A Functional Variant rs1820453 in YAP1 and Breast Cancer Risk in Chinese Population

Wei Chen¹*, Wei Wang²,³, Beibei Zhu¹, Hui Guo², Yu Sun¹, Jie Ming², Na Shen¹, Zhi Li², Zhenling Wang¹, Lifeng Liu¹, Bingxi Cai¹, Jiayu Duan¹, Jiaoyuan Li¹, Cheng Liu¹, Rong Zhong¹, Weiguo Hu¹, Tao Huang²*, Xiaoping Miao¹*

¹ State Key Laboratory of Environment Health (Incubation), Ministry of Education Key Laboratory of Environment & Health, Ministry of Environmental Protection Key Laboratory of Environment and Health (Wuhan), and Department of Epidemiology and Biostatistics, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, 2 Department of Breast and Thyroid Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, 3 Department of Breast Surgery, Hubei Cancer Hospital, Wuhan, China, 4 Department of Oncology, Renmin Hospital of Wuhan University, Wuhan, China

Abstract

**Background:** To investigate the association between rs1820453 which located in the promoter region of yes-associated protein 1 (YAP1) gene and breast cancer (BC) risk.

**Method and Findings:** We conducted a hospital-based case-control study including a total of 480 BC cases and 545 cancer-free controls in Chinese population. Then the expression quantitative trait locus (e-QTL) analysis was performed to explore the possible function of rs1820453 to the YAP1 gene expression. The association between rs1820453 and BC risk was significantly identified with the odds ratio (OR) was 1.27 (95% confidence interval (CI) =1.03-1.57) under allelic model when adjusted by age and menopausal status. In addition, the correlation analysis of rs1820453 and YAP1 expression level found that this variant was significantly associated with the gene expression in Chinese population. When compared with level of mRNA expression of the AA genotype (6.011±0.046), the mRNA expression level in CC genotype (5.903±0.026) was statistically lower (P=0.024).

**Conclusion:** The results from this study suggested that rs1820453 A>C change may affect the gene expression and contribute to the risk of developing BC in Chinese population though larger sample-size studies along with functional experiments were anticipated to warrant the results.

Introduction

Breast Cancer (BC) as the most commonly diagnosed cancer among female in the world has been one of the most important global public health problem with much attention has been paid on it [1]. Much effort has been executed to explore the association between BC risk and genetic variants as genetic factor was considered to be a important risk factor for BC susceptibility [2-4]. Though, population-based studies have estimated that high penetrance mutations of BRCA1 and BRCA2 account for about 15% of the familial aggregation of BC [5]. However, genetic variants that contribute to the susceptibility of developing sporadic BC are still unclear [6].

Yes-associated protein 1 gene (YAP1) located at 11q22, a site of frequent loss of heterozygosity in sporadic BC [7,8] have been indicated that may act as a tumor suppressor [9]. The protein YAP1 gene encoded was a transcriptional coactivator which was important in P73-dependent apoptosis [10,11]. It has been reported that YAP1 interact with p53-binding protein-2 (ASPP2) which was an important regulator of the apoptotic activity of p53 [12,13].

Several studies have shown that YAP1 may bind to the p53 family member p73 and was critical for the DNA damage induced in BC cells and some other types of neuronal apoptosis [11,14,15]. YAP1 loss by siRNA may protect BC cells from DNA damage-mediated apoptosis which then may promote the developing of tumor. Furthermore, YAP1 has also
been reported to stabilize the P73 protein of the post-
translation stage [16,17].

It was demonstrated that YAP1 protein expression was
decreased or lost in breast cancers [9]. In addition, Yuan M et
al, have found that BC cells with YAP1 silencing show
increased migration and invasion which then enhanced tumor
growth.

Intriguingly, a recent research identified a YAP1 variant,
rs1820453, was associated with survival of small-cell lung
cancer patients and the variant A>C change created a
transcriptional factor binding site which then resulted in the
down regulation of YAP1 expression [18]. Since YAP1 protein
expression was frequently found to be decreased in breast
cancers and rs1820453 A>C may down-regulate the YAP1
expression; we have interesting to know whether this variant is
associated with BC risk. Therefore, we carried out a hospital-
based case-control study to investigate the association
between rs1820453 and BC risk.

Materials and Methods

Ethics Statement

After written informed consent was obtained, 2 ml peripheral
venous blood sample and the characteristic data were
collected from each participant at the recruitment. The age and
menopausal status of each subjects and the ER and PR status
of each patients were collected. However, due to the poor
memory of some participants, the characteristics of age of
menarche and family history, which were originally expected to
be collected, were not completed finally. In addition, the blood
samples were stored in the -80°C refrigerator before the DNA
was extracted and the samples were transformed by the cold
chain. This study was approved by the ethics committee of
Union Hospital of Huazhong University of Science and
Technology and Tongji Medical College, Huazhong University
of Science and Technology.

Study subjects

First, 506 cases and 576 cancer-free controls were asked to
participate in the study. But 18 cases and 21 controls were
excluded because of their declining to the research. Therefore,
488 cases and 555 controls were included for genotyping. All
subjects were genetically unrelated Chinese Han women living
in Wuhan city and surrounding regions. Patients were
consecutively recruited between June 2009 and December
2011 at the Union Hospital of Huazhong University of Science
and Technology, Wuhan, China. All cases were
histopathologically confirmed without any previous radiotherapy
and chemotherapy before they were included in this study; and
there is no restriction about age and type of BC or disease
stage. Controls were cancer-free individuals randomly selected
among the health check-up persons at the same hospital in the
same time period as cases were enrolled. In addition, the
controls were frequency matched to the cases for age (± 5
years).

Genotyping

Genomic DNA was extracted from the whole blood sample of
all participants using the RelaxGene Blood System DP319-02
(Tiangen, Beijing, China) according to the manufacturer’s
directions. The quantity and quality of DNA was assessed by
the NanoDrop 2000 spectrophotometer. The genotype of
rs1820453 was determined by the TaqMan SNP Genotyping
Assay (Applied Biosystems, Foster city, CA) using the 7900HT
Fast Real-Time PCR System (Applied Biosystems, Foster city,
CA). To ensure quality control, 5% duplicated samples were
randomly selected to evaluate the reproducibility and with 100%
concordance. Moreover, genotyping was performed
without knowledge of case or control status. The call rate of
genotyping was 98.3 %, and 480 patients and 545 controls
were finally included for subsequent statistical analyses.

Statistical analyses

Hardy-Weinberg equilibrium (HWE) for rs1820453 was
evaluated by goodness-of-fit $\chi^2$ test for genotypes in the control
group. Difference in distribution of demographic characteristics
between cases and controls were evaluated by $\chi^2$ test and t
test where appropriate. The association between BC risk and
rs1820453 was assessed by the odds ratio (OR) along with 95%
confidence interval (95 % CI) using unconditional
multivariate logistic regression analysis with adjustment for age
and menopausal status. All statistical analyses were performed
using the SPSS 12.0 software with two-sided $P$ value less than
0.05 was considered to be statistical significance.

The genotype and mRNA expression data analysis from
HapMap database

The genotype-phenotype analysis was carried out to explore
the possible function of rs1820453 using the SNPexp database
which was available online (http://app3.titan.uio.no/biotools/
help.php?app=snpexp) [19] after significant association of
rs1820453 and BC risk was found. The genotype data were
from the HapMap phase ii release 23 data set including 270
individuals from 4 populations. Among the 270 individuals, 45
were unrelated Chinese Han population from Beijing (CHB), 45
were Japanese in Tokyo (JPT), 90 were Yoruba in Ibadan,
Nigeria (YRI) and 90 were Utah persons from northern and
western Europe (CEU). The mRNA expression data were from
EBV-transformed B lymphoblastoid cell lines from the same
populations.

Results

Results of case-control study

Characteristics of study subjects. The distributions of
demographic characteristics of the participants were presented
in Table 1. A total of 1025 subjects including 480 cases and
545 controls were analyzed in the current study. The mean age
was 48.30 years ($\pm$9.91) and 48.87 years ($\pm$12.51) for case and
control group, respectively; and no significant difference ($P=$
0.417) were identified. In addition, there were more
postmenopausal persons in controls but no significant

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control group, respectively; and no significant difference ($P=$
0.417) were identified. In addition, there were more
postmenopausal persons in controls but no significant
distribution difference ($P= 0.094$) of menopausal status between cases and controls was found.

**Association analysis.** The genotypes distribution of rs1820453 for cases and controls and the association between this variant and BC risk were summarized in Table 2. The genotypes in control group were in agreement with the HWE ($P= 0.942$). When considered the A allele as reference, the C allele was significantly associated with increased BC risk with the OR=1.27 (95 % CI=1.03-1.57) after adjusted by age and menopausal status under logistical regression analyses. The persons who carried the CC genotype had significantly increased BC risk when compared to those who carried AA genotype ($P= 0.009$; OR=2.05, 95 % CI=1.19-3.53). Moreover, the significant association between rs1820453 and BC risk were also identified in recessive and additive models with the OR of 1.98 (95 % CI=1.16-3.38) and 1.26 (95 % CI=1.03-1.54), respectively. When classified BC cases according to the Estrogen Receptor (ER) and Progesterone Receptor (PR) status, significant association of BC risk and rs1820453 was only remained in ER-PR+ specified BC subgroup but not in ER+/PR+ subgroup ($P =0.004$, OR=1.53, 95 % CI=1.14-2.03 and $P =0.191$, OR=1.18, 95 % CI=0.92-1.50 under allelic model for ER-PR- subgroup and ER+/PR+ subgroup, respectively).

### Results of the Genotype and mRNA Expression Analysis From the HapMap Database

The correlation analysis of rs1820453 and YAP1 mRNA expression in 44 (1 missing) unrelated Chinese Han population found that when compared with the levels of mRNA expression in 26 cell lines with rs1820453 AA genotype (6.01±0.046), 2 cell lines with the CC genotype had statistically lower levels of mRNA expression (5.90±0.026, $P= 0.024$) (Figure 1). However, we did not identify significant association of YAP1 mRNA expression and rs1820453 in the 270 all population HapMap lymphoblastoid cell lines with the mRNA expression levels in 129 cell lines with AA genotype, 102 cell lines with AC genotype and 37 cell lines with CC genotype were 6.07±0.060, 6.07±0.085, and 6.08±0.085; respectively (Figure 2). The all results suggested that rs1820453 A>C change may be the risk factor for BC susceptibility in Chinese through influencing the YAP1 gene expression.

### Discussion

In the present case-control study consisted of 480 cases and 545 controls, we identified that rs1820453 A>C change significantly associated with increased BC risk in Chinese population with the OR of 1.27 under the allelic model ($P= 0.023$). Then the genotype-phenotype correlation analysis of rs1820453 and YAP1 gene mRNA expression level found that the mRNA expression level was significant lower in cell lines with CC genotype than the cell lines with the AA genotype in Chinese population ($P= 0.024$). But it was worth noting that the association of rs1820453 A>C change and YAP1 gene mRNA expression was not significantly identified in the all four populations ($P$ for additive model= 0.858). All of these suggested that rs1820453 may be the risk factor for BC through influencing the activity of YAP1 gene in Chinese but not in other populations. Certainly, more population-based studies in other ethnic populations are anticipated to further explore the association of rs1820453 and BC risk.

The protein YAP1 encoded by YAP1 gene as a transcriptional coactivator played an important role in the P73-driven apoptosis pathway. It has been reported that the function of P73 was critical for cellular responding to the cytotoxic chemotherapy and the P73 level in tumor cells reduced by siRNA or genetic mutations may lead to strong reduction of apoptosis induced by DNA damage [11,20]. On the other hand, YAP1 as the downstream apoptotic gene among the P73- dependent apoptotic signaling was crucial in stabilizing and enhancing P73 activity. Therefore, the variants in YAP1 gene which may result in the change of YAP1 activity may finally impact the DNA-damage induced apoptosis pathway.

The rs1820453 located in the promoter region of YAP1 gene and the variant A>C change may cause down regulation of...
YAP1 which in turn might weaken the P73-dependent apoptosis of cancer cells and suppress the chemotherapy-induced cancer cell death. All of which then resulted in faster cancer progression. The gene report experiment from Wu C et al, found that rs1820453 A>C change may affect YAP1 promoter activity; and the rs1820453 A-containing promoter had higher transcriptional activity. The cells containing rs1820453 A allele drove a significantly higher gene expression when compared to the cells containing rs1820453 C allele [18].

In addition to the interaction with P73-driven tumor suppression pathway, present study had found that YAP1 displayed as a protein that integrate another RASSF1A-driven tumor suppressor pathway with the P73 pathway [15].

Though we first identified that rs1820453 was significantly associated with increased BC risk in the current study; some limitations also should be acknowledged. First, this was a hospital-based case-control study which may bring in selection bias during the participants recruitment. Moreover, the sample size of this study was relatively small. Therefore, it would be important to confirm the results in larger and prospective studies. In addition, environmental risk factors were also important in the developing of BC. However, we did not analysis the environmental effect or the gene-environment interaction effect due to the lacking of information on exposure of environmental risk factors. And the estimated effect of rs1820453 on BC risk was not adjusted by some confounding characteristics such as age of menarche and family history owing to the uncompleted collection of the relevant information. Furthermore, though the e-QTL analysis used the HapMap data identified significant association between the mRNA expression level of YAP1 and rs1820453 genotypes, the sample size was relatively small, especially the CC genotypes in Chinese was just 2. Meanwhile, we did not perform more functional experiments to further support the results because of the deficiency of samples.

In conclusion, our case-control study and e-QTL analysis identified rs1820453 was associated with BC risk. The results provided a basic insight of this variant contribute to the development of BC.

Figure 1. The mRNA expression level of YAP1 gene in unrelated CHB. doi: 10.1371/journal.pone.0079056.g001
susceptibility of BC though functional analyses and larger sample size studies were needed to warrant the results.

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Author Contributions

Conceived and designed the experiments: WH TH XM.
Performed the experiments: WW HG YS ZL ZW BC JL CL.
Analyzed the data: WC BZ NS RZ. Contributed reagents/materials/analysis tools: JM LL JD. Wrote the manuscript: WC WW TH XM.

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