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# *PON1 Q192R* polymorphism (rs662) is associated with childhood embryonal tumors

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**Abstract** Genetic susceptibility and environment exposures are associated risk factors in carcinogenesis. Gene polymorphisms that decrease the activity of detoxifying carcinogen substances may modify the effect of exposures. We investigated whether the polymorphisms *PON1* rs662 (Q192R), and *PON1* rs854560 (L55M) would be associated with embryonal tumors in Brazilian children. Blood samples from 163 children with embryonal tumors and 342 as control group were genotyped by TaqMAN real-time PCR assays. Logistic regression was used to evaluate the association between the polymorphisms of cases and controls groups, adjusted by skin color and age strata. When all tumors were taken together, the presence of the *PON1* rs662 (Q192R) variant genotype (RR) was associated with an increased risk of developing embryonal tumors ($OR = 2.80$, 95 % CI 1.12–7.02). The presence of at least one variant *PON1* rs662 R allele increased the risk of developing Wilmś Tumor although without statistical power. However, it was observed a significant association of *PON1* rs662 (Q192R) variant genotype (RR) with retinoblastoma ($OR = 4.08$, 95 % CI 1.13–14.97), whereas the *PON1* rs854560 (L55M) polymorphism was not associated with any tumor. These results indicate that PON1 polymorphisms may have an influence on the risk of developing embryonal tumors.

**Keywords** Embryonal tumors · Genetic susceptibility · *PON1* polymorphisms · Wilms tumor · Neuroblastoma · Retinoblastoma

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## Introduction

The enzyme Paraoxonase 1 (PON1) detoxifies activated some organophosphorus pesticides, for instance, chlorpyrifos oxon, diazoxon and paraoxon. Newborns have substantially lower levels of PON1 than adults, leaving them more vulnerable to organophosphate exposures. [1, 2] The health consequences of inadequate use of pesticide and exposure to organophosphate compounds, and pyrethroids have been demonstrated in Brazil [3]. Recently the maternal pesticide exposure related to agricultural activities demonstrated that organophosphate and other non-specified pesticides were strongly associated with acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML) in early childhood. [4].

*PON1* gene has three polymorphisms considered determinants of PON1 activity levels, so far, Q192R (rs662),

L55M (rs854560) and *PON1* C-108T (rs705379). These polymorphisms cause variation in the enzyme levels or activity resulting in different individual ability to metabolize pesticides and have been investigated in the risk of childhood brain tumors (CBTs) [4]. We have previously demonstrated that the variant allele (M) of *PON1* L55M (rs854560) was associated with an increased risk of developing childhood ALL, whereas the variant allelle (R) of *PON1* Q192R (rs662) was associated with decreased risk. [5] Furthermore, variations on incidence rates of embryonal tumors are observe in different Brazilian settings, particularly incidence rates for neuroblastoma and retinoblastoma, warranting to further studies. [7] Therefore, we have speculated that *PON1* polymorphisms would modulate the risk of developing childhood cancer. In this context, the distribution of *PON1* polymorphisms we investigated and tested whether there is association with embryonal tumors in Brazilian children.

## Methods

### Subjects

Biological samples from 505 Brazilian children were genotyped. There were 163 embryonal tumors, classified as Wilms' Tumor ($n = 72$), sporadic retinoblastoma ($n = 37$), neuroblastoma ($n = 34$), and medulloblastoma ($n = 18$), aged less than 7 years old. Patients were part of a series of cases between 2008 and 2012 from four institutions in the southeast region of Brazil.

The control group consisted of 342 samples from unselected healthy children as donation of the National Cord Blood Bank (http://www.inca.gov.br/). These samples are from the same geographic region (Rio de Janeiro and São Paulo) and collected in the same period of the diagnostic cases. The criteria for race distribution was assessed according to the Instituto Brasileiro de Geografia e Estatística (http://www.ibge.gov.br/home), which relies on skin color self-definition and gathered, into two major groups: white and non-white. The exclusion criteria for both groups of cases and controls were presence of congenital anomalies and samples from another geographic region besides Rio de Janeiro and São Paulo. Demography characteristics are shown in Table 1. Genomic DNA was isolated from peripheral blood or buccal cells with QIAamp DNA Blood Mini Kit (Qiagen®, USA) or OrageneDNA (Genotek, Ontario, Canada) respectively, according to manufacturer's instructions. *PON1* polymorphisms (rs662 and rs854560) were detected by allelic discrimination using TaqMAN® probes (TaqMAN® SNP Genotyping Assays–Applied Biosystems, Foster City, CA) [4].

**Table 1** Demographic characteristics and allele frequencies of PON1 variants in patients with embryonal tumors and control group in Brazil, 2008–2013

| | *N* | Embryonal tumors *n* (%) | Control group *n* (%) | *p* value |
|---|---|---|---|---|
| Total | 505 | 163 (32.3) | 342 (67.7) | |
| Gender | | | | |
| Males | 255 | 89 (54.6) | 166 (48.5) | 0.21 |
| Females | 250 | 74 (45.4) | 176 (51.5) | |
| Skin color[a] | | | | |
| White | 297 | 104 (64.4) | 151 (44.1) | <0.01 |
| Non-white | 154 | 54 (35.6) | 150 (55.9) | |
| Tumor Subtypes | | | | |
| Wilms Tumor | | 74 (44.2) | – | |
| Neuroblastoma | | 34 (20.8) | – | |
| Medulloblastoma | | 18 (11.0) | – | |
| Retinoblastoma | | 37 (24.0) | – | |
| Allele frequencies | | | | |
| *PON1* Q192R | | 0.51 | 0.46 | |
| *PON1* L55M | | 0.28 | 0.24 | |

*n* number of cases

[a] Two cases of embryonal tumors and 42 of control group were not classified

### Ethical aspects

All Brazilian collaborating institutions approved the study, and written informed consent were obtained from the parents.

### Statistical analysis

Allele frequencies were derived by gene counting. The Chi square ($\chi^2$) method was used for comparisons of PON1 alleles and genotype distributions across population subgroups. The 95 % confidence intervals (95 % CI) were determined for pair-wise comparisons of allele frequencies between subgroups. The $\chi^2$ test for goodness-of-fit was used to check whether the distribution of PON1genotypes in the overall population deviated from the Hardy–Weinberg equilibrium. Statistical significant value was defined as $p \leq 0.05$. Unconditional binary logistic regression models estimated the adjusted odds ratios (ORs) and 95 % CIs of the association between polymorphism and tumor risk after adjusting for skin color and age at diagnosis. Data were adjusted by self-reported skin color (categorized as whites and non-whites). All statistical analyses were performed using SPSS Statistic 18 (Chicago, IL) except by the comparison of haplotype frequencies between the patient and control groups that were carried out using GraphPad Prism software. Haplotypes were determined as follows: PON192Q:PON55L haplotype represents all genotypes

**Table 2** *PON1* genotyping frequencies distribution of patients with embryonal tumors and controls and risk estimates according to tumor type, Brazil, 2008–2013

| PON1 Genotype | Control group | Embryonal tumors | | Wilms Tumor | | Neuroblastoma | | Medulobastoma | | Retinoblastoma | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* (%) | *N* (%) | OR[a] (CI95 %) | *N* (%) | OR[a] (CI95 %) | N (%) | OR[a] (CI95 %) | *N* (%) | OR[a] (CI95 %) | *N* (%) | OR[a] (CI95 %) |
| rs662 | | | | | | | | | | | |
| QQ | 104 (30.9) | 36 (22.2) | 1.0[b] | 16 (21.6) | 1.0[b] | 9 (26.5) | 1.0[b] | 4 (23.5) | 1.0[b] | 7 (18.9) | 1.0[b] |
| QR | 160 (47.6) | 85 (52.5) | 1.53 (0.70–3.34) | 42 (56.8) | 2.02 (0.67–6.10) | 18 (52.9) | 1.15 (0.38–3.52) | 7 (41.2) | 0.16 (0.01–2.64) | 18 (48.6) | 2.13 (0.61–7.44) |
| RR | 72 (21.4) | 41 (25.3) | 2.80 (1.12–7.02) | 16 (21,6) | 1.74 (0.39–7.66) | 7 (20.6) | 2.42 (0.58–10.04) | 6 (35.3) | 0.80 (0.06–10.11) | 12 (32.4) | 4.08 (1.13–14.97) |
| QR + RR | 232 (69.0) | 123 (77.8) | 1.88 (0.91–3.92) | 58 (78.4) | 2.07 (0.70–6.21) | 25 (73.5) | 1.37 (0.49–3.83) | 13 (76.5) | 0.44 (0.06–3.41) | 30 (81.0) | 2.56 (0.83–7.90) |
| rs854560 | | | | | | | | | | | |
| LL | 177 (52.7) | 85 (54.5) | 1.0 | 38 (52.0) | 1.0 | 16 (50.0) | 1.0 | 9 (60.0) | 1.0 | 22 (61.2) | 1.0 |
| LM | 134 (39.9) | 56 (36.0) | 1.01 (0.52–1.96) | 30 (41.1) | 1.67 (0.62–4.47) | 10 (31.2) | 0.77 (0.25–2.38) | 4 (26.7) | 1.86 (0.23–14.92) | 12 (33.3) | 0.69 (0.26–1.84) |
| MM | 25 (7.4) | 15 (9.5) | 1.14 (0.37–3.52) | 5 (6.8) | 0.83 (0.13–5.21) | 6 (18.8) | 2.42 (0.62–9.44) | 2 (13.3) | 2.20 (0.13–37.66) | 2 (5.5) | 0.25 (0.30–2.03) |
| LM + MM | 159 (47.3) | 71 (45.5) | 1.03 (0.56–1.92) | 35 (47.9) | 1.44 (0.58–3.57) | 16 (50.0) | 1.15 (0.44–3.01) | 6 (40.0) | 2.13 (0.33–13.61) | 14 (38.8) | 0.60 (0.23–1.52) |

Abbreviations: *n* number of cases, *OR* odds ratio, *CI* confidence intervals

[a] adjusted by skin color and age strata

[b] 1.0, wild-type genotypes used as the reference

combinations containing Q and L alleles (QQ/LL; QR/LL; QQ/LM; QR/LM). The frequency of this haplotype was estimated in patients and control group and the percentages of each group were compared in order to evaluate if there were any statistical difference between them through $\chi^2$ test. The same calculations were done to the other haplotypes (PON192Q:PON55M; PON192R:PON55L; PON192R:PON55M) for all tumor types.

## Results

The *PON1* rs662 (Q192R) and *PON1* rs854560 (L55M) variant allele frequencies in cases and controls groups are shown in Table 1. Genotype frequencies did not significantly differ from Hardy–Weinberg equilibrium in control group. Genotype distribution across both cases and controls groups are shown in Table 2, together with the adjusted ORs. When all tumors were taken together, the presence of the *PON1* rs662 (Q192R) variant genotype was associated with an increased risk of developing embryonal tumors ($OR = 2.80$, 95 % CI 1.12–7.02). When the tumors were stratified, the significant association of *PON1* rs662 (Q192R) variant genotype with retinoblastoma ($OR = 4.08$, 95 % CI 1.13–14.97) was observed. Conversely, the *PON1* rs854560 (L55M) variant allele was not statistically significant associated with embryonal tumors. The presence of at least one variant R allele was two fold increased the risk in Wilmś tumor without statistical significance. In addition to SNP analysis, haplotypes were evaluated for association with embryonal tumors. The difference of haplotype frequencies between cases and controls were not statistical significant (data not shown).

## Discussion

In the present study, the genotype frequencies of the *PON1* rs662 (Q192R) and *PON1* rs854560 (L55M) variants in a series of Brazilian children with embryonal tumors were assessed. To the best of our knowledge, no previous study has evaluated the association of these two *PON1* variants with Wilms tumor, neuroblastoma, and retinoblastoma. These embryonal tumors, that afflict very young children, probably originate from immature tissue during fetal life. Defects in tissue growth pathways would generate tumor genesis during the prenatal/postnatal period and, probably are modulated by the genetic susceptibility. In this sense, these tumors may share some etiological factors and can be analyzed in the same group.

Several environmental factors have been explored with the association with childhood cancers [8–10] .Transplacental exposures can increase risk of malignances that

initiate during fetal development [11, 12]. Infant leukemia with abnormalities that occur during fetal hematopoiesis have been associated with maternal environmental exposure during pregnancy and with genetic susceptibility of xenobiotic system involved mainly in pesticides and medicines compounds [12–14]. Epidemiological observational studies have also demonstrated the risk association of pesticides exposures with Wilms tumor [15, 16], neuroblastoma development [17] and retinoblastoma [18]. Genetic susceptibility to metabolic genes has been demonstrated to confer risk that modulates the mechanistic pathway leading to leukemia in these settings. [6, 14] It is likely that genetic susceptibility play a role in the etiology of these embryonal tumors. Retinoblastoma is associated with germ line mutations in *RB1* gene in about 40 % of cases, while in 5–10 % of Wilms tumor mutations *WT1* germ line mutation are attributable to genetic predisposition [19].

Previous studies performed by Searles Nielsen et al. [8] showed that *PON1* C-108T allele was statistically significant associated with CBTs in children whose mothers reported chemical treatment of the home for pests during pregnancy or childhood ($OR = 2.6$; 95 % CI, 1.2–5.5) [3]. The *PON1* C-108T is a promoter-region SNP associated with enzyme levels [5]. The *PON1* C-108T polymorphism is in strong linkage disequilibrium with *PON1* rs854560 (L55M). [5, 21] In further studies, the same researchers observed strong interaction between genotype and insecticide exposure during childhood., however, no association between CBT and the single coding region SNP *PON1* rs662 (Q192R) [6].

Our data suggests that *PON1* rs662 (Q192R) is associated with an increased risk of retinoblastoma. This result contrast with our previous leukemia study in which the presence of *PON1* Q192R variant allele conferred a decreased risk of childhood leukemia [6]. There is a high interindividual variation of PON1 activity from birth to 7 years of age. PON1 activities increased over time and reached a plateau between 6 months and 7 years of age in most, but not all, individuals. [2] It is also known that Q and R alloenzyme metabolize different substrates at different rates. Common pesticides are usually better metabolized by R alloenzyme [2, 20, 21]. The co-existence of the two *PON1* polymorphisms (rs662 and rs854560) was not a risk factor for embryonal tumor. However, it may be due to the small numbers of subjects with these two polymorphisms together.

Although an accurate estimate of pesticide exposure was not performed in these settings, speculation remains. These exposures may affect the risk, even in retinoblastoma, in which other mutation plays a major role. Brazil has one of the highest incidence rates of retinoblastoma and clearly deserves special attention [7].

The present investigation is part of ongoing research on risk factors that influence the development of childhood neoplasias. This study has some limitations: i) the small numbers of some embryonal tumors, especially medulloblastoma; and ii), the lack of information about pesticides exposure limits our data interpretation, and the no information about the selection bias, such as, smoking, alcohol drinking and pesticide exposure during maternal pregnancies among the case group. In the control group, smoking and alcohol drinking are variables for exclusion criteria to collect the cord blood.

These results encourage further investigations on *PON1* polymorphims and the risk of embryonal tumors especially when exposure to pesticides occurs. These may contribute to a better understanding of embryonal tumors etiology.

**Conflict of interest** All authors disclose that no financial or personal relationships with other individuals or organizations have inappropriately influenced this study.

**Authors' contributions** GMV, BAAG, BDC contributed to study design, data collection, interpretation, and manuscript writing. MSPO has supported the study, discussed data interpretation, and reviewed the manuscript. RMV and BAAG contributed equally to all laboratory work. LCST performed statistical analysis. The Brazilian Embryonal Study group members provided clinical and biological data collection. All authors read and approved the manuscript. BDC and MSPO have scholarships grants from CNPq # 311511/2009-0 and # 309091/2007-1, respectively.

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