 2006).

Polymorphisms reported in the PON1 gene, include 2 amino acid polymorphisms, leucine/methionine (L/M) at position 55 and glutamine/arginine (Q/R) at position 192. In addition to these two

polymorphisms in the coding region, five polymorphisms are found in the non-coding region of the gene (Leviev & James, 2000), at positions: – 108 (C/T), –126 (G/C), –162 (A/G), –832 (G/A) and –909 (C/G).

Of these, the polymorphism at position –108 contributes most significantly for variation in PON1 expression (Brophy et al., 2001). The *PON1 Q192R* polymorphism and the variability in its expression are estimated to result in a greater than 60-fold inter-individual difference in rates of chlorpyrifos detoxification in humans (Li et al., 1993), thereby influencing dose response to chlorpyrifos.

The Q/R polymorphism at position 192, significantly affects the catalytic efficiency of PON1 (hydrolysis of paraoxon), which is greatest with purified PON1 from individuals with *PON1 192 RR* genotypes and is least with *PON1 192 QQ* genotypes (Davies et al., 1996). However, the ability of PON1 to protect against lipid peroxidation is greatest in individuals with *PON1 192 QQ* genotypes and least with *PON1 192 RR* genotypes (Aviram et al., 1998; Mackness et al., 1998).

The *PON1 Q192R* polymorphism is substrate-dependent. PON1R192 isozyme was initially known to hydrolyse paraoxon more readily than PON1Q192 (Adkins et al., 1993; Humbert et al., 1993) but the latter isoform was found to hydrolyse diazoxon, sarin, and soman more rapidly than PON1R192 in vitro (Davies et al., 1996). However the PON1R192 isoform has a better affinity for diazoxon (Li et al., 2000) and hydrolyses chlorpyrifos oxon at a slightly higher rate than the PON1Q192 isoform both in vitro and in vivo (Li et al., 2000; Richter & Furlong, 1999).

Interaction between PON1 Gln192Arg polymorphism, type 2 diabetes and exposure to herbicides

Association between PON1 polymorphisms and development of CHD or complications of DM, as well as other disorders have been extensively studied while interaction with exposure to xenobiotics was not much considered previously. Murata et al. (2004) reported that PON1 Gln192Arg polymorphism was not related to development of T2D, but with development of its microangiopathic complications.

However, in Mauritius, a previous cross-sectional study was undertaken in 2005 on 73 agricultural workers who were mostly exposed to pesticides to look at the prevalence of diabetes in that occupational group and check for a possible relationship between the *PON1 Gln192Arg* polymorphism and T2D. That study demonstrated a possible interaction between *PON1 Gln192Arg* polymorphism and T2D in a male population exposed to pesticides. There was an increased frequency of heterozygote individuals and a trend towards a lower proportion of individuals homozygous for the *Arg 192* allele ($p<0.05$) in participants presenting with abnormal glucose metabolism (type 2 diabetes or impaired glucose tolerance), compared to participants with normal glucose metabolism (Peermamode, 2005).

Aims of our study:

The present study was an attempt to replicate our previous findings, this time in workers exposed to herbicides as these are more widely used than pesticides in Mauritius. If an association was confirmed between the *PON1 Gln192Arg* polymorphism and T2D in persons exposed to xenobiotics, this could help to provide further insight into the pathophysiology of that multifactorial disorder, and have an immediate impact on measures to be taken for the prevention of diabetes in our agricultural economy.

POPULATION, MATERIALS AND METHODS

Recruitment of participants in our cross-sectional Study

We tried to replicate findings of a previous study that demonstrated a possible interaction between *PON1 Gln192Arg* polymorphism and type 2 diabetes in a male population exposed to pesticides ((n=73, p=0.045) (Peermamode, 2005). In this cross-sectional replication study, we aimed to study the prevalence of type 2 diabetes and confirm interaction between the *PON1 Gln192Arg* polymorphism and occupational exposure to herbicides. Inclusion criteria in our study was being a sprayerman employed in the sugar industry and using herbicides.

Sample size considerations:

Gorroochurn *et al.* (2007) demonstrated that if the p-value of the initial study (p=0.045) is only slightly less than the nominal alpha (α=0.05), the sample size required for the replication study must be larger to achieve a replication power of 80%. Using the following formula as proposed by Gorroochurn *et al.* (2007):

$$n_2 \approx n_1 \{[z_{crit} + \Phi^{-1}(p_{REP})]/z_1\}^2$$

n_1 = population size of initial study,

n_2 = population size of replication study,

$z_{crit} = \Phi^{-1}(1 - \alpha/2)$,

$z_1 = \Phi^{-1}(1 - p_1/2)$ where p_1 is p-value of initial study,

p_{REP}: replication power,

Φ^{-1}: inverse standard normal distribution function.

We calculated that the sample size for replication in our cross-sectional study should consist of a minimum of 142 participants to achieve a replication power of 80%, at a significance level α= 0.05.

Approval by Ethics committee

We sent our research proposal together with the information sheet and form for informed consent on 17th September 2007 to the Research Ethics Committee of the University of Mauritius (UOMREC) to request approval for ethical clearance. Conditional clearance subject to some comments was obtained on 9th October 2007, and final ethical clearance was granted on 8th January 2008 following a meeting with members of the UOMREC and our written response to their comments.

Several days prior to recruitment, we held group information sessions in the two sugar estates as advised by the Research Ethics Committee of the University of Mauritius, providing information on the aim of our study, its procedures and answering questions from potential participants. We also distributed information sheets to potential participants to give them sufficient time to read and understand before volunteering to attend morning research clinics in a fasting state at dispensaries of Mon Desert Alma or FUEL sugar estates. More than 90% of those who fitted inclusion criteria and attended the group information sessions actually attended appointments for research clinics. We were able to recruit a total of 181 male labourers exposed to herbicides, after they gave written consent.

Phenotyping:

All participants were submitted to the same morning research clinic investigations by a 2 member-team (Miss Annick HEBE, specialised nursing officer from SSR Resource Centre and Dr Meera MANRAJ, Registered Medical Practitioner), in a 12 to 14 hours fasting state, after they gave written informed consent.

Urine and Blood Collection:

Glycosuria was checked on early morning urine samples brought by participants in a special container provided to them for this purpose, using Boehringer Mannheim Uristix strips, and results were recorded as nil, +, ++ and +++.

Blood was collected in a fasting state from veins in the antecubital fossa by a qualified nurse for characterisation of glucose and lipid profile and for DNA extraction. Whole venous blood samples for fasting plasma glucose (FPG) were collected in 2 mL fluoride oxalate tubes for all participants.

An Oral Glucose Tolerance Test (OGTT) was carried out only for those individuals who had no history of diabetes and who did not present with glycosuria in the early morning urine samples. After initial blood sampling in a fasting state, research participants drank 75g anhydrous glucose dissolved in 250 mL of water. Blood was sampled 2 hours after the glucose load to assay 2 hour plasma glucose concentrations.

Fasting venous blood samples were collected in a 5 mL heparin substrate tubes for the following assays: Total cholesterol, HDL cholesterol (LDL cholesterol was calculated), triglycerides, uric acid, urea, creatinine, and liver enzymes (AST, ALT):

A total of 10 to 20 mL of whole blood was collected in tubes containing ethylenediamine tetra-acetic acid (EDTA) substrate for DNA extraction and for both haemoglobin and haematocrit assays. All samples were kept on ice and transported in a cooler box until they were processed in the laboratory.

Anthropometric measurements

Height was measured to the nearest centimetre (cm), with participants standing without shoes, feet close to a wall where a rigid measuring tape was fixed. Weight of subjects was measured in light clothing, without footwear on a calibrated balance scale, and recorded to the nearest kilogram (kg). Body mass index (BMI) was calculated as weight in kg/(height in m)^2 and given in kg/m^2.

Waist and hip girths were measured using a measuring tape to the nearest centimetre (cm) with people lightly clothed. Waist was measured halfway between the umbilicus and xiphoid process and hip was measured as the widest circumference over the great trochanters.

Blood pressure (BP):

BP was measured after 15 minutes of rest using a standard mercury sphygmomanometer (ACCOSON, England) and recorded as the average of 2 measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) from both arms.

Electrocardiogram (ECG)

A standard 12-lead ECG was carried out at rest by Miss Annick Hebe on all participants. Additional recordings were carried out such as re-recording of inferior leads in deep inspiration, or high lateral recordings, or posterior leads recording depending on the findings of the previous standard leads. ECGs were interpreted by Dr Meera Manraj and in case of doubts about the clinical findings or interpretation of the ECG, patients were referred to the occupational physician, Dr Roger Tennant for follow-up.

Clinical data

All participants were submitted to a standard questionnaire where they were interviewed on the following:

Date of birth, ethnicity, level of education, place of work, number of years in present employment

Exposure to herbicides:

- Number of years of exposure to herbicides.
- Number of hours of exposure per week to herbicides.
- Use of protective equipment during working hours assessed as: "regular use" if labourer used equipment seriously, "irregular use" if he removed equipment when feeling hot or when sugarcane plants were tall and "no use" for those who never used the protective equipment provided.
- Additional exposure to herbicides/pesticides after normal working hours.

Smoking habits (classified as "current smoker", "ex-smoker" and "never") and alcohol intake which was recorded as number of units of alcoholic drink consumed per week (1 unit of alcohol being equivalent to 330 ml of beer or a small glass of wine or 1 peg/tot of strong spirits).

- Occasional drinker, <1 unit/day
- Moderate drinker, 1–2 units/day
- Heavy drinker, >2 units per day

Personal or Family History of high blood pressure, diabetes mellitus, or ischaemic heart disease.

Clinical examination:

All participants benefited from clinical examination with emphasis on the cardiovascular and respiratory systems, carried out by Dr Meera Manraj. In case of abnormalities, patients were referred to Dr Roger Tennant, the occupational physician of the company for further investigations. Patient counselling was also performed by Dr Meera Manraj, especially on importance of healthy lifestyle including proper nutritional habits and avoidance of smoking and excessive alcohol consumption.

Biochemical analyses

Samples collected on heparin substrate were centrifuged and plasma was frozen until assays were carried out by Medical Laboratory Technicians of SSR Centre, using the Automated Biochemistry Analyser (COBAS Mira Plus) of Central Laboratory of Victoria Hospital, for total cholesterol, HDL cholesterol, triglycerides, uric acid, urea, creatinine, AST and ALT, were assayed. Glucose was assayed using a glucose oxidase method while total cholesterol, HDL cholesterol, triglycerides and uric

acid were assayed by enzymatic colometric methods. Urea, AST and ALT were assayed using a UV enzymatic kinetic method and creatinine was assayed by an end – point colorimetric method. LDL cholesterol was calculated in mmol/L using the Friedewald formula (Friedewald *et al.*, 1972):

LDL cholesterol in mmol/L=Total cholesterol – HDL cholesterol – Triglyceride/2.2

Haemoglobin was determined by a colorimetric method and haematocrit was read on a haematocrit reader after centrifugation using a haematocrit microcentrifuge at the SSR Resource Centre laboratory.

Genotyping

Blood samples collected on EDTA for DNA extraction were centrifuged and pellets were subjected to red blood cell lysis on the same day. The resulting white blood cell pellets were kept at -20 °C for DNA extraction in batches at a later stage by Medical Laboratory Technicians of SSR Centre.

Genotyping for *PON1 Gln192Arg* polymorphism, using PCR-RFLP (Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism) techniques, was performed blind with regard to the clinical status of study participants, in the SSR Resource Centre Laboratory by Mrs. Solange Lee Kwai Yan, medical laboratory technician.

PCR reactions

PCR reactions were performed with the following primers using a Perkin-Elmer GeneAmp PCR System 9600 (Perkin Elmer Corporation, Norwalk, USA), at an annealing temperature of 60°C, with the following primers (Forward 5' TAT TGT TGC TGT GGG ACC TGA 3' and Reverse 5' CAC GCT AAA CCC AAA TAC ATC TC 3'). PCR reactions were carried out in 20 µl volume with 6 µl Template DNA (10 ng/ µl) and 14 µl of the following mix (1 µl primer mix (both 5'and 3'primers) at 5 µM, 1 µl dNTP at 2.5 mM (Boehringer), 2 µl of Perkin-Elmer Buffer (10 X), 0.2 µl of Taq polymerase (5 U/µl), 2 µl MgCl2 at 12.5 mM, and 8 µl H2O.

Enzymatic cleavage of amplicons:

After PCR reactions we carried out a precipitation procedure to decrease the total volume of unwanted ions (which could potentially interfere with the enzymatic cleavage) before adding the digestion mix. We precipitated 15 µL PCR product with 80 µL cold absolute ethanol, that mixture was left overnight at –20 °C and then centrifuged at 3300 rpm for 1 hour at 4°C before discarding ethanol and allowed to dry before adding a volume of 15 µl of a digestion mix. The precipitated PCR product was resuspended in 15 µL digestion mix composed of 0.5 µl (2 u/µL) of restriction enzyme AlwI, 1.5 µl of Buffer (10X) and 13 µl H2O. The restriction mix was incubated for 4 hours at 37 °C.

Digested PCR products were genotyped via electrophoretic size-specific separation on agarose gel in horizontal electrophoretic tanks (Fischer Scientific) filled with Tris-Acetate-EDTA (TAE) 1X buffer. The 15 µl digested PCR products were loaded with 5 µl Ficoll Blue (loading buffer type II, Sambrook *et al.*, 1989) onto gels which were composed of 4% MetaPhor agarose (FMC Bioproducts, Rockland, USA), TAE 1X and 3 µl ethidium bromide (10 mg/ml, Sigma, St Louis, USA) per 100 ml of TAE 1X. The fragments were visualised on an ultraviolet transilluminator after electrophoresis at 80 V/cm for 60 min, and experiments were documented with Polaroid 667 photos for each gel. Electrophoretic patterns

for each polymorphism are shown in Figure 1: wild-type *Gln192* allele was visualised as 99 bp bands while *Arg192* allele yielded 69 and 30 bp bands.

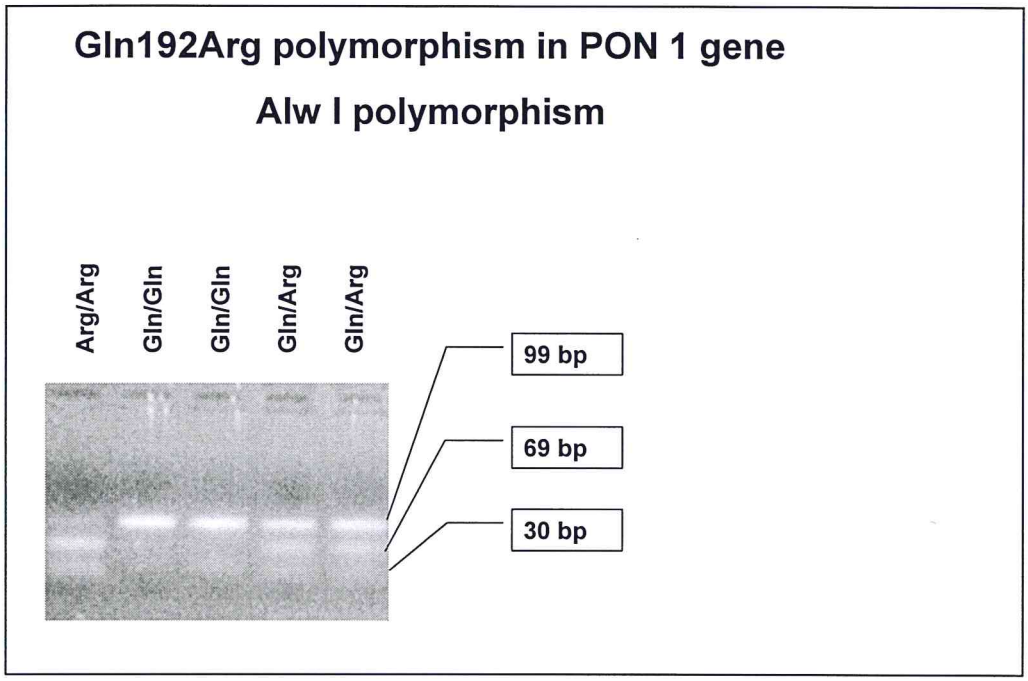

Figure 1: Polaroid photo of agarose gel, after electrophoresis of PON1 PCR products that were digested by restriction enzyme Alw I

Expected alleles for PON1 Gln192Arg or Q192R polymorphism:
Gln192 or Q allele: no restriction site; Arg192 or R allele: one restriction site.
Homozygous Gln/Gln or QQ genotype: Expected size is 99 bp (amplicon).
Homozygous Arg/Arg or RR genotype: 2 fragments are expected, 69 and 30 bp.
Heterozygous Gln/Arg or QR genotype, 3 fragments are expected: 99, 69 and 30 bp.

Phenotype definitions:

Disorders of hyperglycemia:

We used the WHO/IDF (2006) definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia to define type 2 diabetes (T2D), and intermediate disorders of glucose metabolism such as Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT).

T2D was defined as:

Fasting Plasma Glucose $\geq$ 7 mmol/L and/or 2 Hour Plasma Glucose (post glucose load) $\geq$ 11.1 mmol/L.

IFG was defined as:

Fasting Plasma Glucose $\geq$ 6.1 and < 7 mmol/L and 2 Hour Plasma Glucose (post glucose load) < 7.8 mmol/L.

IGT was defined as:

Fasting Plasma Glucose < 7 mmol/L and 2 Hour Plasma Glucose (post glucose load) $\geq$ 7.8 mmol/L and <11.1 mmol/L.

Hypertension:

Hypertension was defined using the Seventh Report of the Joint National Committee (2004) classification:

Hypertension was defined as Systolic BP (SBP) $\geq$ 140 mmHg or Diastolic BP (DBP) $\geq$ 90 mmHg.

Statistical analyses:

Data collected was entered and analysed using Epi Info™ (Version 3.4.1, CDC, Atlanta). For comparison of categorical data between groups, 2 x 2 contingency tables were constructed and Yates corrected Chi-square tests were performed. We used logistic regression models to calculate multivariate-adjusted odd ratios (ORs), and Cornfield 95% Confidence limits for OR for each independent variable.

Quantitative variables with a normal distribution were compared between groups using ANOVA parametric test for inequality of population means. However, when the variances were not homogenous as demonstrated by Bartlett's test for inequality of population variances, or if quantitative variables were not normally distributed, the non-parametric Kruskal-Wallis H test was applied.

We used the gene counting method to estimate allele frequencies for polymorphisms in affected and non-affected subgroups and we verified whether there was a deviation of observed genotype frequencies from those predicted by Hardy-Weinberg Equilibrium (HWE) using the Chi-Square Goodness of Fit.

A p-value $\leq$0.05 was considered to be significant.

RESULTS

We recruited a total of 181 male labourers who were occupationally exposed to herbicides, 86 from Mon Desert Alma estate and 95 from FUEL sugar estate, belonging to different ethnic groups. The majority of participants were of Asian Indian ancestry (115 North-Indian, 42 South-Indian), 22 participants who belonged to the "Creole" group and 2 who were of mixed origin. None of the 24 labourers in the Creole/mixed origin group were diabetic, so we did not include them in analyses comparing genotype proportions between those who were affected by type 2 diabetes and those who were not.

Overall prevalence of type 2 diabetes:

Overall prevalence of type 2 diabetes in the 157 participants of Indian origin was 19%, this was similar to figures expected for a male population of similar age, based on results from the latest NCD Survey carried out in 2004. Prevalence of T2D was 29% in the 42 participants of South-Indian origin, and 16.5% in 115 participants with North-Indian ancestors, this difference was not significant (p-value=0.26).

Prevalence of abnormal glucose metabolism (T2D and intermediate disorders of glucose metabolism such as IFG or IGT) was 38% in participants of South-Indian origin and 36% in those of North-Indian origin. Prevalence of hypertension was 29% in participants of South-Indian origin and 38% in those of North-Indian origin.

We compared some characteristics between groups of participants with or without T2D

Results are presented in Table 1 below.

As can be seen from Table 1, diabetic labourers were older, were more likely to be hypertensive and to have a self-reported family history of diabetes (a parent or a sibling) than their non diabetic counterparts. The duration in number of years of exposure to herbicides seemed to be longer in diabetic labourers (p=0.03).

There was a trend towards higher BMI, larger waist girths and higher triglyceride concentrations in diabetic participants but between group differences were not significant.

Use of protective equipment was similar in diabetic and non-diabetic individuals (p=0.48).

Smoking habits (p=0.15) and alcohol intake (p=0.78) were similar in diabetic and non-diabetic labourers (results no shown).

Allelic and Genotypic proportions between groups:

Gln192 allele frequency was significantly higher in the diabetic participants (70%) compared to non-diabetic participants (52%), $p<0.01$.

All genotype proportions in groups of diabetic and non diabetic labourers were in Hardy-Weinberg Equilibrium.

Table 1: Comparison of some characteristics between diabetic and non diabetic participants

	Diabetic n=30	Non diabetic n=127	p-value
	Mean ± SD or Median [25th-75th percentile] or %		
Age (years)	49.2 ± 5.0	44.8 ± 5.2	0.0001
Triglyceride (mmol/L)	1.92 [1.01- 3.68]	1.54 [1.04- 2.19]	0.13
HDL Cholesterol (mmol/L)	1.32 ± 0.35	1.42 ± 0.41	0.24
Waist (cm)	90.8 ± 12.6	87.1 ± 10.0	0.08
BMI (kg/m2)	26.06 ± 4.98	24.62 ± 4.12	0.10
HBP	70%	27.2%	0.00003
Number of Years of Exposure to herbicides	15.7 ± 9.6	12.1 ± 7.4	0.03
Adequate use of protective equipment	54%	41%	0.48
Family History of Diabetes	77%	32%	0.00002
Gln192 or Q Allele	70%	52%	0.01

Genotype distribution between diabetic and non diabetic individuals in this cross sectional study of labourers occupationally exposed to herbicides are shown in Figure 2, the same distribution is shown in Figure 3 for individuals occupationally exposed to pesticides in the previous 2005 cross-sectional study. Genotype distributions between hypertensive and non hypertensive individuals exposed to herbicides in the present study are shown in Figure 4.

Genotype proportions in groups defined according to diabetic status were different in labourers occupationally exposed to herbicides again. The QQ homozygous genotype was more frequent in diabetic individuals (50%) compared to those without diabetes (24%) while QR and RR genotypes were less frequent in diabetic individuals (50%) compared to those without diabetes (76%), $\chi 2=7.93$, df=2, p=0.02. Participants in our cross-sectional study with homozygous QQ genotype were 3 times more likely to be diabetic than if they had the other two genotypes (QR or RR), OR=3.10, Cornfield 95% Confidence Interval [1.36-7.05], p=0.007. This pattern was different from previous findings in the smaller group of labourers exposed to pesticides, studied in 2005, in whom genotype distributions were different (more heterozygotes and less QQ homozygotes in diabetic individuals).

We also found a significant association between *PON1 Q192R* genotypes and hypertension in our cross-sectional study. Proportion of heterozygotes was lower (36%) in 55 hypertensive individuals than in 100 normotensive participants (61%), proportion of homozygous RR individuals was higher (31%) in hypertensive labourers compared to normotensive individuals (12%), $\chi 2=11.30$, df=2, p=0.0035. This association between the *PON1 Q192R* polymorphism and hypertension persisted when we restricted genotypic association analyses to the 125 non-diabetic labourers, ($\chi 2=12.68$, df=2, p=0.0018). RR individuals were 3 times more likely to be hypertensive than individuals with other genotypes, OR=3.28, Cornfield 95% Confidence Interval for OR: [1.43 – 7.53], p=0.005. However no allelic association with seen with hypertension.

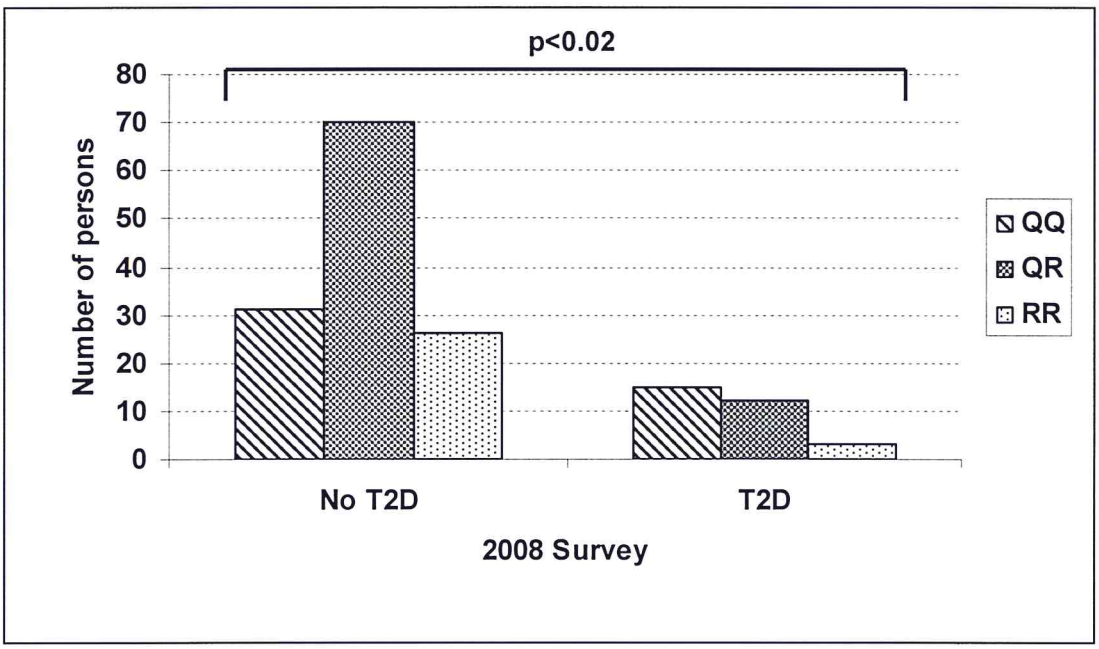

Figure 2: Genotype distributions for the PON1 Q192R polymorphism in diabetic and non diabetic labourers exposed to herbicides.

Results are shown here for 157 labourers working in the sugar industry. Proportion of homozygotes for the Gln192 or Q allele was significantly higher in diabetic individuals exposed to herbicides, while proportions of heterozygotes and homozygotes for the Arg192 or R allele were lower than in non diabetic individuals (p<0.02).

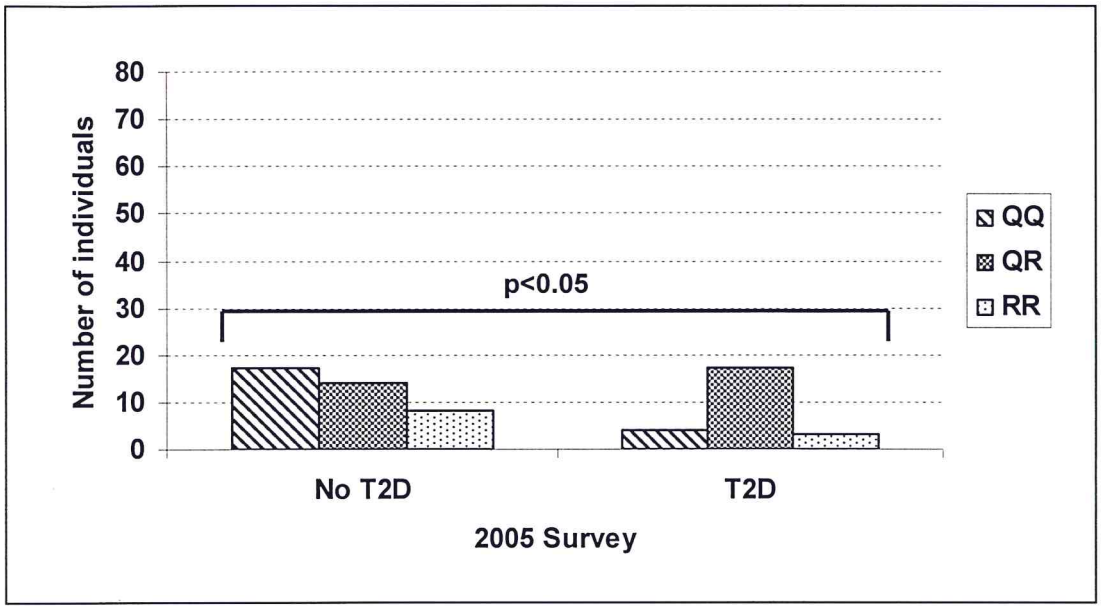

Figure 3: Genotype distributions for the PON1 Q192R polymorphism in diabetic and non diabetic labourers exposed to pesticides (2005 cross-sectional survey in 2005).

We show here results of genotype studies in 73 labourers exposed to pesticides who were followed up at the Occupational Health Unit of the Ministry of Health and Quality of Life. Proportion of heterozygote individuals was higher in diabetic labourers while proportion of homozygotes for the Q allele was lower than in non diabetic labourers exposed to pesticides. These results were of borderline significance.

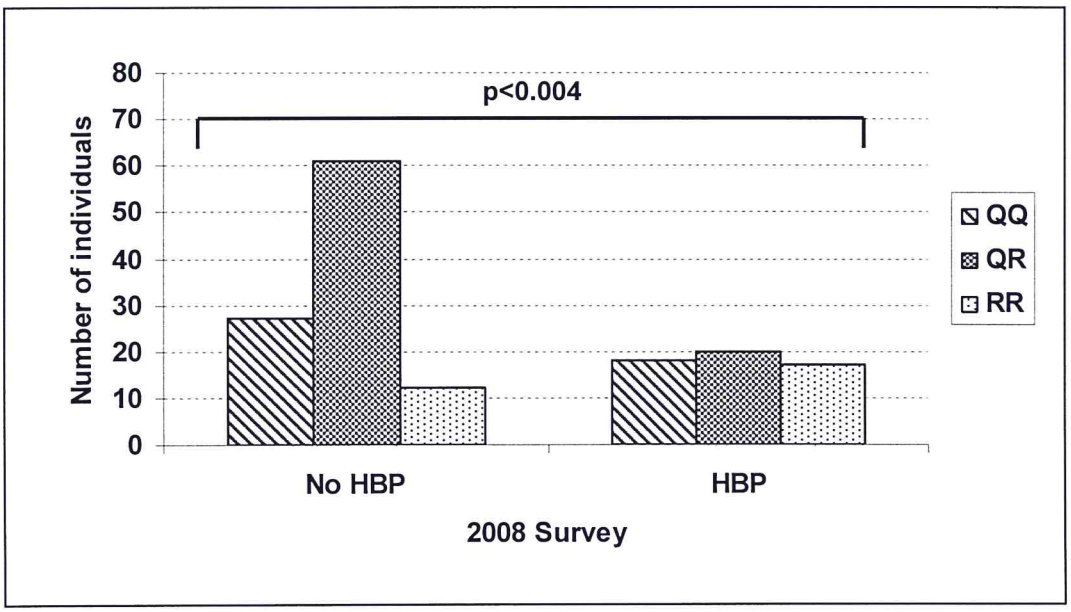

Figure 4: Genotype distributions for the PON1 Q192R polymorphism in hypertensive and non hypertensive labourers exposed to herbicides

We show genotype distributions in groups defined according to the blood pressure status of labourers working in the sugar industry who are exposed to herbicides, irrespective of their diabetic status. Genotype proportions were significantly different with a lower proportion of heterozygotes and higher proportion of homozygotes for the Arg192 or R allele ($p<0.004$) in hypertensive individuals compared to those with normal blood pressure.

We used logistic regression models to calculate multivariate-adjusted odd ratios (ORs), and Cornfield 95% Confidence limits for OR for objectively assessed risk factors to type 2 diabetes in labourers occupationally exposed to herbicides.

Association between duration of exposure to herbicides in years and diabetes was not significant when age was taken into account.

We studied age, hypertension and QQ genotype as multivariate terms in relation to diabetes in our cross-sectional study, and observed the following results described in Table 2:

Table 2: Unconditional Logistic Regression analyses with diabetes as dependent variable:

TERM	Odds Ratio	95% Confidence Interval	p-value
Age	1.14	1.04-1.25	0.006
Hypertension	4.95	1.91-12.82	0.001
QQ Genotype	3.57	1.37-9.25	0.009

Exposed individuals with the QQ genotype (50% in diabetic individuals) were 3.5 times more likely to be diabetic, taking into account their age and hypertensive status.

Given the prevalence of the QQ genotype in our study population (29%) and in the overall Mauritian population (30%) (Manraj, 2002), the population attributable risk % (PAR%) for this genotype would not negligible in a population exposed to organophosphates (Pe):

PAR%= [(Pe) (OR-1)] / [1+(Pe)(OR-1)], where Pe would be the % of population exposed to organophosphate compounds occupationally (or environmentally).

DISCUSSION:

Only male labourers were included in our cross-sectional studies as female labourers are usually not exposed to xenobiotics in the sugar industry or in other agricultural activities in Mauritius. This absence of direct occupational exposure to organophosphates in Mauritian women helps to prevent risks of gestational diabetes in those who are at a child-bearing age (Saldana, 2007) and risks of smaller head circumference in the newborn for women with low serum paraoxonase levels (Berkowitz et al, 2004), with potential neurological defects.

Only about half of the male labourers occupationally exposed to herbicides reported adequate use of protective equipment (overall, gloves, mask + helmet, and boots) provided to them by their employers, those who did not use them adequately complained of being uncomfortable when sugar cane was tall and weather was too warm to wear the overall or helmet. Even those who did not wear regularly the protective equipment provided were aware that exposure to herbicides without protection could be harmful but they were under the delusion that regular daily milk intake would protect them from harmful effects of exposure to herbicides.

Prevalence of diabetes in our cross-sectional study was as expected for a population of same age, confirming our previous findings in 2005. Moreover diabetes was not more frequent in those who had no adequate level of protection. Exposure to pesticides and herbicides in our study individuals does not seem to increase the risk of developing type 2 diabetes, as described in occupationally exposed individuals in Australia (Beard et al, 2003) or in US chemical workers exposed to dioxin (Longnecker & Michalek, 2000). The current findings should be interpreted with caution because of the cross-sectional nature of our studies, and because our sample size was much smaller than the Australian or U.S study where the study design was moreover adequate to draw conclusions on incidence of diabetes.

In the present study, unlike the previous one in 2005, we found both allelic association and genotypic association between the *PON1 Q192R* polymorphism and type 2 diabetes in the 157 labourers exposed to herbicides. The Q allele was associated with increased likelihood of being diabetic ($p=0.01$) and the QQ genotype was associated with increased likelihood of being diabetic in male labourers exposed to herbicides ($p=0.02$), these findings seem more consistent that those in 2005 where sample size was smaller.

These results are however in contradiction with those in 2005, where the RR genotype was more frequent in diabetic labourers exposed to pesticides, another type of xenobiotics. Our present study was thus not truly a replication study, the same polymorphism was studied but occupational exposure was with different organophosphate compounds.

These apparently discordant findings could be explained by the fact that pesticides and herbicides can be hydrolysed differently by different PON1 192 isozymes. In vitro studies have indeed shown differences in catalytic efficiency between the two isozymes depending on the substrate:

- PON1R192 isozyme was initially known to hydrolyse paraoxon more readily than PON1Q192 (Adkins et al., 1993; Humbert et al., 1993)
- But PON1Q192 was found to hydrolyse diazoxon, sarin, and soman more rapidly than PON1R192 in vitro (Davies et al., 1996).
- However the PON1R192 isoform hydrolyses chlorpyrifos oxon at a slightly higher rate than the PON1Q192 isoform both in vitro and in vivo (Li et al., 2000; Richter & Furlong, 1999).

Labourers in our study were exposed to several types of herbicides, which add to the complexity of genotype/substrate interactions, this is further compounded by the fact that metabolism of POPs in mammalian systems is hard to control; the half-life of the compounds ranging from 7 to 10 years in humans (DeVito et al, 1995).

One unexpected finding in our cross-sectional study was the association between the *PON1 Q192R* polymorphism and hypertension. Labourers with RR genotypes were more likely to be hypertensive, and less likely to be diabetic. The effect of the polymorphism on hypertension was independent from that on T2D, as seen in subgroups analyses. The *PON1 Q192R* polymorphism has therefore pleiotropic effects, different alleles carry inverse effects for intermediate phenotypes which confer higher susceptibility to coronary heart disease (CHD). This can cause null overall effects of the polymorphism on CHD.

We had studied the polymorphism and CHD in previous case-control studies (364 male CHD v/s 170 healthy male controls), no association was found with CHD or T2D in men. However we found an association in studies of women of North-Indian ancestry, with a gene-gender interaction on both CHD and T2D (Manraj, 2003). We also found an association with hypertension in the South-Indian subgroup (n=174), where carrying the RR genotype increased odds of being hypertensive, OR=2.71 [1.16<OR<6.41], p=0.02 (Manraj, 2002).

Association between the RR genotype and hypertension was described in an Italian case-control study (Marra et al, 2006) and in participants to the British Women's Heart and Health cohort study (Lawlor et al, 2004). The latter study also included a meta-analysis of studies assessing the association between the *PON1 Q192R* polymorphism and coronary heart disease in men and women, there was no robust evidence that the *PON1 Q192R* polymorphism was associated with CHD risk.

Given the different phenotypes observed with different genotypes in occupationally exposed labourers, it seems that genotype-environment interactions can influence susceptibility to disease. This

mechanism is different from epigenetic modifications (DNA methylation/demethylation and molecular modifications to chromatin), which increasingly are thought to provide a link between the environment and alterations in gene expression which can lead to disease susceptibility (Jirtle & Skinner, 2007; Edwards & Myers, 2007).

Our present study has some limitations, we did not measure PON1 activities or POPs concentrations in study participants, this could have brought more light in the functional aspects of the *PON1 Q192R* polymorphism, and on gene-environment interactions.

A prospective study of the relation between herbicide exposure and diabetes is warranted in bigger occupational cohorts, with serum measurements of POPs and assessment of PON1 activity, together with genotype studies, as both exposure and disease have substantial prevalence, and the public health impact could be important.

CONCLUSION:

Prevalence of type 2 diabetes was not higher in labourers occupationally exposed to herbicides compared to men of the same age in the Mauritian population. However, participants in our cross-sectional study who were homozygous QQ for *PON1 Q192R* polymorphism were 3 times more likely to be diabetic while carriers of the RR genotype for the same polymorphism were 3 times more likely to be hypertensive. Further studies are needed to clarify and confirm these findings.

LIST OF REFERENCES:

- ADKINS, S., GAN, K.N., MODY, M., LA DU, B.N., 1993. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. *Am. J. of Hum. Genet*, 52, 598–608.
- ATTERBERRY, T.T., BURNETT, W.T., CHAMBERS, J.E., 1997. Age-related differences in parathion and chlorpyrifos toxicity in male rats: target and nontarget esterase sensitivity and cytochrome P450 mediated metabolism. *Toxicology and Applied Pharmacology*, 147, 411- 418.
- AVIRAM, M., BILLECKE, S., SORENSON, R., BISGAIER, C., NEWTON, R., ROSENBLAT, M., EROGUL, J., HSU, C., DUNLOP, C., LA DU, B.N., 1998. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective active of human paraoxonase alloenzymes Q and R. *Arterioscler Thromb Vasc Biol*, 10, 1617–1624.
- BEARD, J., SLADDEN, T., MORGAN, G., BERRY, G., BROOKS, L., McMICHAEL, A., 2003. Health Impacts of Pesticide Exposure in a Cohort of Outdoor Workers. *Environmental Health Perspectives*, 111, 724–730.
- BERKOWITZ, G.S., WETMUR, J.F., BIRMAN-DEYCH, E., OBEL, J., LAPINSKY, R.H., GODBOLD, J.H., HOLZMAN, I.R., WOLFF, M.S., 2004. In utero pesticide exposure, maternal paraoxonase activity and head circumference. Environmental Health Perspectives, 112, 388-391.
- BILLECKE, S., DRAGANOV, D., COUNSELL, R., STETSON, P.,WATSON, C., HSU, C., 2000. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate ester. Drug Metab. Dispos.; 28, 1335-1342.
- BLATTER, M.C., JAMES, R.W., MESSMER, S., BARJA, F., POMETTA, D., 1993. Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. Identity of K-45 with paraoxonase. *European Journal of Biochemistry*, 211, 871– 879.
- BROPHY, V.H., JAMPSA, R.L., CLENDENNING, J.B., MCKINSTRY, L.A., FURLONG, C.E., 2001. Effects of 50 regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am. J. of Hum. Genet*, 68, 1428–1436.
- CALVERT, G.M., SWEENEY, M.H., DEDDENS, J., WALL, D.K., 1999. Evaluation of diabetes mellitus, serum glucose, and thyroid function among United States workers exposed to 2,3,7,8 tetrachlorodibenzo-*p*-dioxin. *Occupational and Environmental Medicine*, 56, 270–276.
- COSTA, L.G., MCDONALD, B.E., MURPHY, S.D., OMENN, G.S., RICHTER, R.J., MOTULSKY, A.G., FURLONG, C.E., 1990. Serum paraoxonase and its influence on paraoxon and chlorpyrifos-oxon toxicity in rats. *Toxicology and Applied Pharmacology*, 103, 66-76.
- COSTA, L.G. 2001. Pesticide exposure: Differential risk for neurotoxic outcomes due to enzyme polymorphisms. *Clinics Occup. Environm. Med.*; 1, 511-523.
- COSTA, L.G., COLE, T.B., FURLONG, C.E., 2006. Gene-Environment Interactions: Paraoxonase (PON1) and Sensitivity to Organophosphate Toxicity. *Lab Med.* 2006; 37(2):109-114.
- DAVIES, H.G., RICHTER, R.J., KEIFER, M., BROOMFIELD, C.A., SOWALLA, J., FURLONG, C.E., 1996. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. *Nat. Genet.*, 14, 334–336.
- DEPARTMENT OF VETERANS AFFAIRS, 2001. Herbicide/Dioxin Exposure and Type 2 Diabetes. Washington, D.C.: National Academy Press.
- DEVITO, M.J., BIRNBAUM, L.S., FARLAND, W.H., GASIEWICZ, T.A, 1995. Comparisons of estimated human body burdens of dioxin-like chemicals and TCDD body burdens in experimentally exposed animals. *Environmental Health Perspectives*, 103:820–831
- ECOBICHON, D.J., STEPHENS, D.S., 1972. Perinatal development of human blood esterases. *Clinical Pharmacology and Therapeutics*, 14(1), 41-47.
- EDWARDS, T.M., MYERS, J.P., 2007. Environmental exposures and gene regulation in disease etiology. *Environmental Health Perspectives*, 115, 1261-1270.
- FISHER, B.E., 1999. Most unwanted. *Environmental Health Perspectives*, 107, A18–A23.

- FRIEDEWALD, W.T., LEVY, R.I. & FREDRICKSON, D.S, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical. Chemistry*, 18, 499-502.
- GORROOCHURN, P., HODGE, S.E., HEIMAN, G.A., DURNER, M., GREENBERG, D.A., 2007. Non-replication of association studies: "pseudo-failures" to replicate? *Genetics in Medicine*, 9(6), 325-31.
- HUMBERT, R., ADLER, D.A., DISTECHE, C.M., HASSETT, C., OMIECINSKI, C.J., FURLONG, C.E., 1993. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat. Genet.*, 3(36), 73–76.
- JIRTLE, R.L, SKINNER, M.K. 2007. Environmental epigenomics and disease susceptibility. *Nature Rev. Genet.*, 8, 253-262
- LAWLOR, D. A., DAY, I.N.M., GAUNT, T.R., HINKS, L.J., BRIGGS, P.J., KIESSLING, M., TIMPSON, N., SMITH, G.D., EBRAHIM, S. 2004. The association of the PON1 Q912R polymorphism with coronary heart disease: findings from the British Women's Heart and Health cohort study and a meta-analysis. *BMC Genetics*, 5: 17. Available from http://www.biomedcentral.com/1471-2156-5-17
- LEE, D.H., LEE, I.K., SONG, K., STEFFES, M., TOSCANO, W., BAKER, B.A., JACOBS, D.R., JR., 2006. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes. *Diabetes Care*, 29 (7), 1638–1644.
- LEVIEV, I., JAMES, R.W., 2000. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. *Arterioscler Thromb Vasc Biol*, 20, 516–521.
- LI, W-F., COSTA, L.G., FURLONG, C.E., 1993. Serum paraoxonase status: a major factor in determining resistance to organophosphates. *Journal of Toxicology and Environmental Health,* 40(2-3), 337-346.
- LI, W-F., FURLONG, C.E., COSTA, L.G., 1995. Paraoxonase protects against chlorpyrifos toxicity in mice. *Toxicology Letters*, 76, 219-226.
- LI, W-F., COSTA, L.G., RICHTER, R.J., HAGEN, T., SHIH, D.M., TWARD, A., LUSIS, A.J., FURLONG, C.E., 2000. Catalytic efficiency determines the *in vivo* efficacy of PON1 for detoxifying organophosphates. *Pharmacogenetics,* 10, 767-779.
- LONGNECKER, M.P., MICHALEK, J.E., 2000. Serum dioxin level in relation to diabetes mellitus among Air Force veterans with background levels of exposure. *Epidemiology*, 11(1), 44–48.
- MACKNESS, M.I., 1989. `A'-Esterases - Enzymes looking for a role? *Biochemical Pharmacology*, 38, 385-390.
- MACKNESS, M.I., ARROL, S., ABBOTT, C., DURRINGTON, P.N., 1993. Protection of lowdensity lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis*, 104, 129 –135.
- MACKNESS, B., MACKNESS, M.I., ARROL, S., TURKIE, W., DURRINGTON, P.N., 1998. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *FEBS Letters*, 423, 57– 60.
- MANRAJ, M., 2002. Insulin Resistance: Search for candidate genes with special reference to patients with early myocardial infarction. *PhD thesis*, University of Mauritius.
- MANRAJ, M. 2003. "Gender-related Difference on the Influence of the Paraoxonase *GLN192ARG* Polymorphism on Coronary Heart Disease in the Indo-Mauritian population" *Proceedings of the 11th International GASAT conference*, 216-222
- MARRA, M., MARCHEGIANI, F., ANTONICELLI, R., SIROLLA, C., SPAZZAFUMO, L., OLIVIERI, F., FRANCESCHI, C., TESTA, R., PAOLISSO, G., JAMES, R.W., BOEMI, M., PARATI, G., 2006. The PON1 192RR genotype is associated with a higher prevalence of arterial hypertension. J. Hypertens., 24 (7), 1293-1298
- MINISTRY OF HEALTH & QUALITY OF LIFE, 2006. *MAURITIUS NON-COMMUNICABLE DISEASES SURVEY 2004* [online]. Available from: http://www.gov.mu/portal/goc/moh/file/ncd/yr2004m.pdf [Accessed on 31st August 2007].

- MUTCH, E., BLAIN, P.G., WILLIAMS, F.M., 1992. Interindividual variations in enzymes controlling organophosphate toxicity in man. *Human & Experimental Toxicology*, 11, 109-116.
- PEERMAMODE, J.K., 2005. Study of the interaction between PON1 Gln192Arg polymorphism and exposure to herbicides and pesticides on the prevalence of type 2 diabetes in agricultural workers in Mauritius. *Thesis (BSc). University of Mauritius*, Supervisors: Dr M. Manraj & Dr P. Ramdin.
- MURATA, M., MARUYAMA, T., SUZUKI, Y., SARUTA, T., IKEDA, Y., 2004. Paraoxonase 1 Gln/Arg polymorphism is associated with the risk of microangiopathy in Type 2 diabetes mellitus. *Diabetic Medicine*, 21(8), 837-844.
- RICHTER, R.J., FURLONG, C.E., 1999. Determination of paraoxonase (PON1) status require more then genotyping. *Pharmacogenetics*, 9, 745–753.
- SALDANA, T.M., BASSO, O., HOPPIN, J.A., BAIRD, D.D., KNOTT, C., BLAIR, A., ALAVANJA, M.C.R., SANDLER, D.P., 2007. Pesticide Exposure and Self-Reported Gestational Diabetes Mellitus in the Agricultural Health Study. *Diabetes Care*, 30, 529–534.
- SHIH, D.M., GU, L., XIA, Y.R., NAVAB, M., LI, W-F., HAMA, S., CASTELLANI, L.W., FURLONG, C.E., COSTA, L.G., FOGELMAN, A.M., LUSIS, A.J., 1998. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature*, 394(16), 284-287.
- SORENSON, R.C., BISGAIER, C.L., AVIRAM, M., HSU, C., BILLECKE, S., LA DU, B.N., 1999. Human serum paraoxonase/ arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids: apoprotein A-1 stabilizes activity. *Arterioscler Thromb Vasc Biol.* , 19, 2214-2225.
- WORLD HEALTH ORGANISATION/INTERNATIONAL DIABETES FEDERATION, 2006. *DEFINITION AND DIAGNOSIS OF DIABETES MELLITUS AND INTERMEDIATE HYPERGLYCAEMIA*. Geneva: *World Health Organisation Press*.