Acta virologica **61**: 445 – 452, 2017 doi:10.4149/av_2017_407

Relationship between PPP1R15A gene polymorphism (rs611251) and Epstein-Barr virus-associated tumors

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Received March 6, 2017; accepted October 10, 2017

Summary. – Protein phosphatase 1, regulatory subunit 15A (PPP1R15A), also known as growth arrest and DNA damage-inducible protein GADD34, plays a vital role in promoting cell death and the unfolded protein response (UPR). In order to explore whether the SNP (rs611251) of PPP1R15A gene has a role in different types of Epstein-Barr virus (EBV)-associated tumors, we detected the PPP1R15A gene rs611251 polymorphism in 195 cases of EBV positive tumors (93 lymphomas, 48 gastric carcinomas, 54 nasopharyngeal carcinomas), 208 cases of EBV-negative tumors (136 gastric carcinoma, 19 nasopharyngeal carcinomas, 53 lymphomas) and 113 peripheral blood samples from healthy individuals. Compared with normal controls, the wild type TT and allele T of rs611251 showed higher frequency in gastric carcinoma (GCs), nasopharyngeal carcinomas (NPCs) and lymphomas. However, there was no significant difference between EBV-associated gastric (EBVaGC) and EBVnGC, EBV-positive NPCs and EBV-negative NPCs, EBV-related lymphomas and EBV-negative lymphomas in rs611251 of PPP1R15A. In conclusion, the PPP1R15A rs611251 polymorphism was significantly related to three kinds of tumors. Nevertheless, EBV has no obvious effect on PPP1R15A rs611251 polymorphism of NPC, GC and lymphoma. What's more, the genotype TT and allele T could be risk factors for NPC, GC and lymphoma. Our study explores the relationship between PPP1R15A gene polymorphism (rs611251) and EBV-associated tumors for the first time. PPP1R15A gene SNP (rs611251) have association with multiple tumor types, which may provide some new clues to the detection and treatment of tumors.

Keywords: PPP1R15A; GADD34; rs611251; gene polymorphism; Epstein-Barr virus; tumor

Introduction

Epstein-Barr virus (EBV) was the first human virus to be directly implicated in carcinogenesis and is a prevalent gamma-herpes virus infecting more than 90% of the human population worldwide. Although EBV coexists with most human hosts, persists asymptomatically for life and remains latent in B cells following resolution of infection, it has the potential to be a serious opportunistic pathogen. It has been reported that in some individuals EBV leads to the development of various malignancies, especially several human lymphoid and epithelial tumors, including lymphoma, nasopharyngeal carcinoma (NPC) and gastric cancer (GC) (Thompson *et al.,* 2004; Akiba *et al.,* 2008). In the pattern of EBV latent infection, three integral membrane proteins (LMP1, LMP2A and LMP2B), two non-coding poly-adenylated RNAs (EBER 1 and 2) and 6 nuclear proteins (EBNAs 1, 2 3A, 3B, 3C and LP) are expressed. LMP1 is a well-known classical oncoprotein and has been proven to be associated with NPC and lymphoma pathogenesis, whereas it cannot be found in GC (Truong *et al.,* 2009; Chang *et al.,* 2016). EBNA3C is essential for

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Abbreviations: PPP1R15A = protein phosphatase 1, regulatory subunit 15A; GADD34 = DNA damage-inducible protein; SNP = single nucleotide polymorphism; EBV = Epstein-Barr virus; EBVaGC = EBV-associated gastric; EBVnGCs = EBV-negative gastric carcinoma; GC = gastric carcinoma; NPC = nasopharyngeal carcinomas; EBER = EBV-encoded small RNA

proliferation of EBV-infected B-lymphocytes as an EBV-encoded nuclear protein.

PPP1R15A is a member of a group of genes whose transcript levels are increased following stressful growth arrest conditions and treatment with DNA-damaging agents. This gene induces growth arrest and the expression of DNA damage-inducible protein 34 (GADD34). It has been reported that the activation of GADD34 is a downstream event in apoptotic signaling pathways and may directly contribute to the apoptotic process (Hollander *et al.,* 2001; Liu *et al.,* 2015). However, more recent work has demonstrated that expression of GADD34 promoted growth of lung carcinoma and revealed the exact immunological mechanisms (Liu *et al.,* 2016). The reduction of GADD34 expression significantly suppressed tumor, resulted in decreased accumulation of myeloid-derived suppressor cells (MDSCs) and T-cells. The inhibition of GADD34 also reduced secretion of vascular epithelial growth factor α and transforming growth factor β by MDSCs (Isobe *et al.,* 2016). It has been observed that EBNA3C can interact with GADD34 to counteract the unfolded protein response, which maintains protein homeostasis by governing the processing capacity of the endoplasmic reticulum (ER) to manage ER client loads (Garrido *et al.,* 2009; Young *et al.,* 2016). Moreover, it has been reported that GADD34 expression enhanced colorectal cancer (CRC) tumorigenesis (Tanaka *et al.,* 2015). The SNP (rs557806) of PPP1R15A gene may improve the identification of metastatic colorectal cancer (mCRC) patients sensitive to bevacizumab regimens and improve personalized treatment of mCRC. This SNP might be a candidate biomarker on the basis of correlations with clinicopathologic parameters and clinical responses (Roh *et al.,* 2016).

SNPs have been the focus of much interest and debate in recent years regarding their possible role in the development of various types of cancer (Wu *et al.,* 2013). It is widely accepted that the significant SNPs can be used as biomarkers for diagnosis and to evaluate the risk and prognosis of some diseases including cancers (Pipan *et al.,* 2015; Xu *et al.,* 2015; Kuang *et al.,* 2016). However, there is little information available in literature about PPP1R15A gene polymorphism. The role of PPP1R15A in EBV-associated carcinomas including GC, NPC and lymphomas has not yet been explored. The purpose of this study was to assess the polymorphism (rs611251) of PPP1R15A in GC, NPC and lymphomas in Northern China. Furthermore, an association between the PPP1R15A gene polymorphism (rs611251) and susceptibility to Epstein-Barr virus-associated carcinomas was also explored.

Materials and Methods

Specimens. This study was approved by the Medical Ethical Committee of the Medical College, Qingdao University. All patients participating in the present study gave informed consents for the use of tissue samples for research. We collected 146 lymphoma tissues, 184 GC tissues and 73 NPC tissues from the Department of Pathology of the Affiliated Hospital of Qingdao University, which is located in Shandong Province of northern China. None of the patients received chemotherapy, radiotherapy or hormone therapy. One hundred and thirteen peripheral blood samples, which were collected from Qingdao Blood Center, were classified as a control group. The individuals in this group have no history of cancer and did not receive any medications. EBV-associated tumors were confirmed with EBV-encoded small RNA (EBER) 1 *in situ* hybridization, as described previously (Tokunaga *et al.,* 1993).

DNA extraction. DNA was extracted from fresh tumor tissues and whole-blood samples using the traditional phenol-chloroform method with proteinase K digestion (Wallace, 1987). The QIAamp DNA FFPE Tissue Kit (QIAGEN GmbH, Hilden, Germany) was used to extract DNA from paraffin-embedded carcinoma tissues. The extracted DNA was stored at -20˚C until the experiment was carried out.

Methods of genotyping and statistical analysis. SNPs were genotyped using Sequenom Mass-ARRAY technology, and genotypic frequencies in controls were tested in accord with Hardy-Weinberg equilibrium using Fisher's exact test ($\chi^2 = 0.32$, $P > 0.05$). Statistical analysis was conducted using SPSS 18.0 statistical software (SPSS, Chicago, IL, USA). The Chi-square test was used to compare differences between various types of tumor cases and controls regarding genotype and allele frequencies of rs611251 in each group. The odds ratio (OR) and 95% confidence intervals (CI) were calculated with unconditional logistic regression analysis. The odds ratio (OR) and *P* values were used to assess the correlation between genotype and the risk among different types of tumor in present research. A two-sided *P*-value <0.05 was defined as a significant threshold.

Results

The distribution of genotype and allelic frequency of PPP1R15A (rs611251) in GC

The PPP1R15A gene SNP (rs611251) was successfully genotyped and analyzed in 184 GC specimens including 48 EBVaGCs and 136 EBVnGCs. The results showed that the frequency of genotype TT and allele T of rs611251 was significantly higher in GC cases than in controls. Furthermore, the allele C showed a decreased risk of GCs. The genotypes and variants of PPP1R15A gene in the GCs are listed in Table 1. To explore the relationship between EBV infection and PPP1R15A gene polymorphism (rs611251) in GC development, we analyzed differences in the PPP1R15A genotype (rs611251) distribution between EBVaGC and EBVnGC. However, there was no significant difference between them (Table 2). Compared with controls, the genotype TT and allele T frequency of rs611251 was significantly higher in

Table 1. Distribution of PPP1R15A polymorphisms in GC and control

Genotype/allele PPP1R15A(rs611251)	GC n = 184 (%)	Control n = 113 (%)	OR (95%CI)	*P*
Genotypic frequencies				
TT	155 (84.24%)	84 (74.34%)	1.00	
TC	27 (14.67%)	26 (23.01%)	0.56 (0.31-1.03)	0.059
CC	2 (1.09%)	3 (2.65%)	0.36 (0.06-2.21)	0.351
Recessive model				
Others	182 (98.91%)	110 (97.35%)	1.00	
CC	2 (1.09%)	3 (2.65%)	0.40 (0.07-2.45)	0.372
Dominant model				
TT	155 (84.24%)	84 (74.33%)	1.00	
Others	29 (15.76%)	29(25.66%)	0.54 (0.30-0.97)	0.037*
Allelic frequencies				
T	332 (91.46%)	194 (85.84%)	1.00	
C	31 (8.54%)	32 (14.16%)	0.57 (0.34-0.96)	0.032*

GC: gastric carcinoma; OR: odd ratio; 95%CI: 95% confidence interval; *: statistical significance.

Table 2. Distribution of PPP1R15A polymorphisms in EBVaGC and EBVnGC

Genotype/allele PPP1R15A(rs611251)	EBVaGC n = 48 (%)	EBVnGC n = 136 (%)	OR (95%CI)	*P*
Genotypic frequencies				
TT	38 (79.17%)	117 (86.03%)	1.00	
TC	9 (18.75%)	18 (13.24%)	0.65 (0.27-1.57)	0.334
CC	1 (2.08%)	1 (0.73%)	0.33 (0.02-5.32)	0.436
Recessive model				
Others	47 (97.92%)	135 (99.26%)	1.00	
CC	1 (2.08%)	1 (0.74%)	0.35 (0.02-5.68)	0.439
Dominant model				
TT	38 (79.17%)	117 (86.02%)	1.00	
Others	10 (20.83%)	19 (13.97%)	0.62 (0.27-1.46)	0.262
Allelic frequencies				
T	85 (88.54%)	248 (92.53%)	1.00	
C	11 (11.46%)	20 (7.46%)	0.62 (0.29-1.35)	0.229

EBVaGC: EBV-associated gastric carcinoma; EBVnGC: EBV-negative gastric carcinoma; OR: odd ratio; 95%CI: 95% confidence interval.

EBVnGCs. Furthermore, the TC genotype and allele C showed a decreased risk of EBVnGCs. No significant difference was found between EBVaGCs and control (Table 3).

The distribution of genotype and allelic frequency of PPP1R15A (rs611251) in NPC

The PPP1R15A gene SNP (rs611251) was successfully genotyped and analyzed in 73 NPC specimens including 54 EBV-positive NPCs and 19 EBV-negative NPCs. The frequency of genotype TT and allele T was significantly higher in EBV-positive NPCs and EBV-negative NPCs than that in controls. Furthermore, the genotype TC and allele C showed a decreased risk for NPCs. The genotypes and variants of latent genes in the NPCs are listed in Table 4. However, no significant difference was found between EBV-positive NPCs and EBV-negative NPCs (Table 5).

The distribution of genotypic and allelic frequency of PPP1R15A (rs611251) in lymphomas

The PPP1R15A gene SNP (rs611251) was successfully genotyped and analyzed in 146 lymphoma specimens including 93 EBV-positive lymphomas and 53 EBV-negative lymphomas. The results showed that TT genotype and allele T frequency of rs611251 was significantly higher in EBV-positive lymphomas

Table 3. Distribution of PPP1R15A polymorphisms in EBVnGC, EBVaGC and control

Genotype/allele PPP1R15A(rs611251)	EBVnGC n = 136 (%)	Control n = 113 (%)	EBVaGC n = 48 (%)
Genotypic frequencies			
TT	117 (86.03%)	84 (74.34%)	38 (79.17%)
TC	18 (13.24%)	26 (23.01%)	9 (18.75%)
OR(95%CI)	0.50 (0.26-0.97)		0.77 (0.33-1.79)
P	0.0370*		0.54
CC	1 (0.73%)	3 (2.65%)	1 (2.08%)
OR(95%CI)	0.24 (0.02-2.34)		0.74 (0.07-7.32)
P	0.314		1.00
Recessive model			
Others	135 (99.26%)	110 (97.35%)	47 (97.92%)
CC	1 (0.74%)	3 (2.65%)	1 (2.08%)
OR(95%CI)	0.27 (0.03-2.65)		0.78 (0.08-7.70)
P	0.332		1.00
Dominant model			
TT	117 (86.02%)	84 (74.34%)	38 (79.17%)
Others	19 (13.97%)	29 (25.66%)	10 (20.83%)
OR(95%CI)	0.47 (0.25-0.90)		0.76 (0.34-1.72)
P	0.020*		0.51
Allelic frequencies			
T	248 (92.53%)	194 (85.84%)	85 (88.54%)
C	20 (7.46%)	32 (14.16%)	11 (11.46%)
OR(95%CI)	0.49 (0.27-0.88)		0.79 (0.38-1.63)
P	0.016*		0.52

EBVnGC: EBV-negative gastric carcinoma; EBVaGC: EBV-associated gastric carcinoma OR: odd ratio; 95%CI: 95% confidence interval; *: statistical significance.

Table 4. Distribution of PPP1R15A polymorphisms in NPC and control

Genotype/allele PPP1R15A(rs611251)	NPC(+) n = 54 (%)	Control n = 113 (%)	NPC(-) n = 19 (%)
Genotypic frequencies			
TT	48 (88.89%)	84 (74.34%)	19 (100%)
TC	5 (9.26%)	26 (23.01%)	0 (0%)
OR(95%CI)	0.34 (0.12-0.93)		0.76 (0.69-0.85)
P	0.030*		0.013*
CC	1 (1.85%)	3 (2.65%)	0 (0%)
OR(95%CI)	0.58 (0.06-5.77)		0.97 (0.93-1.01)
P	1.00		1.00
Recessive Model			
Others	53 (98.15%)	110 (97.35%)	19 (100%)
CC	1 (1.85%)	3 (2.65%)	0 (0%)
OR(95%CI)	0.69 (0.07-6.81)		0.97 (0.94-1.00)
P	1.00		1.00
Dominant Model			
TT	48 (88.89%)	84 (74.34%)	19 (100%)
Others	6 (11.11%)	29 (25.66%)	0 (0%)
OR(95%CI)	0.36 (0.14-0.93)		0.74 (0.67-0.83)
P	0.031*		0.013*
Allelic frequencies			
T	101 (93.52%)	194 (85.84%)	38 (100%)
C	7 (6.48%)	32 (14.16%)	0 (0%)
OR(95%CI)	0.42 (0.18-0.99)		0.86 (0.81-0.91)
P	0.041*		0.007*

NPC (+): EBV-positive NPC; NPC(-): EBV-negative NPC; OR: odd ratio; 95%CI: 95% confidence interval; *: statistical significance.

Table 5. Distribution of PPP1R15A polymorphisms in NPC (+) and NPC (-)

Genotype/allele PPP1R15A(rs611251)	NPC(+) n = 54 (%)	NPC(-) n = 19 (%)	OR (95%CI)	*P*
Genotypic frequencies				
TT	48 (88.89%)	19 (100%)	1.00	
TC	5 (9.26%)	0 (0%)	0.91 (0.83-0.99)	0.316
CC	1 (1.85%)	0 (0%)	0.98 (0.94-1.02)	1.000
Recessive model				
Others	53 (98.15%)	19 (100%)	1.00	
CC	1 (1.85%)	0 (0%)	0.98 (0.95-1.02)	1.000
Dominant model				
TT	48 (88.89%)	19 (100%)	1.00	
Others	6 (11.11%)	0 (0%)	0.89 (0.81-0.98)	0.329
Allelic frequencies				
T	101 (93.52%)	38 (100%)	1.00	
C	7 (6.48%)	0 (0%)	0.94 (0.89-0.98)	0.190

NPC (+): EBV-positive NPC; NPC (-): EBV-negative NPC; OR: odd ratio; 95%CI: 95% confidence interval.

Table 6. Distribution of PPP1R15A polymorphisms in lymphoma and control

Genotype/allele PPP1R15A(rs611251)	Lymphoma (+) n = 93 (%)	Control n = 113 (%)	Lymphoma (-) n = 53 (%)
Genotypic frequencies			
TT	80 (86.02%)	84 (74.34%)	47 (88.68%)
TC	13 (13.98%)	26 (23.01%)	5 (9.43%)
OR(95%CI)	0.53 (0.25-1.09)		0.34 (0.12-0.96)
P	0.082		0.034*
CC	0 (0.00%)	3 (2.65%)	1 (1.89%)
OR(95%CI)	0.97 (0.93-1.01)		0.60 (0.06-5.89)
P	0.247		1.000
Recessive model			
Others	100 (100%)	110 (97.35%)	52 (98.11%)
CC	0 (0.00%)	3 (2.65%)	1 (1.89%)
OR(95%CI)	0.97 (0.94-1.00)		0.71 (0.07-6.94)
P	0.249		1.000
Dominant model			
TT	80 (86.02%)	84 (74.33%)	47 (88.68%)
Others	13 (13.98%)	29 (25.66%)	6 (11.32%)
OR(95%CI)	0.47 (0.23-0.97)		0.37 (0.14-0.96)
P	0.038*		0.035*
Allelic frequencies			
T	173 (93.01%)	194 (85.84%)	99 (93.40%)
C	13 (6.99%)	32 (14.16%)	7 (6.60%)
OR(95%CI)	0.46 (0.23-0.90)		0.43 (0.18-1.01)
P	0.020*		0.046*

Lymphoma (+): EBV positive lymphoma; Lymphoma (-): EBV negative lymphoma; OR: odd ratio; 95%CI: 95% confidence interval; *: statistical significance.

and EBV-negative lymphomas than that in controls. Furthermore, the TC genotype and allele C showed a decreased risk of two groups. The genotypes and variants of PPP1R15A genes in the lymphomas are listed in Table 6. Moreover, no significant difference was found between EBV-positive lymphomas and EBV-negative lymphomas (Table 7).

Table 7. Distribution of PPP1R15A polymorphisms in Lymphoma (+) and Lymphoma (-)

Genotype/allele PPP1R15A(rs611251)	Lymphoma (+) n = 93 (%)	Lymphoma (-) n = 53 (%)	OR (95%CI)	*P*
Genotypic frequencies				
TT	80 (86.02%)	47 (88.68%)	1.00	
TC	13 (13.98%)	5 (9.43%)	0.66 (0.22-1.95)	0.445
CC	0 (0.00%)	1 (1.89%)	1.02 (0.98-1.06)	0.375
Recessive model				
Others	100 (100%)	52 (98.11%)	1.00	
CC	0 (0.00%)	1 (1.89%)	1.02 (0.98-1.06)	0.346
Dominant model				
TT	80 (86.02%)	47 (88.68%)	1.00	
Others	13 (13.98%)	6 (11.32%)	0.79 (0.28-2.21)	0.646
Allelic frequencies				
T	173 (93.01%)	99 (93.40%)	1.00	
C	13 (6.99%)	7 (6.60%)	0.94 (0.36-2.44)	0.900

Lymphoma (+): EBV-positive lymphoma; Lymphoma (-): EBV-negative lymphoma; OR: odd ratio; 95%CI: 95% confidence interval.

Discussion

In the present study, we first investigated PPP1R15A gene polymorphism (rs611251) in lymphomas, NPCs and GC specimens. The rs611251 is located at the genomic position of 48873829 on chromosome 19. The mutation of this site is T-C nucleotide mutation, which leads to Val-Ala mutation. We found that the distribution of wild genotype TT and allele T showed a higher frequency in lymphomas, NPCs and GC samples, which may be a risk factor for these tumors. The allele C showed a decreased risk of lymphomas, NPCs and GC samples than that in controls. Consequently, the mutation of this site may be a protective factor for NPC, GC and lymphoma. However, little is known about the relationship between SNP (rs611251) with EBV-associated tumors.

It is well known that NPC is closely related to EBV infection. Expression of the oncogene LMP1 has been associated with NPC pathogenesis (Baichwal *et al.,* 1988). In the present study, we found that the distribution of genotype TT and allele T showed a higher frequency both in EBV-positive NPCs and EBV-negative NPCs compared with the normal controls. The allele C also showed a decreased risk of EBV-positive NPCs and EBV-negative NPCs. It indicated that genotype TT and allele T may be a risk factor for NPCs.

GC is a heterogeneous disease that is largely influenced by *H. pylori* infection, environmental factors and genetic susceptibility. Epidemiological studies have shown that 95% of the adults are infected with EBV infection, though half of the world's population suffers from *H. pylori* infection. However, only a small proportion of them progresses to chronic atrophic gastritis and ultimately GC (EBVaGC accounts for 1.3–20.1% in GC and 1–2% *H. pylori*-infected cases develop GC) (Scholte *et al.,* 2002; Akiba *et al.,* 2008; Uozaki *et al.,* 2008; Lee *et al.,* 2009). Furthermore, genetic factors may play key important roles in GC development.

Our results showed that the distribution of genotype TT and allele T of PPP1R15A (rs611251) had higher frequency in EBVnGC samples, while EBVaGCs does not reflect significant difference compared with normal controls. This difference may be related to the affection of EBV. However, the statistical analysis indicated that EBVaGCs and EBVnGCs had no significant difference with each other, but significant difference was found between GCs and controls. The genotype TT and allele T of PPP1R15A (rs611251) in GCs may be a risk factor for GCs.

In the present study we found that EBV-positive NPCs have significant difference compared with normal controls ($P < 0.05$), but the difference between EBVaGCs and normal controls was not significant ($P > 0.5$). NPC and GC were both EBV-associated epithelial carcinomas (Imai *et al.,* 1994), however, they have different latency types of EBV. Different latency types correlate with specific EBV-associated malignancies (Babcock *et al.,* 2000). It has been reported that oncogene LMP1 inhibits the differentiation of human epithelial cells. It is also related to cancer development, growth, invasion, metastasis, and the epithelial-mesenchymal transition (Dawson *et al.,* 1990, Yoshizaki *et al.,* 2002). Some authors have reported that LMP1 can be detected in 95.6% of NPC tissues and is associated with NPC pathogenesis, whereas the expression of LMP1 was not found in gastric cancer or in nonneoplastic gastric tissue (Baichwal *et al.,* 1988; Tsang *et al.,* 2003; Truong *et al.,* 2009). Hence, the different expression of LMP1 maybe a way to explain our results and these findings require further study.

EBV is associated with various lymphoproliferative disorders, such as Burkitt's lymphoma, Hodgkin's lymphoma, T

cell lymphoma, B cell lymphoma in immunodeficiency, and lymphoepithelioma-like carcinoma of the parotid gland (Young *et al.,* 1992; Ambinder *et al.,* 1994). Our study verified that genotype TT and allele T were also risk factors for lymphoma.

It was reported that overexpression of GADD34 enhanced the ability of EBNA3C to co-activate EBNA2, which could activate the LMP1 promoter. EBNA3C is essential to initiate B-cell growth, as well as ongoing B-cell transformation. Moreover, EBNA3C can interact with GADD34 that is upregulated in response to viral infection as well as ER-stress in both nuclear and cytoplasmic compartments and counteracts the unfolded protein response. In addition, the interaction also requires the domination of the HSV-1 ICPγ34.5, which shares homology with GADD34 in the PP1-binding domain (He *et al.,* 1997; Garrido *et al.,* 2009). However, no significant difference was found between three kinds of EBV-associated tumors and corresponding EBV-negative tumors. EBV infection may not play a vital role in the SNP (rs611251) of PPP1R15A. The exact mechanism is not yet clear. Further in-depth study and analysis will be necessary under rather more naturalistic conditions to confirm the relationship between PPP1R15A gene polymorphisms and EBV-associated tumors. Our results are the first to identify that PPP1R15A gene SNP (rs611251) has association with multiple tumor types, which may provide some new clues to the detection and treatment of tumors.

Conclusions

Our present results indicate that the PPP1R15A rs611251 polymorphism was significantly related to three kinds of tumors (NPC, GC and lymphoma). The genotype TT and allele T could be risk factors for NPC, GC and lymphoma. Nevertheless, EBV has no obvious effect on PPP1R15A rs611251 polymorphism.

Acknowledgements. This work was supported by National Natural Science Foundation of China (NSFC 81571995), and Natural Science Foundation of Shandong Province (ZR2015HM069).

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