

## **Association of IL-6 -634C/G polymorphism and the risk of osteosarcoma in a Chinese population.**

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### **Abstract**

**In this study, we will investigate the interleukin-6 (IL-6) -634C/G polymorphism and the risk of osteosarcoma in the ethnic group Han of China. The subjects in this study consisted of 191 patients with primary sporadic osteosarcoma and 207 cancer-free controls. The IL6-634 G/C polymorphism was determined by PCR followed by restriction fragment-length polymorphism analysis. High risk of osteosarcoma was found in the CG genotype of IL6 -634 G/C polymorphism (OR=1.88, 95% CI 1.23-2.85). However, no significant association was found between GG genotype and osteosarcoma risk (OR=1.62, 95% CI 0.78-2.69). When CG and GG carriers were grouped together, higher osteosarcoma risk was detected (OR=1.81, 95% CI 1.21-2.71). The G allele was significantly associated with risk of osteosarcoma (OR=1.66, 95% CI 1.20-2.28). In conclusion, this study found a significant association between the IL6 -634 G/C polymorphism and osteosarcoma risk.**

**Keywords:** Interleukin-6, Osteosarcoma, Risk.

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### **Introduction**

Osteosarcoma is one of the most common primary osteotumor affecting young adults with an incidence of approximately 0.4 per 100,000 population per year [1]. It is most common between the ages of 10-19, and the incidence rate for the condition ranges between one and five cases per one million people [2].

Studies have revealed that the tumorigenesis and malignant progression of osteosarcoma are significantly associated with genetic and epigenetic mechanisms [3].

Interleukin-6 (IL-6) is a pro-inflammatory cytokine which plays a central role in host defense mechanisms [4]. IL-6 levels are altered in cancer patients, especially after chemotherapy and local radiotherapy.

Anderson et al. found that IL-6 activate STAT signaling in bystander cells that maintain EWS/FLI1 expression [5]. Lissat et al. suggest that IL6 contributes to ES tumor progression by increasing resistance to apoptosis in conditions of cellular stress, such as serum starvation, and by promotion of metastasis [6].

Qi et al. indicated that IL-6 -174 G/C genotype was associated with risk for development and metastasis of osteosarcoma in Chinese Han population [7]. In this study, we will investigate the IL-6 -634C/G polymorphism and the risk of osteosarcoma in the ethnic group Han of China.

### **Methods**

#### ***Study population***

A case-control study was designed to assess the role of IL-6 -634C/G polymorphism and the risk of osteosarcoma. The subjects in this study consisted of 191 patients with primary sporadic osteosarcoma and 207 cancer-free controls. All participants filled and signed an informed consent form. Controls with any kinds of cancer were excluded.

#### ***Sample collection***

Genomic DNA was extracted from circulating leukocytes using commercial DNA isolation kits (Tiangen Biotech, Beijing, China). Briefly, the red blood cells, as well as the nuclei of leukocytes, were lysed. Subsequently, proteins were precipitated, followed by the precipitation of DNA using isopropanol. The DNA pellet was washed with ethanol. Finally, DNA was rehydrated with the DNA rehydration solution and preserved in liquid nitrogen.

#### ***Genotyping method***

The IL6-634 G/C polymorphism was determined by PCR followed by restriction fragment-length polymorphism analysis. The PCR product (180 bp) was amplified using forward primer 5'-GAGACGCCTTGAAGTAACTG-3' and reverse primer 5'-AACCAAAGATGTTCTGAACTGA-3' with Taq Polymerase (Promega, USA) in the presence of 1.5 mM

Mg<sup>2+</sup>. Cycling parameters were: denaturation at 95°C (45 s), annealing at 48°C (45 s), extension at 72°C (60 s) for 40 cycles with final extension at 72°C for 10 min. Digestion was performed with Bsr BI (New England Biolabs, USA), at 37°C for 3 h. The C allele has no Bsr BI cleavage site, whereas the PCR product is cleaved into two fragments of 120 and 60 bp in the presence of the G allele.

### Statistical analysis

We used Statistical Product and Service Solutions 23.0 software (SPSS, Inc., USA) for all analyses. For continuous data, the two independent samples t-test were used for comparisons of two groups. Chi-square test was used for categorical data. Differences of genotype distributions between controls and cases were evaluated by the Chi-square test. Odds Ratios (ORs) and their 95% CIs were also calculated. A two-tailed  $p < 0.05$  was considered statistically significant.

### Results

Distribution of demographic factors is shown in Table 1. There were 191 patients with osteosarcoma and 207 entered into the final analyses. There were no significant differences in the distributions of age and sex ( $p > 0.05$ ). Compared with controls, the cases group had higher family history of cancer ( $p < 0.01$ ).

**Table 1.** Characteristics of the cases and controls.

Characteristics	Case (n=191)	Control (n=207)	P value
Age (y)	28.5 ± 13.1	27.3 ± 17.9	0.76
Sex			
Male	150	158	0.58
Female	41	49	
Family history of cancer			
Yes	139	88	<0.01
No	52	119	
Tumor location			
Extremities	162		
Non-extremities	29		
Metastasis			
Yes	75		
No	116		

The genotype distributions of the SNP are presented in Table 2. The genotype distributions of SNP were in accordance with Hardy-Weinberg equilibrium in the control group ( $p > 0.05$ ). High risk of osteosarcoma was found in the CG genotype of IL6 -634 G/C polymorphism (OR=1.88, 95% CI 1.23-2.85). However, no significant association was found between GG genotype and osteosarcoma risk (OR=1.62, 95% CI 0.78-2.69). When CG and GG carriers were grouped together, higher osteosarcoma risk was detected (OR=1.81, 95% CI 1.21-2.71).

The G allele was significantly associated with risk of osteosarcoma (OR=1.66, 95% CI 1.20-2.28).

**Table 2.** The genotype and allele frequencies of IL-6 -634C/G polymorphism.

Genotype	Case	Control	OR	95% CI	P value
CC	85	124	1		NA
CG	88	68	1.88	1.23-2.85	0.003
GG	17	15	1.62	0.78-2.69	0.19
CG+GG	105	83	1.81	1.21-2.71	0.002
C	258	316	1		NA
G	122	90	1.66	1.20-2.28	0.001

### Discussion

We investigated the association between the IL6 -634 G/C polymorphism and osteosarcoma risk. High risk of osteosarcoma was found in the CG genotype of IL6 -634 G/C polymorphism. However, no significant association was found between GG genotype and osteosarcoma risk. When CG and GG carriers were grouped together, higher osteosarcoma risk was detected. The G allele was significantly associated with risk of osteosarcoma.

Wang et al. found that CC genotype of IL-6 gene polymorphisms at positions of -174 and -572 may denote potential high risk of endometrial adenocarcinoma [8]. Zidi et al. suggested that IL-6 variants rs1800795 and rs1474348 are major risk factors of CC susceptibility and evolution among Tunisian women [9]. Liu et al. found that IL-6 rs1800795 polymorphism may enhance the susceptibility to prostate cancer in African-American men [10]. Bandy et al. demonstrates that there is a strong association between the IL-6 -174G/C promoter single nucleotide polymorphism and a decreased risk of colorectal cancer in ethnic Kashmiri population [11]. Zhang et al. indicated that suggested that IL-6R rs2228145 and IL-6 rs10499563 genotype were associated with decreased risk of gastric cancer for the individuals with negative and positive *Helicobacter pylori* infection [12].

This study has some limitations. As with all case-control studies, there is a potential for differential recall bias, with cases more likely to recall a history of these medical conditions as a result of their osteosarcoma. Additionally, the results were not adjusted by other clinical factors.

### Conclusion

This study found a significant association between the IL6 -634 G/C polymorphism and osteosarcoma risk.

### Conflicts of Interest

None.

## References

1. Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. *Cancer Treat Res* 2009; 152: 3-13.
2. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. *CA Cancer J Clin* 2014; 64: 83-103.
3. Ambros V. The functions of animal microRNAs. *Nature* 2004; 431: 350-355.
4. Hirano T. Interleukin-6 and its relation to inflammation and disease. *Clin Immunol Immunopathol* 1992; 62: 60-65.
5. Anderson JL, Titz B, Akiyama R, Komisopoulou E, Park A, Tap WD, Graeber TG, Denny CT. Phosphoproteomic profiling reveals IL6-mediated paracrine signaling within the Ewing sarcoma family of tumors. *Mol Cancer Res* 2014; 12: 1740-1754.
6. Lissat A, Joerschke M, Shinde DA, Braunschweig T, Meier A, Makowska A, Bortnick R, Henneke P, Herget G, Gorr TA, Kontny U. IL6 secreted by Ewing sarcoma tumor microenvironment confers anti-apoptotic and cell-disseminating paracrine responses in Ewing sarcoma cells. *BMC Cancer* 2015; 15: 552.
7. Qi Y, Zhao C, Li H, Zhang B, Tada K, Abe H, Tada M. Genetic variations in interleukin-6 polymorphism and the association with susceptibility and overall survival of osteosarcoma. *Tumour Biol* 2016; 37: 9807-9811.
8. Wang HY, Zhang JJ, Zheng XY, Liu JH, Li YW. Association between IL-6 Gene (-174 & -572 G/C) polymorphisms and endometrial adenocarcinoma risk. *Pathol Oncol Res* 2016; 22: 825-829.
9. Zidi S, Stayoussef M, Alsaleh BL, Gazouani E, Mezlini A, Ebrahim BH, Yacoubi-Loueslati B, Almawi WY. Relationships between common and novel interleukin-6 gene polymorphisms and risk of cervical cancer: a case-control Study. *Pathol Oncol Res* 2017; 23: 385-392.
10. Liu TZ, Guo ZQ, Wang T, Cao Y, Huang D, Wang XH. Meta-analysis of the role of IL-6 rs1800795 polymorphism in the susceptibility to prostate cancer: Evidence based on 17 studies. *Medicine (Baltimore)* 2017; 96: 6126.
11. Banday MZ, Balkhi HM, Sameer AS, Chowdri NA, Haq E. Strong association of interleukin-6 -174G/C promoter single nucleotide polymorphism with a decreased risk of colorectal cancer in ethnic Kashmiri population: A case control study. *Tumour Biol* 2017; 39: 1010428317695940.
12. Zhang JZ, Liu CM, Peng HP, Zhang Y. Association of genetic variations in IL-6/IL-6R pathway genes with gastric cancer risk in a Chinese population. *Gene* 2017; 623: 1-4.

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