

# Genetic Polymorphism of Interleukin 10 Gene and Sasang Constitution in Bell's Palsy Patients

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We hypothesized that the IL10 gene is important candidate in the development of Bell's palsy and specific genotypic and allelic variations should be associated with Bell's palsy in the Korean population. In this study, we assessed the SNP (single-nucleotide polymorphism) of IL10 in patients with Bell's palsy. 62 patients with Bell's palsy were selected from the subjects who visited for the Bell's palsy service of the department of acupuncture & moxibustion, college of Oriental Medicine, Daegu Haany University from May 2002 to May 2003. Pyrosequencing was performed for genetic analyses. There was no statistically significant genotypic distribution difference between control and Bell's palsy group. And there was not statistically significant allelic frequency difference between control and Bell's palsy group. In this study the IL10 genotypemight not be the risk factor of Bell's palsy patients in Korean. studies will be necessary for the exact genetic markers. Establishment of more systemic approach and high quality of prospective cohorts will be necessary for the good prediction of genetic markers.

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**Key words :** Single Nucleotide Polymorphisms (SNP), Bell's Palsy, Interleukin 10 (IL10), Sasang Constitution, minisequencing

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## Introduction

Most of peripheral 7th cranial nerve palsies remain without an identified aetiology, and will be diagnosed as idiopathic or Bell's palsy. Some characteristics of this condition may be feature of a viral infection. Recently an increased interest and focus on the possible herpes simplex virus (HSV) aetiology in idiopathic facial paralysis has been seen. Infection of the facial nerve with HSV induces edema and paralysis of the nerve.<sup>1,2)</sup> In recent some study the interleukin 6 (IL6), interleukin 8 (IL8) and Tumor necrosis factor-alpha (TNF- ) levels were significantly higher in the Bell's palsy than in the control. As in every infectious and immune reactions, cytokines should also be involved in Bell's palsy.<sup>2)</sup> For many cytokines and their receptors, genetic variants have been described.<sup>3-7)</sup> Gene expression can be regulated by a number of genetic elements located in the 5-upstream region of the gene. Variances in this upstream sequence can result in different level of gene expression. IL10 is one of the immunomodulatory

cytokines and it has anti-inflammatory capabilities.<sup>8)</sup> To date, genetic polymorphism in the 5'-flanking region of the IL10 gene has not been described in Bell's palsy. We hypothesized that the IL10 gene is important candidate in the development of Bell's palsy and specific genotypic and allelic variations should be associated with Bell's palsy in the Korean population. In this study, we assessed the SNP (single-nucleotide polymorphism) of IL10 in patients with Bell's palsy.

## Subjects and Methods

### 1. Study population

62 patients with Bell's palsy were selected from the subjects who visited for the Bell's palsy service of the department of acupuncture & moxibustion, college of Oriental Medicine, Daegu Haany University from May 2002 to May 2003. Diagnosis of Bell's palsy was made by neurologic examination, Additional otolaryngologic and radiological examinations such as cranial or temporal magnetic resonance imaging or temporal bone computed tomography were performed in case of requirement. The control group consisted of 104 healthy volunteers who visited for the health examinations at Jehan medical center in Daegu from May 2002 to May 2003. They had no history of chronic systemic disease, drug use, or facial

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paralysis. The physical examination was normal in the controls. They did not have an acute infection in the past 1 month. Ultimately, 168 Koreans were enrolled in the current analysis.

## 2. Blood sample collection

Blood samples were obtained from the antecubital vein without regarding to the time of the last meal. This study was approved by the ethics review committee of the medical research institute, Jehan medical center. Informed consent was obtained from all subjects.

## 3. DNA preparation and genotyping

Blood samples from all subjects were obtained for DNA extraction and collected in EDTA tube. Genomic DNA was extracted using DNA isolation kit for Mammalian Blood (Boehringer Mannheim, IN, USA). The extracted DNA was amplified by polymerase chain reaction (PCR). The IL10 gene (113-bp) was amplified using 25 ng of DNA, 5 pmol of each primer. IL10 forward was 5'-GGGTAAAGGAGCCTGGAACAC-3' and IL10 reverse was 5'-GGGTGGGCTAAATATCCTCAAAGT-3'. The PCR amplification was performed by using 0.5 unit Taq polymerase (HT Biotechnology Ltd., Cambridge, United Kingdom).

The 30 ul of PCR reaction mixtures were 10 mM Tris-HCl, pH 9.0, 1.5 mM magnesium chloride, 50 mM potassium chloride, 0.1% Triton-X 100, 0.01 % [v/v] stabilizer, 0.25 mM of each deoxynucleotide triphosphate (dNTP), 0.1 M of each oligonucleotide primer. The PCR steps were denaturation of 5 minute at 95°, 30 cycles of 30 seconds at 95°, 30 seconds at 60°, and 30 seconds at 72° with a Thermocycler (Astech, Fukuoka, Japan). The reverse primer was biotinylated to allow the preparation of single-stranded DNA. The quality of PCR products was controlled by 1.5% of agarose gel electrophoresis. DNA Preparation for pyrosequencing was performed according to manufacturer's standard protocol (Pyrosequencing AB, Uppsala, Sweden)<sup>9</sup>.

The streptavidin sepharose beads (Streptavidin Sepharose HP, Amersham Pharmacia Biotech, Uppsala, Sweden) were immobilized to PCR products. The sequencing primer of IL10 was 5'-CTGGCTTCCTACAG-3' and it was designed so that the terminal residue hybridized to the base immediately adjacent to the A/C mutation from Pyrosequencing AB ([http:// www.pyrosequencing.com](http://www.pyrosequencing.com))<sup>9</sup>. By incubation at room temperature for 10 minutes, 20 ul of biotinylated PCR products were immobilized onto streptavidin-coated sepharose beads, the immobilized PCR products were transferred to a Millipore 96-well filter plate (Millipore, Bedford, MA, USA).

Vacuum was used to eliminate the different solutions and reagents to obtain pure, single-stranded DNA while the beads

remained in the wells<sup>10</sup>. In 55 ul of 4 M acetic acid containing 0.35 uM of IL10 sequencing primer the beads with the immobilized template were resuspended. Then the 45 ul of suspension was transferred to a PSQ 96 plate (Pyrosequencing AB, Uppsala, Sweden)<sup>11</sup>. By using PSQ 96 Sample Prep Thermoplate (Pyrosequencing AB, Uppsala, Sweden) the PSQ 96 plate containing the samples was heated at 90° for 5 minutes for sequencing primer annealing, and moved to room temperature for 10 minutes.

Then the PSQ 96 Plate was placed into the process chamber of the PSQ 96 instrument (Pyrosequencing AB, Uppsala, Sweden)<sup>12</sup>. The enzymes, substrates, and nucleotides were dispensed from a reagent cassette into the wells by using the PSQ 96 SNP Reagent Kit (Pyrosequencing AB, Uppsala, Sweden). The light was generated when a nucleotide is incorporated into a growing DNA strand<sup>13</sup>. From this process the polymorphism of the IL10 was genotyped automatically.

## 4. Statistical analysis

To compare the age of Bell's palsy patients and controls Student's t-test was used. To compare sex, the distribution of the genotypes and the frequencies of alleles between Korean Bell's palsy patients and controls 2 tests was used. AS statistical package SAS program (release 8.2, SAS Institute Inc.) was used.

# Results

## 1. Characteristics of the subjects

The characteristics of the Bell's palsy patients and controls are shown in Table 1. There was no significant difference between the patients and controls as for age ( $p=0.603$ ) but there was significant difference as for sex ( $p<0.001$ ). Mean age of the controls and patients was 44.5 12.8 and 46.1 13.9 years. The number of male / female of the controls and patients was 23 / 81 and 33 / 29.

**Table 1. Clinical characteristics of patients and controls**

	Controls (n = 120)	Patients (n = 108)	P value
Male / Female	87 / 33	66 / 42	0.061
Age, mean±SD, y	45.6 ± 15.1	44.2 ± 15.2	0.492

Chi-square test was used to compare values of patients and controls for sex. Age was compared by Student's t test.

## 2. IL10 genotype Distribution

There was no statistically significant genotypic distribution difference between control and Bell's palsy group ( $p=0.052$ , OR (95% CI) ; 2.23 (0.98-5.98)). The frequencies of

A/A homozygotes and A/C heterozygotes among control subjects were 91 (87.5%) and 13 (12.5%). The frequencies of A/A and A/C among the Bell's palsy patients were 47 (75.8%) and 15 (24.2%). These results are shown in Table 2.

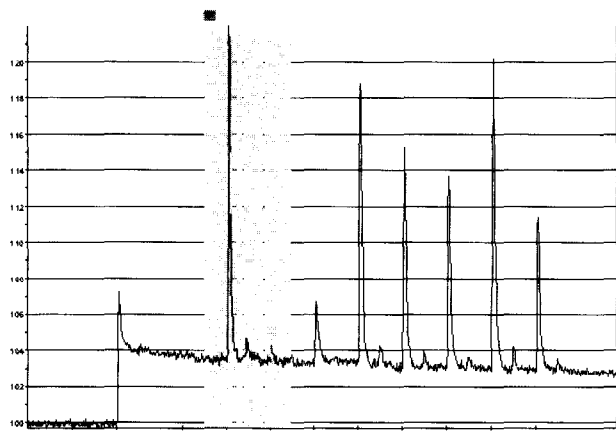


Fig. 1. Pyrosequencing result of A/A homozygote genotype of the IL10 gene.

Table 2. Genotype and allele frequencies of polymorphism in IL10 gene in patients and control

	Genotype (%)				Allele Frequency (%)		
	AA	AC	CC	P value	A	C	P value
Controls (n = 120)	106(88.3)	14(11.7)	0(0)	0.720	226(94.2)	14(5.8)	0.728
Patients (n = 108)	97(89.8)	11(10.2)	0(0)		205(94.9)	11(5.1)	
	Sasang Constitution						
Soyang-person (n = 39)	36(92.3)	3(7.7)	0(0)	0.199	75(96.1)	3(3.9)	0.197
Soeum- person (n = 35)	32(91.4)	3(8.6)	0(0)	0.225	67(95.7)	3(4.3)	0.221
Taeum-person (n = 34)	29(85.3)	5(14.7)	0(0)	0.643	63(92.6)	5(7.4)	0.191

Values are % Chi-square tests were used to compare genotype and allele frequencies between controls and patients also between controls and Sasang Constitution of the patients.

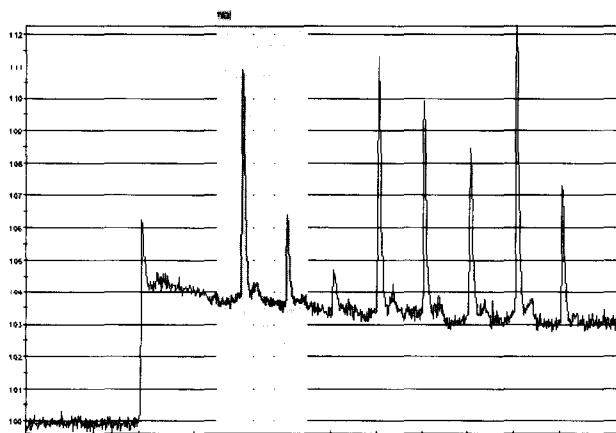


Fig. 2. Pyrosequencing result of A/C heterozygote genotype of the IL10 gene.

### 3. Allele Frequencies Distribution

There was not statistically significant allelic frequency difference between control and Bell's palsy group ( $p=0.064$ , OR (95% CI) ; 2.06 (0.94-4.49)). The allelic frequency of A and C was 195 (93.8%) and 13 (6.2%) among the control subjects and 109 (87.9%) and 15 (12.1%) in Bell's palsy patients, respectively.

## Discussion

Normal facial movement is required for speaking, chewing, swallowing and protecting the eye. Bell's palsy is an acute peripheral monosymptomatic facial palsy with undetectable causes. The aetiology of Bell's palsy remains unclear although genetic, vascular, infective and immunological causes have all been provided<sup>14</sup>). Recently, it has been suggested that Bell's palsy is caused by reactivation of HSV in the geniculate ganglia<sup>15,16</sup>). Bell's palsy causes most cases of unilateral and acute facial palsy. Symptoms improve in nearly all patients with Bell's palsy, but some patients are left with cosmetic deficits or malfunction<sup>17</sup>.

In Bell's palsy, there is an inflammatory reaction which is compressing the facial nerve in the fallopian canal, particularly in the labyrinthine segment. In decompression surgeries, it is common to encounter an inflammatory reaction in this labyrinthine segment. It is likely that there is demyelination in Bell's palsy. This inflammation or demyelination is possibly caused by HSV infection<sup>2,18</sup>). Cytokines take important part in the infectious and inflammatory processes.

They serve to transduce antigen specific signals, and focus inflammatory response where it is needed. That is, as in other disease states, some of the cytokines play a role in Bell's palsy, but importance of this role has not been established delicately to date. Interleukines and TNF- $\alpha$  are the cytokines which are released by certain cells (mononuclear phagocytes, endothelial cells, epithelial cells, T cells, natural killer cells, etc.), and act in the generation of immune response, inflammation, acute phase reaction, fever, etc.<sup>18</sup>

In one study, The IL-6, IL-8 and TNF- levels were significantly higher in the Bell's palsy than in the control ( $p<0.01$  and  $p=0.017$ , respectively) while the IL-1 $\beta$  and IL-2 $\gamma$  levels were similar in both groups. The mean IL-6 levels were 8.631 and 5.305 pg/ml in the patients and controls, respectively. The mean IL-8 levels were 9.363 and 60.8 pg/ml in the patients and controls, respectively. The TNF- levels were 10.162 and 4.308 U/ml in the patients and controls, respectively, which were statistically significant ( $p<0.01$ ).<sup>18</sup> IL10 is one of the immunomodulatory cytokines. IL10 is produced by a variety of cell types, including monocytes and B cells.<sup>19</sup>

It is an up-regulator of B lymphocyte production and differentiation,<sup>20</sup> but has anti-inflammatory abilities that can directly down-regulate TNF, IL-1, IL-8 and interferon-production.<sup>21</sup> Three of IL10 SNPs have been studied in some detail: -1082(G to A), -819(C to T) and -592(C to A)<sup>22-24</sup>.

Genetic factors appear to contribute to virtually every human disease, conferring susceptibility or resistance, affecting the severity or progression of disease, and interacting with environmental influences. In trying to get the information about genetic variation is important for understanding how genes function or malfunction, and how genetic and functional variation are related. In this study, we investigated polymorphism in the IL10 gene region in Korean Bell's palsy patients. Our data did not provide any evidence for in vivo functional regulation of IL10 in between Bell's palsy subjects and control participants.

The overall analysis revealed no significant interactions between genotype ( $p=0.052$ ). The frequencies of A/A and A/C among control subjects were 91 (87.5%) and 13 (12.5%). The frequencies of A/A and A/C among the Bell's palsy patients were 47 (75.8%) and 15 (24.2%). And our data failed to show any allelic frequency difference between Bell's palsy and control Korean ( $p=0.064$ ). The allelic frequency of A and C was 195 (93.8%) and 13 (6.2%) among the control subjects and 109 (87.9%) and 15 (12.1%) in Bell's palsy patients, respectively.

Genetic factors and environmental factors are both critical in the development of Bell's palsy. So far it is very difficult to apply the results from genetic studies to clinic patients. There are some limitations of this study. Firstly, the IL10 serum of the patients was not taken, which makes the information somewhat heterogenous. And the sample size was too small. In the further studies, these limitations should be improved. The cytokine IL10 may not be pathogenetic factors in Bell's palsy. But further studies including different cytokine gene can be a useful for predicting Bell's palsy.

## Conclusion

In this study the IL10 genotypemight not be the risk factor of Bell's palsy patients in Korean. Further studies will be necessary for the exact genetic markers. Establishment of more systemic approach and high quality of prospective cohorts will be necessary for the good prediction of genetic markers.

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