Genetic influence on stress response in cancer patients

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Genetic influence on stress response in cancer patients

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Kang, Jee In

December 2010

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<ABSTRACT>

Genetic influence on stress response in cancer patients

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(Directed by Professor Kee Namkoong)

Cancer patients who have to adapt to a long process with multiple stressful events show various stress responses. Genetic components may contribute to individual differences of stress response and risk for development of stress-related psychiatric problems. The present study aimed to investigate the influence of FK506 binding protein 5 (FKBP5) gene polymorphisms regulating the hypothalamic-pituitary-adrenal (HPA) axis on individual distress levels in cancer patients faced with a stressful situation. To elucidate predicting values of distress level, the present study used a prospective design.

A total of 130 patients (90 males, 40 females) who were newly diagnosed with advanced gastric cancer and supposed to receive the first-line chemotherapy were included and 93 patients (63 males, 30 females) were followed up at 6 week after 2 cycles of chemotherapy. Distress level and coping patterns were measured by the Hospital Anxiety and Depression Scale (HADS) and Mini-Mental Adjustment to Cancer (Mini-MAC) scale. For genetic factors, three single nucleotide polymorphisms of FKBP5 rs1360780,

rs9296158 and rs9470080 were genotyped.

The FKBP5 rs9296158 and rs9470080 had a group-by-time interaction effect for HADS-anxiety and HADS-depression. In addition, the step-wise linear regression analyses showed that FKBP5 gene polymorphisms and specific coping patterns were significant predictors of anxiety and depression at follow-up. In particular, FKBP5 rs9296158 and rs9470080 were significant predictors of the changes in HADS-anxiety and HADS-depression scores over time.

Our findings indicate that the genetic components such as FKBP5 gene polymorphisms may play a crucial role in anxiety and depression following prolonged stress exposure.

Key words: cancer, stress, depression, gene, FKBP5, HPA axis

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I. INTRODUCTION

People respond to stress differentially. Moreover, individual stress response is different even to the same stressful situation [1]. After experiencing similar traumatic life events, not all individuals develop stress-related psychiatric disorders such as mood and anxiety disorders [2]. The determinants of these individual differences are not clearly defined. According to the diathesis-stress model [3], there are genetic or biological predispositions and environmental stressors that combine to manifest as abnormal behaviors and mental illness. Considerable evidence supports the concept that susceptibility to stress-related psychiatric disorders such as mood and anxiety disorders is due to the combined effect of genes and the environment [4-6]. A recent review of post-traumatic stress disorder (PTSD) showed that interactions between genetic and neurobiological factors and environmental factors affect vulnerability and resilience following trauma exposure [2].

Biological aspect of stress response is characterized by regulation of

the hypothalamic-pituitary-adrenal (HPA) axis [7, 8]. The HPA axis activation results in glucocorticoid secretion from the adrenal cortex, and glucocorticoid feedback mediated by glucocorticoid receptors (GR) regulates the HPA system [9]. While activation of HPA axis is essential for the successful adaptation to a real threat and maintain homeostasis, chronic elevation of glucocorticoids results in changed sensitivity of GR [10, 11]. Change of GR sensitivity consequent to chronic stress exposure has been implicated in the hippocampal vulnerability and pathophysiology of major depression [10, 12-14]. Taken together, GR-mediated HPA axis regulation seems to serve as a key interface between chronic stress and the development of stress-related disorders. Therefore, to understand individually differential phenotypes after chronic stress, it is important to clarify moderating factors to contribute to the HPA axis dysregulation.

The stress-related disorders are known to be considerably heritable [6, 15]. In particular, dysfunction in HPA axis was proposed as one of the most heritable biological markers of major depression [16]. Also, genetic predisposition contributes to the stress regulatory HPA axis [11, 17, 18]. Therefore, genetic variations may play a role in the dysregulated HPA axis responses to stressors and subsequently contribute to development of stress-related disorders. However, the role of particular genes in the underlying stress responses and the psychopathologies are not well understood so far.

GR-related genes regulating the HPA axis are a potent candidate for differences of individual HPA axis responses and vulnerability to stress-related psychiatric problems [19]. In particular, genetic variants of FK506 binding protein 5 (FKBP5), the major regulatory protein of the HPA axis have received growing interest [20-22]. FKBP5, a co-chaperone of hsp90 binds to the GR and modulates glucocorticoid sensitivity [20]. A FKBP5 overexpression in New World Monkeys has been reported to be associated with GR insensitivity [23]. FKBP5 gene is located on chromosome 6p21 and the gene variants are

known to facilitate altered GR sensitivity, leading to decreased efficiency of the negative feedback of HPA axis and dysregulated stress response in healthy individuals [24]. A recent cohort study showed that the FKBP5 rs1360780 was associated with depression status and FKBP5 is an interesting gene target for depression and treatment response [21]. In addition, a study of PTSD suggested that FKBP5 gene variants moderate the effects of early life stress on the stress hormone system, thus developing adult PTSD symptoms [25]. Taken together, these suggest that FKBP5 gene variants may contribute to dysregulated biological responsivity and then leading to vulnerable phenotypes such as depression and anxiety in the face of long-lasting stressors.

Although there have been numerous studies of stress response after chronic stress exposure, most of them were conducted using animal model and in vitro experiments. It is not easy to examine biological stress responsivity and behavioral stress responses under similar stress situations in human real life. One of the most distressing events in human life is a disease. In particular, cancer, a chronic life-threatening illness can be a multi-dimensional trauma and causes high stress responses [26]. Therefore, cancer patients have to suffer a long process of adaptation to multiple stressful events such as chemotherapy during treatment courses [27-30]. A previous study showed that 47.2% and 57% of patients with gastrointestinal cancer had high distress on anxiety and depression subscales of the Hospital Anxiety and Depression Scale (HADS) [31]. Specially, gastric cancer is the most prevalent cancer and the second main cause of death by cancer in East Asia [32]. Furthermore, many patients with gastric cancer tend to be in an non-cured advanced stage at the first diagnosis due to its vague symptoms, and patients with advanced gastric cancer have a poor prognosis and short survival of only 7~9 months with chemotherapy [33]. For these reasons, people with advanced gastric cancer cannot help but think the danger to befall them and face stressful situations, thus showing to high stress responses.

The purpose of the present study was to elucidate the influence of FKBP5 gene polymorphisms regulating the HPA axis on distress levels in advanced gastric cancer patients faced with a similar stressful situation. To improve our knowledge about predictive values of psychological distress level, using a prospective design, initial psychological distress in patients newly diagnosed with advanced gastric cancer was examined and the distress level was followed up at 6 week after 2 cycles of chemotherapy.

II. MATERIALS AND METHODS

1. Participants

Participants included 130 patients (90 males, 40 females) with advanced non-resectable gastric cancer from the outpatient clinic in the Yonsei Cancer Center and Oncology Clinic at Gangnam Severance Hospital. All of them were participants for the clinical trial of specific combination chemotherapy. They were newly diagnosed with advanced gastric cancer or recently recurred gastric cancer, and then they have made treatment decision just lately for the palliative chemotherapy. In addition, they were 1) outpatients with histologically confirmed metastatic or locally advanced adenocarcinoma of the stomach, 2) 18 to 60 years, 3) aware of the diagnosis of cancer, 4) able to understand the study and respond to the scales 5) patients with no prior chemotherapy and other advanced disease. Patients were excluded if they were applied to the Eastern Cooperative Oncology Group (ECOG) performance status score 3 or above. Participants with any neurological disorders were also excluded. The ethnicity of all participants was Korean. Written informed consent was obtained from all subjects prior to the beginning of the study, and the protocol was approved by the Institutional Review Board.

Initial psychiatric evaluation was performed within 4 weeks after recognizing advanced gastric cancer, and 1 week before 1st chemotherapy. Follow-up evaluation was conducted at 6 week after completing 2 cycles of chemotherapy (1 cycle = 3 weeks). The self-report of distress levels and adjustment styles and genotyping were performed. Of participants, 93 patients (63 males, 30 females) have received chemotherapy of at least 2 cycles and been followed up. Demographic and clinical data were collected from interview with oncologist and research nurse and medical chart.

2. Measures

A. Hospital Anxiety and Depression Scale (HADS)

Levels of depression and anxiety were defined as a phenotype of stress response. For assessing the distress level, HADS was used. This was designed to measure psychological distress of patients in medical and surgical settings including cancer patients [34]. The HADS is a 14-item instrument that reflects two dimensions; anxiety (7 items) and depression (7 items). Each item is rated on a 4-point Likert scale from 0 to 3, with a maximum of 21 for anxiety and depression, respectively. For each subscale, a score of between 8-10 identified possible cases, and a score of 11 or more the probable cases of a clinically meaningful anxiety or depression [34]. The HADS has been previously validated for the Korean population [35].

B. Mini-Mental Adjustment to Cancer (Mini-MAC) scale

Since coping with illness can affect distress levels, individual coping patterns were assessed with Mini-MAC scale. The Mini-Mac scale is a widely used disease-specific questionnaire which is designed to measure mental adjustment and coping styles to cancer [36]. The Mini-MAC scale consists of 29 items using a 4-point Likert-type scale and represents five dimensions of Fighting Spirit, Hopeless/Helplessness, Anxious Preoccupation, Fatalism, and Cognitive Avoidance [36]. Fighting Spirit dimension with 4 items is characterized by a determination to fight the illness and the adoption of an optimistic attitude. Hopeless/Helplessness dimension with 8 items is related to feelings of giving up and engulfment by knowledge of the diagnosis and a pessimistic attitude. Anxious Preoccupation dimension with 8 items is characterized by constant preoccupation with cancer and feelings of devastation, fear and apprehension. Fatalism dimension with 5 items measures a tendency to accept unavoidable

situations such as putting oneself in the hands of God. Cognitive Avoidance dimension with 4 items measures a tendency to block off or ignore problem or emotions. The Korean version of the Mini-MAC has been shown to have overall good reliability and validity in Korean population [37].

C. ECOG performance status scale

The ECOG performance status scale was used to measure physical ability of patients [38]. This is an observer scale for the daily living ability of cancer patients which is graded from 0 to 4. Zero indicates that the patient is able to carry out all normal activities, and 4 indicates that the patient is completely disabled.

D. Adverse effects related to chemotherapy

Adverse effects were measured for all possible events such as nausea, vomiting and abnormality of laboratory findings. Evaluation of adverse effects were performed by research nurses and graded from 0 to 4 regarding each dimension (NCI-CTC criteria, version 2.0). Zero indicates no occurrence of adverse event and 4 indicates the occurrence of the most serious adverse event. In the analyses of our data, we included only 6 dimensions out of various adverse effects, which have been expected to occur frequently and significantly affect psychological distress. They were nausea, vomiting, fatigue, anorexia, abdominal pain, and other serious adverse events.

3. Genotyping

A blood sample through venipuncture was donated by participants, and the genomic DNA was isolated using standard techniques. Three SNPs of the FKBP5 locus, rs1360780, rs9296158 and rs9470080 were targeted. The genotyping was screened using single base primer extension assay using the ABI PRISM SNaPShot Multiplex kit according to manufacturer's instructions (ABI, Foster City, CA, USA). The forward and reverse primer pairs used for the assay were 5′-CCTGAAAAGATTATCTGATGC-3′ (forward) and 5′-GCAAAGTCTCCACTGTTTCT-3′ (reverse) for the rs1360780, 5′-AAAAGGGT AGAACGCTTTAGA-3′ (forward) and 5′-ATTCCATG CCCAATAAAAC-3′ (reverse) for the rs9296158, and 5′-ATGAGCCACTGTGTCCAG -3′ (forward) and 5′-AACCAAACTTT CCAGATGAA-3′ (reverse) for the rs9470080. The genomic DNA flanking the SNP was amplified with PCR reaction with the forward and reverse primer pairs and standard PCR reagents in 10 microliter reaction volume, containing 10ng of genomic DNA, 0.5pM of each oligonucleotide primer, 1 microliter of 10X PCR Gold buffer, 250μM dNTP, 3mM $MgCl_2$ and 0.25 unit i-StarTaq DNA Polymerase (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea). The PCR reactions were carried out as follows: 10 minutes at 95℃ for 1 cycle, and 35 cycles on 95℃ for 30 seconds, 55℃ for 1 minute, 72℃ for 1 minute followed by 1 cycle of 72℃ for 10 minutes. After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP) and exonuclease I (USB Corporation, Cleveland, OH, USA) at 37℃ for 75 minutes and 72℃ for 15 minutes to purify the amplified products. One microliter of the purified amplification products were added to a SNaPshot Multiplex Ready reaction mixture containing 0.15pmols of genotyping primer for primer extension reaction. The primer extension reaction was carried out for 25 cycles of 96℃ for 10 seconds, 50℃ for 5 seconds, and 60℃ for 30

seconds. The reaction products were treated with 1 unit of SAP at 37℃ for 1 hour and 72℃ 15 minutes to remove excess fluorescent dye terminators. One microliter of the final reaction samples containing the extension products were added to 9 microliter of Hi-Di formamide (ABI, Foster City, CA). The mixture was incubated at 95℃ for 5 minutes, followed by 5 minutes on ice and then analyzed by electrophoresis in ABI Prism 3730xl DNA analyzer. Analysis was performed using Genemapper software (version 4.0; Applied Biosystems).

4. Statistical analysis

Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview/) [39] was used to determine the linkage disequilibrium structure of the SNPs and to test for Hardy-Weinberg Equilibrium for genotype frequencies. The *t* test was used to evaluate group differences on continuous variables. To compare the HADS scores between the initial and follow-up points, paired-sample t-test was used. In addition, Pearson's correlation coefficient was used to explore relationships between clinical variables and patients' distress levels.

The primary analysis was to examine the main effect of the FKBP5 SNPs on anxiety and depression levels measured by HADS. To determine if there was an effect of genotype group or time for distress level, one-way analysis of variance (ANOVA) with repeated measures was carried out to determine the significant change over time among 3 genotype groups for each SNP. The HADS scores at the two time points of initial phase before chemotherapy and follow-up phase after 6 weeks were the two levels on the within-group factor. The three groups according to each genotype were the three levels on the between-group factor.

Next, step-wise multiple linear regression analyses were conducted to determine whether three SNPs of FKBP5 gene are predictors of anxiety and depression symptoms in response to long-lasting stress in cancer patients. The

dependent variables of the regression analyses were each of follow-up scores of HADS-anxiety and HADS-depression, and the degree of change on HADS-anxiety and HADS-depression scores over time. As the independent variables, three SNPs of the FKBP5 gene and clinical variables identified as significant in correlation analyses were included. Because the repeated measures ANOVA were conducted for three SNPs, a Bonferroni correction was performed to correct for multiple testing in ANOVA with the level of significance set to alpha $< 0.05/3=0.017$. In other analyses, the significance was accepted at $p<0.05$. All tests were two-tailed. The data were analyzed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

III. RESULTS

1. Characteristics of participants

The participants comprised 90 males and 40 females. The median age of the participants was 60 years (range 20-77 years). All participants were patients with advanced gastric cancer who were diagnosed pathologically as gastric adenocarcinoma and supposed to start chemotherapy for clinical trial. No participants had taken previous chemotherapy. ECOG performance status of most participants was 0 (14.6%) or 1 (83.8%). At the initial assessment, the mean scores of HADS-anxiety and HADS-depression were 6.4 (4.0) and 7.6 (4.0), respectively, and the mean scores of Mini-MAC scale were 14.0 (4.0), 20.2 (5.1), 13.7 (2.6), 12.0 (2.0) and 10.7 (2.1) for Hopeless/Helplessness, Anxious Preoccupation, Fatalism, Fighting Spirit and Cognitive Avoidance, respectively. No significant differences were observed between male and female (all $p>0.05$).

After 2 cycles of chemotherapy, 93 patients (63 males, 30 females) were available for follow-up assessment. Their median age was 61 years (range 20-77 years). Their mean scores of HADS-anxiety and HADS-depression at follow-up point were 6.5 (5.4) and 8.5 (5.1), respectively. Demographic and clinical variables were presented in Table 1.

Among participants, patients newly diagnosed with metastatic gastric cancer were 82.8%, and patients with recently recurred gastric cancer were 17.2%. There were no significant differences of clinical variables including anxiety and depression scores between them (all $p>0.05$).

Table 1. Demographic and clinical characteristics of participants followed up after 6 weeks (N=93)

	Mean ± SD (range) or Number	%
Age (years)	58.1 ± 11.6 (Median 61, range 20-77)	
Male/Female	63/30	67.7/32.3
Body Mass Index	21.6 ± 3.0 (15-29)	
Cancer Type		
Gastric adenocarcinoma	93	100.0
Cancer Stage		
Metastatic AGC	77	82.8
Recurrent AGC	16	17.2
ECOG		
0	11	11.8
1	80	86.0
2	2	2.2
HADS-anxiety (initial)	6.5 ± 4.0 (0-21)	
HADS-depression (initial)	7.7 ± 4.1 (0-19)	
HADS-anxiety (follow-up)	6.5 ± 5.4 (0-28)	
HADS-depression (follow-up)	8.5 ± 5.1 (0-28)	
Mini-MAC Hopeless/Helplessness	13.8 ± 3.9 (8-31)	
Mini-MAC Anxious Preoccupation	20.4 ± 5.0 (8-32)	
Mini-MAC Fatalism	13.8 ± 2.6 (8-20)	
Mini-MAC Fighting Spirit	11.9 ± 2.0 (6-16)	
Mini-MAC Cognitive Avoidance	10.9 ± 2.1 (6-16)	

SD: standard deviation, AGC: Advanced Gastric Cancer, ECOG: Eastern Cooperative Oncology Group performance status scale, HADS: Hospital Anxiety and Depression Scale, Mini-MAC: Mini-Mental Adjustment to Cancer scale

2. C**hanges in the HADS subscales over time and clinical variables related to HADS scores**

In the sample followed up after 6weeks (N=93), there were significantly positive correlations between initial and follow-up levels of HADS-anxiety and HADS-depression ($r=0.59$, $p<0.001$, and $r=0.68$, $p<0.001$, respectively). Paired-sample t-test of HADS scores over time showed that the mean scores of HADS-anxiety at the follow-up point were not significantly different from the initial level of anxiety ($p=0.99$), whereas the mean scores of HADS-depression at the follow-up point were significantly higher than the initial level of depression ($p= 0.038$). When the cut-off value of possible cases was set as 8 and that of probable cases as 11 on the HADS subscale, possible and probable cases of anxiety were 26.9% and 11.8% of participants at the initial assessment and 14.0% and 11.8% of them at the follow-up point. In addition, possible and probable cases of depression were 25.8% and 22.6% of participants at the initial assessment and 35.5% and 22.6% of them at the follow-up point. The numbers of individuals with clinically meaningful anxiety and depression were given in Table 2 and Figure 1.

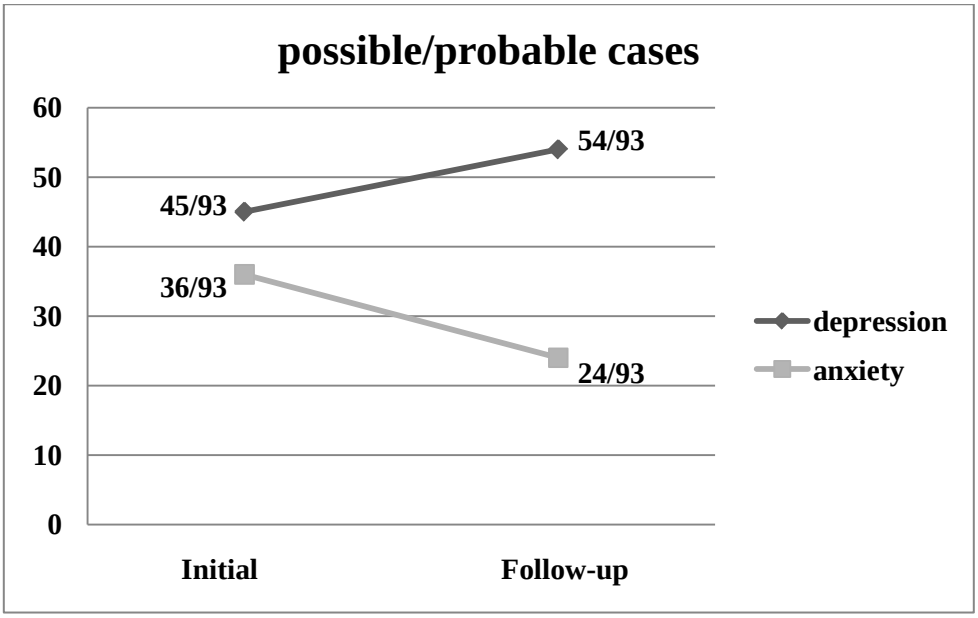
In the Pearson's correlation analyses, adverse effects of chemotherapy had no correlations with HADS-anxiety and HADS-depression at follow-up (all $p>0.05$). ECOG also had no correlations with them ($r=0.10$, $p=0.34$, and $r=0.19$, $p=0.07$, respectively). Time from the initial diagnosis (periods from date first diagnosed with cancer to visit date for initial assessment) was positively correlated with HADS-depression ($r=0.23$, $p=0.028$). Anxious Preoccupation of Mini-MAC scale was significantly positively related to HADS-anxiety and HADS-depression ($r=0.48$, $p<0.001$, and $r=0.33$, $p<0.001$, respectively). Also, Hopeless/Helplessness was significantly positively related to them ($r=0.40$, $p<0.001$, and $r=0.42$, $p<0.001$, respectively). Other clinical variables showed no significant associations with HADS scores ($p>0.05$).

Table 2. Changes in the number of possible or probable cases assessed by of the HADS over time

N= 93	Possible/probable cases	Initial	Follow-up
HADS-anxiety	No. of possible case (%)	25/93(26.9%)	13/93(14.0%)
	No. of probable case (%)	11/93(11.8%)	11/93(11.8%)
HADS-depression	No. of possible case (%)	24/93(25.8%)	33/93(35.5%)
	No. of probable case (%)	21/93(22.6%)	21/93(22.6%)

HADS: Hospital Anxiety and Depression Scale

Figure 1. Line graphs showing changes in the number of the possible/ probable cases with depression or anxiety over time: The number of possible or probable cases of the total numbers of participants are shown at the two time points of initial phase before chemotherapy and follow-up phase after 2 cycles of chemotherapy.



3. **Genotype distributions of participants**

The rs1360780, rs9296158 and rs9470080 polymorphisms of the FKBP5 were genotyped. For the rs1360780 polymorphism, the most prevalent genotype was that with CC (61.3%), followed by that with CT (32.3%), and TT (6.5%). For the rs9296158 polymorphism, the most prevalent genotype was that with GG (54.8%), followed by that with GA (36.8%), and AA (8.6%). Distribution for the rs9470080 was CC (52.7%), CT (38.7%), and TT (8.6%). Genotype frequencies of the three SNPs were in Hardy-Weinberg equilibrium (p=0.60, p=0.63 and p=0.85, respectively). Table 3 lists the genotype distributions of the FKBP5 gene polymorphisms. The three SNPs were in high linkage disequilibrium with r^2 values ranging from 0.70 to 0.89.

In the present analyses, the frequencies of minor alleles of the FKBP5 rs1360780, rs9296158 and rs9470080 were only 6.5%, 8.6% and 8.6% respectively. In the subsequent regression analyses, the genotypes of the three SNPs were coded as 0, 1, or 2 according to the count of the minor allele [25].

Table 3. Genotype and allele frequencies and genotype classification for the FKBP5 gene polymorphisms in participants (N=93)

Genotype/Allele	Subject	
	N	%
FKBP5 rs1360780		
Genotype		
CC	57	61.3
CT	30	32.3
TT	6	6.5
Allele		
C	144	77.4
T	42	22.6
FKBP5 rs9296158		
Genotype		
GG	51	54.8
GA	34	36.8
AA	8	8.6
Allele		
G	136	73.1
A	50	26.9
FKBP5 rs9470080		
Genotype		
CC	49	52.7
CT	36	38.7
TT	8	8.6
Allele		
C	134	72.0
T	52	28.0

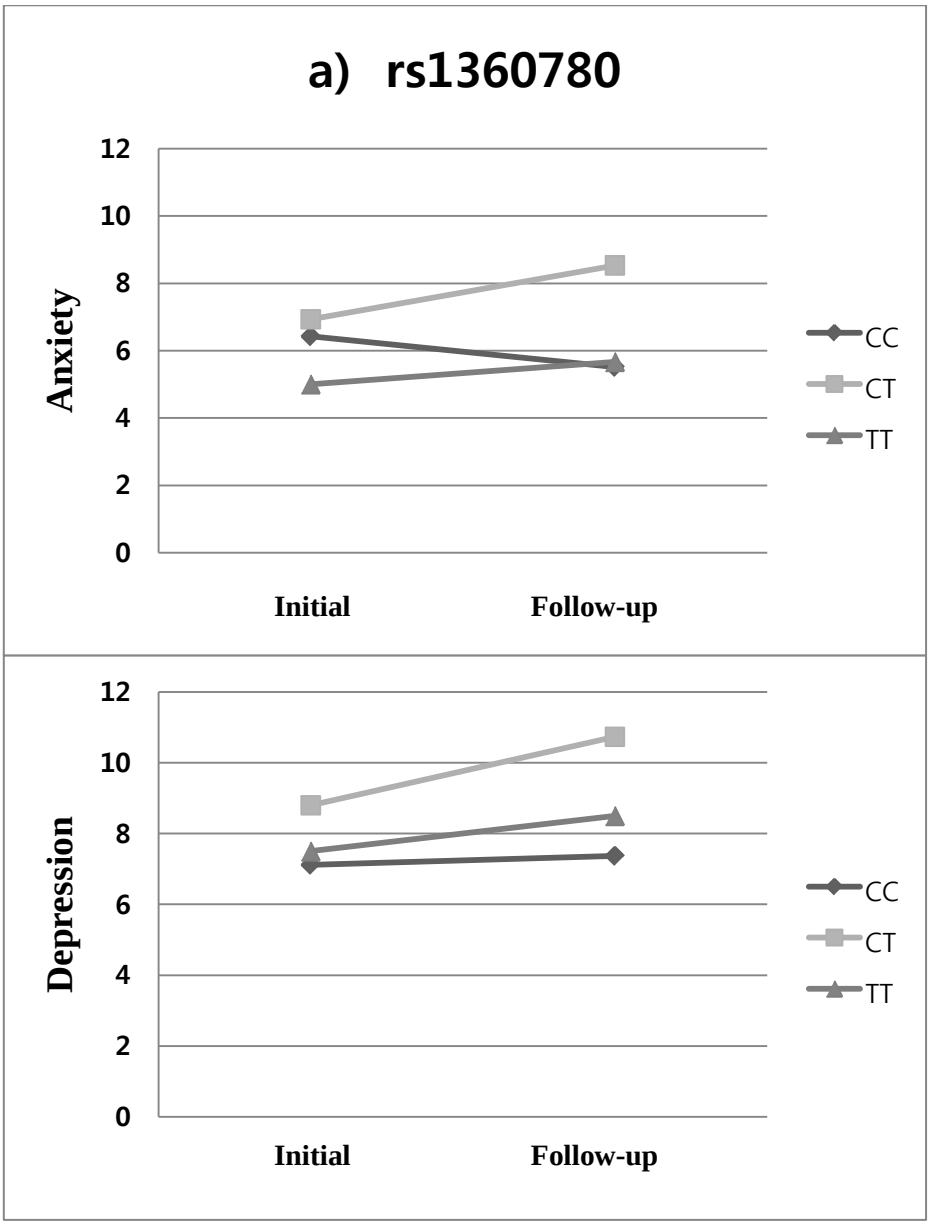
4. Changes in depression and anxiety levels over time according to genotypes of the FKBP5 gene polymorphisms

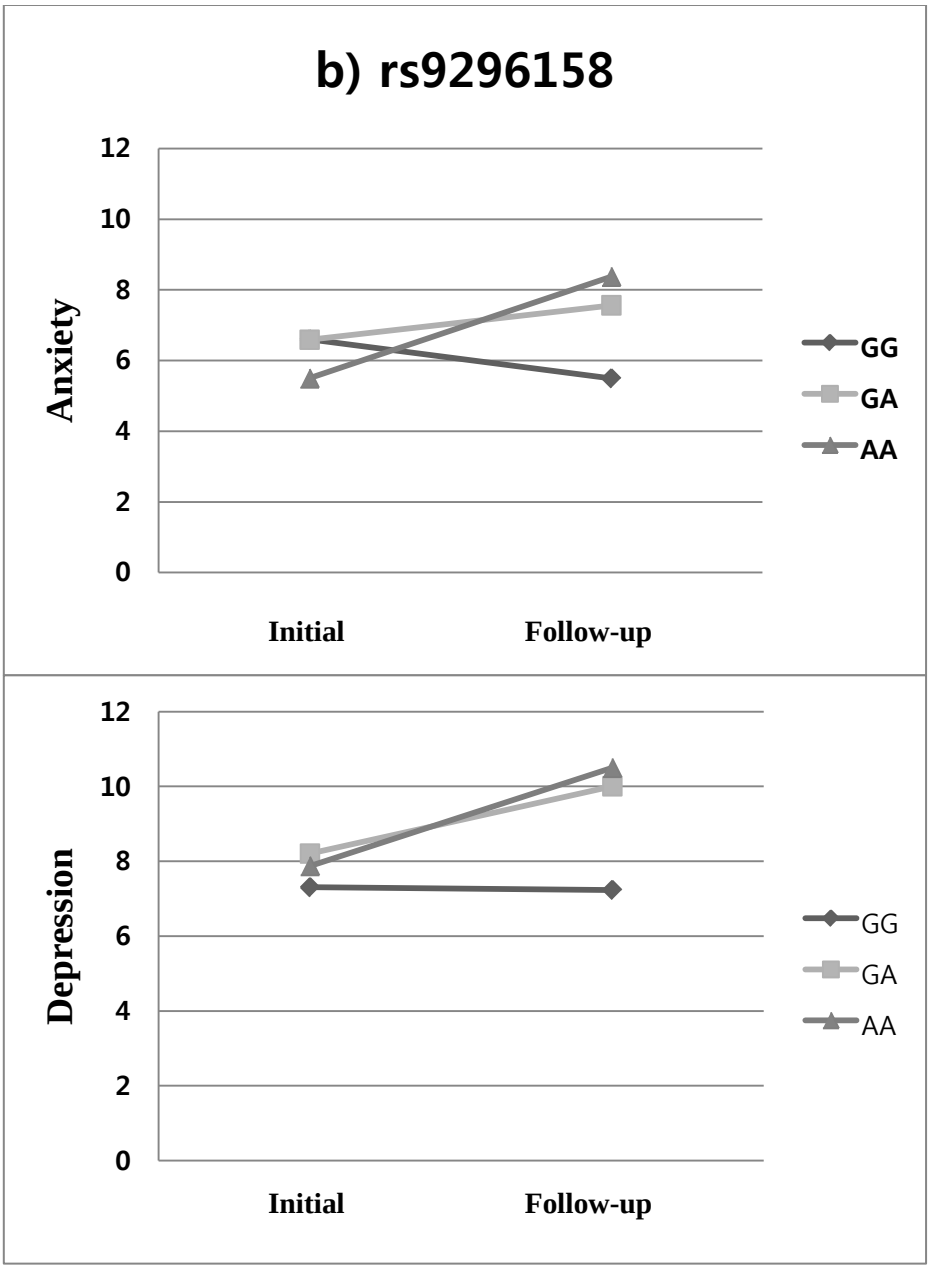
For the rs1360780, repeated measures ANOVA for HADS-anxiety indicated no significant time effects (F=0.45, p=0.50) and between-group difference (F=2.00, p=0.14). However, there was a marginally significant group-by-time interaction in HADS-anxiety (F=3.39, p=0.038). For HADS-depression, the rs1360780 had no significant time effects (F=3.25, p=0.075), but it had a marginally significant between-group difference (F=3.71, p=0.028). There was no significant group-by-time interaction on HADS-depression for the rs1360780 (F=1.93, p=0.15) (Figure 2a).

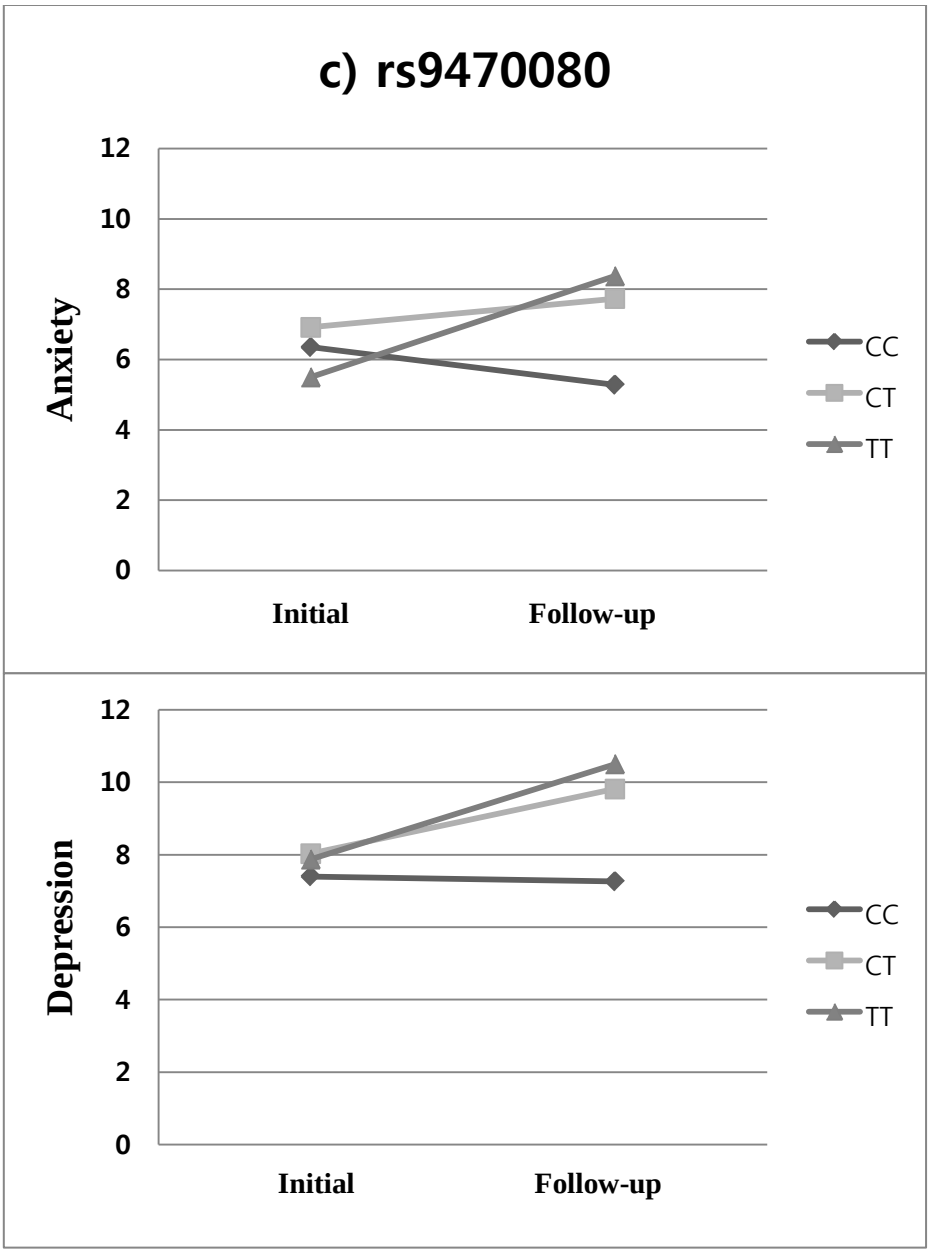
For the rs9296158, repeated measures ANOVA for HADS-anxiety showed no significant differences between periods (F=2.34, p=0.13) and between groups (F=0.65, p=0.52). For HADS-depression, there was no between-group difference (F=2.27, p=0.11), whereas there was a significant effect of time (F=7.85, p=0.006). In addition, the rs9296158 had a significant group-by-time interaction for HADS-anxiety (F=4.38, p=0.015) and there was a marginally significant group-by-time interaction for HADS-depression (F = 3.57, p= 0.032) (Figure 2b).

For the rs9470080, repeated measures ANOVA for HADS-anxiety showed no significant differences between periods (F=2.14, p=0.15) and between groups (F=1.38, p=0.26). For HADS-depression, the rs9470080 had no significant difference between groups (F=1.78, and p=0.17), whereas it had a significant time effect (F=7.64, p=0.007). In addition, the rs9470080 had a marginally significant group-by-time interaction for HADS-anxiety and HADS-depression (F=3.95, p=0.023, and F=3.79, p=0.026, respectively) (Figure 2c).

Figure 2. Changes in anxiety and depression scores according to genotypes between the two time points of initial and follow-up phase; the line graphs showing group differences of the changes in anxiety and depression scores of HADS over time in a) rs1360780, b) rs9296158, and c) rs9470080




b) rs9296158
Anxiety
12
10
8
6
4
2
0
Initial
Follow-up
GG
GA
AA
Depression
12
10
8
6
4
2
0
Initial
Follow-up
GG
GA
AA


c) rs9470080
Anxiety
12
10
8
6
4
2
0
Initial
Follow-up
CC
CT
TT
Depression
12
10
8
6
4
2
0
Initial
Follow-up
CC
CT
TT

5. Regression analysis

In the step-wise linear regression analyses, independent variables included the three SNPs which were coded as 0, 1, or 2 according to the minor allele count, coping styles of Mini-MAC, and time from the initial diagnosis. The result showed that FKBP5 rs9470080 and Anxious Preoccupation dimension of the Mini-MAC were significant predictors of the HADS-anxiety at follow-up. In addition, FKBP5 rs9296158, Hopeless/Helplessness and time from the initial diagnosis were significant predictors of the HADS-depression at follow-up. For the changes in HADS-anxiety and HADS-depression scores, FKBP5 rs9296158 and FKBP5 rs9470080 were significant predictors, respectively. FKBP5 rs9296158 accounted for 8% of changes in HADS-anxiety. In addition, FKBP5 rs9470080 explained 6% of changes in HADS-depression (Table 4).

Table 4. Results from step-wise linear regression for distress levels

	Unstandardized Coefficients B	Standardized Coefficients Beta	p-value
HADS-anxiety (at follow-up)	Adjusted R^2 =0.25,	F=16.10,	p=0.000
FKBP5 rs9470080	1.63	0.20	0.034
Anxious Preoccupation	0.50	0.46	0.000
HADS-depression (at follow-up)	Adjusted R^2 =0.26,	F=11.61,	p=0.000
FKBP5 rs9296158	1.83	0.23	0.012
Hopeless/Helplessness	0.54	0.41	0.000
Time from the initial diagnosis	0.05	0.21	0.022
Changes in HADS-anxiety	Adjusted R^2 =0.08,	F=8.84,	p=0.004
FKBP5 rs9296158	2.02	0.309	0.004
Changes in HADS-depression	Adjusted R^2 =0.06,	F=7.31,	p=0.008
FKBP5 rs9470080	1.60	0.27	0.008

IV. DISCUSSION

The present study prospectively investigated the influence of FKBP5 gene polymorphisms on stress responses in patients newly diagnosed with advanced gastric cancer. The FKBP5 rs9296158 and rs9470080 showed a group-by-time interaction effect for HADS-anxiety and HADS-depression in repeated measures ANOVA. In addition, step-wise linear regression analyses revealed that FKBP5 rs9296158 and rs9470080 were significant predictors of the changes in HADS-anxiety and HADS-depression scores. For the rs1360780, there was a marginally significant group-by-time interaction in HADS-anxiety ($p=0.038$). These findings suggest that the FKBP5 gene polymorphisms regulating HPA-axis may be associated with anxiety and depression levels after prolonged stress exposure during cancer treatment courses.

FKBP5 gene polymorphisms have not been reported before in Korean population, as far as we know. The genotypic distributions were similar to those presented in Japanese populations (The International HapMap Project). Linkage disequilibrium analysis of the rs9296158, rs1360780 and rs9470080 demonstrated that the three SNPs were in tight linkage disequilibrium with each other. The present study also showed that patients with homozygous minor allele of the rs9296158 and rs9470080 had a tendency of higher distress levels after long-lasting stress, although not showing statistically significant group difference (Figure 2). These genetic variants have been previously reported to be associated with vulnerability to psychopathology [22, 25]. Binder et al. reported that these SNPs of FKBP5 has significant interactions with environmental factors such as childhood trauma [25]. In particular, rs9296158 had the most significant interaction effect with environment and subjects carrying minor A allele had significantly higher post-traumatic stress symptoms. A recent study revealed that rs9470080 had significant main effects on suicide attempt and a haplotype of four SNPs of

rs3800373, rs9296158, rs1360780 and rs9470080 increased risk of suicide attempts only among individuals with childhood trauma [22].

In the present study, for the rs1360780, none reached statistically significance. However, due to the low frequency of TT genotype of the rs1360780 (6.5%), when we grouped them into two categories of CC and non-CC (CT + TT) genotype to conserve statistical power, repeated measures ANOVA for of HADS-depression showed a significant time effect (F=6.39, p=0.013) and between-group difference (F=6.49, p=0.013) and a marginally significant group-by-time interaction (F=3.58, p=0.06), in which carriers with minor T allele were associated with higher depression. Homozygous minor T allele of the rs1360780 was reported to have increased FKBP5 protein levels and be related to depression and treatment response [21, 24]. A recent study showed that subjects with TT genotype of the rs1360780 are related to elevated cortisol levels and insufficient and prolonged recovery after repeated psychosocial stress [40]. The authors suggested that minor T allele may contribute to a vulnerability to the stress-related disorders that is related to an impaired recovery from stress. Also, in another study with a large sample, FKBP5 rs1360780 was shown to be related to cortisol reactivity and have a significant interaction with insecure attachment, in which infants with minor T allele had a double-risk for increased cortisol reactivity.[41] Furthermore, the FKBP5 is suggested to be a potential therapeutic target for the prevention and treatment of stress-related psychiatric disorders [20]. When considering linkage disequilibrium of the three SNPs, our findings indicate that FKBP gene may serve as a link through modulating the HPA axis between prolonged stress and the development of stress-related psychiatric disorders.

So far, there is little research of genetic influence on stress-related phenotypes after prolonged real stress exposure in human. The present study was conducted with a relatively homogenous sample exposed to long-lasting stressor with same type in real life. Distress levels were assessed at the two

time points. Because participants were patients newly diagnosed with advanced gastric cancer and supposed to receive chemotherapy for the first time, the point for initial assessment would be related to the time of acute high stress with patients' emotional disturbance from cancer diagnosis and anticipatory fear of chemotherapy. On the other hand, the point for follow-up assessment would be related to period of long-lasting stress during 6 weeks of palliative chemotherapy. For the sample as a whole, paired-sample t-test over time showed that anxiety level at follow-up point was not significantly different from initial state, whereas depression level at follow-up point was significantly higher than initial state (8.5 ± 5.1 vs. 7.7 ± 4.1, $p=0.038$). For the FKBP5 genotypes, as shown in Figure 2, there were no significant differences of initial anxiety and depression levels among the three groups on each SNP, whereas distress level at 6 week follow-up tends to be different depending on FKBP5 genotypes. These findings indicate that the genetic variants of FKBP5 may play an important role in individual vulnerability to prolonged stress response rather than acute stress response. Not surprisingly, this can be explained by alteration of the GR sensitivity after prolonged stress exposure, because FKBP5 expression is associated with receptor sensitivity.

On the other hand, individual cognitive and behavioral responses to cancer have been considered as an important determinant of individual distress levels [42-44]. Accordingly, to approach stress responses to cancer, it may be essential to evaluate patients' adjustment and coping styles to cancer together. When HADS scores at follow-up were examined as a dependent variable in step-wise linear regression analyses, copying styles of Hopeless/Helplessness and Anxious Preoccupation as well as FKBP5 gene polymorphisms were the main factors influencing distress levels in advanced cancer patients. However, when changes in HADS score from initial to follow-up point were considered as a dependent variable, the influence of coping styles disappeared and only genetic factor of FKBP5 remained as a

significant predictor. This indicates that distress level itself is determined by variable factors including coping styles and genetic factors, but individual adaptation following long-lasting stress exposure is modulated by genetic factors regulating HPA axis rather than by coping styles.

Although gene variants regulating FKBP5 expression play an important role in modulating the GR sensitivity and altering HPA axis, this may explain just small portion of manifestation of complex stress response and disease vulnerability. A growing body of research suggests genetic and environmental effects on stress response. Binder et al. showed that child trauma interacts with the FKBP5 gene and leads to adult PTSD symptoms [25]. They reported that the alleles of the FKBP5 gene variants with more FKBP5 protein and mRNA expression had association with GR resistance (i.e. less suppression in the dexamethasone test) in healthy individuals while the same alleles had association with increased GR sensitivity (i.e. a higher dexamethasone suppression ratio) in people with PTSD symptoms related to child abuse [25]. The reversal of the functional association suggests a potential influence of childhood environment on trauma-related psychopathology. In our study, environmental factors such as childhood trauma that might interact to genetic factors for stress responses were not considered. Also, other confounding environmental factors such as family conflict and socioeconomic status were not considered. These are major limitations of the present study.

Another limitation of this study was the relatively small sample size, although our sample consisted of relatively homogenous population exposed to same stressor. Considering the limited sample size, genetic influences of the above mentioned polymorphisms need to be interpreted with caution. In addition, we examined just clinical phenotypes to stress, and did not measure physiological stress responsivity such as cortisol reactivity according to the genotypes. Further research should be undertaken to confirm the influence of the FKBP5 gene polymorphism on physiological stress response. Finally, the

present study examined quantitative distress level just based on self-report and did not classify phenotypes as a categorical clinical disorder using structured interview. Because previous studies of FKBP5 also consisted of people with psychiatric problems who do not seek treatment, the findings cannot be generalized to patients with clinical disorders. For a better understanding of genetic vulnerability of stress-related psychiatric disorders, further studies in the clinical samples with a case-control design are needed.

V. CONCLUSION

In this thesis, the results showed that genetic variations of FKBP 5 and patients' coping patterns to cancer were potent predictive factors for anxiety and depression in patients with advanced cancer. In particular, our findings indicate that genetic components such FKBP5 gene polymorphisms play a crucial role in anxiety and depression following prolonged stress exposure. A better understanding of genetic influences on stress responses might provide insight into the vulnerability of stress-related psychiatric disorders. Although the sample size is small to draw any firm conclusions on this issue, our results are meaningful in that we included relatively homogenous population exposed to real life stressors with similar level and used a prospective design. To confirm genetic influence of FKBP5 on psychological morbidity following prolonged stress, further investigation is required with a larger population.

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< ABSTRACT (IN KOREAN)>

유전적 특성이 암환자의 스트레스 반응에 미치는 영향

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암환자들은 암의 진단 및 치료과정에서 여러 스트레스를 겪으면서 다양한 스트레스 반응을 나타낸다. 유전적 요인은 개개인의 스트레스 반응의 차이와 정신 질환의 취약성에 영향을 줄 것이다. 본 연구는 시상하부-뇌하수체-부신 축을 조절하는 FKBP5 유전자 다형성이 암으로 고통 받고 있는 환자의 불안, 우울과 같은 스트레스 반응에 미치는 영향을 살펴보고자 하였다.

진행성 위암을 새롭게 진단받고 항암치료를 처음 받기로 예정되어 있는 환자를 대상으로 전향적 연구 설계로 진행하였다. 진행성 위암으로 확진된 130명의 암환자(남자 90명, 여자 40명)를 모집하여 진단받은 지 1개월 이내에 기초 평가를 실시하였다. 이들 중 6주 후에 평가 가능했던 93명의 환자(남자 63명, 여자 30명)에 대하여 추적 평가를 실시하였다. Hospital Anxiety and Depression Scale 과 Mini-Mental Adjustment to Cancer Scale 등을 이용하여 우울, 불안, 대처방식 등을 평가하였다. 유전적 요인에 대해서는

FKBP5 유전자의 세 가지 단일염기다형성 rs1360780, rs9296158, rs9470080 을 조사하였다.

FKBP5 유전적 특성과 6주 후의 불안 및 우울 점수의 변화를 분석했을 때 rs9296158 와 rs9470080 은 유전형에 따른 그룹과 시간과의 교호작용효과를 보였다. 또한 단계적 회기 분석 결과, FKBP5 유전자 및 대처방식은 불안, 우울 정도를 예측하였다. 특히, rs9296158 와 rs9470080 은 시간의 경과에 따른 불안 및 우울 점수 변화의 유의한 예측인자로 나타났다.

본 연구결과는 FKBP5 유전자 다형성과 같은 유전적 특성이 암 치료과정에서 스트레스 반응으로 나타나는 불안 및 우울 증상에 중요한 역할을 할 것임을 시사한다.

핵심되는 말: 암, 스트레스, 우울, 유전자, FKBP5, HPA 축