

DEPRESSION IS ASSOCIATED WITH CRP SNPS IN PATIENTS WITH FAMILY HISTORY

Abstract

Objective: The pathogenesis of depression is not fully understood, but studies have suggested that higher circulating levels of C reactive protein (CRP) might relate to depression occurrence. However, due to the highly variability of individual patients' conditions, the results to date are inconsistent. Considering Single nucleotide polymorphisms (SNPs) of CRP gene have also been suggested to predict plasma CRP levels. In the present study, we hypothesize that inherited CRP allelic variations may co-vary with depressive symptomatology.

Methods: We recruited patients with a diagnosis of depression, with or without family depression history. We then detected serum CRP levels, as well as genome CRP SNPs from participants of this project.

Results: We found a significantly higher circulating CRP levels in patients with a positive family history. Furthermore, we also identified certain inherited CRP SNPs (A allele in rs1417938 and C allele in rs1205) which could up-regulate serum CRP levels and thus be associated with depression occurrence.

Conclusion: Our findings raise new evidence for the relationship between circulating CRP level and depression occurrence.

Keywords

• CRP • depression • SNPs • familial inheritance

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1. Introduction

C-reactive protein (CRP) is a conserved protein which has existed for more than 400 million years and is found among vertebrate and invertebrate animals. CRP can strongly react with C polysaccharide of pneumococcus and thus is how its name is derived [1]. CRP was initially identified in the serum of patients with an acute bacterial infection [2]. At the acute inflammation phase, CRP is produced mostly by the liver and its concentration might rise 2000-fold during the first 24-48 hours of infection. Because serum CRP level is barely detectable under healthy conditions and is usually higher than 10 mg/L in acute inflammation conditions; people have long thought a relatively low level of serum CRP (3-10mg/L) doesn't represent any physiological or pathological situations. However, recent studies have found under some chronic inflammation conditions, such as cardiovascular disease, metabolic syndrome or cancer, patients will

usually have a serum CRP level higher than 3mg/L [3]. Furthermore, studies also indicate an increase in serum CRP may be related to some physical activities, such as smoking [4].

It has been widely acknowledged that chronic inflammation is largely involved in depression [5]. Considering elevated CRP is a primary marker for chronic inflammation, two hypotheses in the field have been raised regarding to inflammation and depression [7]: The first hypothesis is depression related neurobiological conditions may lead to enhanced inflammation and CRP levels. In contrast, the second hypothesis states that elevated CRP or inflammation conditions, in turn, may increase the risk of depression. Plenty of investigations had been made to find out more precise mechanism under CRP and depression, however, the results to date were inconsistent and full of controversy.

Interestingly, both CRP polymorphism and depression are about 40% inheritable

[8]. Furthermore, identical twin studies have suggested a potential genetic pathway which may link depression and elevated CRP levels [9]. In addition, there are several CRP genetic polymorphisms which have been found to regulate serum CRP levels. However, although some studies have implicated CRP in depression occurrence; whether and how CRP involved in it still remains controversial. Considering there is evidence suggesting CRP gene polymorphisms and serum CRP level were susceptibility factors for depression in elder people [10], in the present study, we have investigated family inherited depression and calculated its potential relations with certain inherited CRP SNPs. The aim here is to more accurately describe the role of the CRP gene polymorphisms and serum CRP levels in depression, and also to discuss whether circulating CRP levels may contribute to depression occurrence.

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2. Materials and Methods

2.1 Participants and grouping:

This investigation recruited 200 adults (100 positive and 100 control, age from 18-65) who participated in 2th affiliated hospital of Xinjiang Medical University research program (2013/7-2016/7). Collected data included social-demographic measurements; character of personality, temperament, and psychopathology; general social and cognitive functioning; health-impairing attributes of habit and lifestyle; biological measurements of serum makers; and DNA for the study of genetic variation and phenotypes. All included participants fulfilled ICD-10 diagnosis standard.

Exclusions from our participation including: history of chronic kidney or liver disease, atherosclerotic cardiovascular disease, prior years cancer treatment, and major neurological disorders, schizophrenia or other chronic inflammation related illness such as smoking. Other exclusions included pregnancy and medicine history, such as: insulin, glucocorticoid, antiarrhythmic, psychotropic, or prescription weight-loss medications.

Data collections under approved protocols and guidelines of the Ethics Committee of 2th affiliated hospital of Xinjiang Medical University. 100 depression patients with at least one family members also have depression symptom were assigned as family-DEP group (fDEP), in contrast, 100 depression patients without any clear depression family members were assigned as non-fDEP group (non-fDEP).

2.2. Procedure

Prior to the laboratory visit, participants were asked to get through 8 hours fast and also asked to avoid exercise for 12 hours. Participants were also asked to abstain from alcohol for 24 hours. Laboratory samples were all collected in the morning. Upon subjects' arrival, they were asked to complete a brief medical history and medication use interview with the help of nurse. After this interview, we recorded and calculated subjects BMI (kg/m²) as well as drew 40 ml blood sample for later use. This 40 ml blood sample was separated into 2 portion of use. The first portion of blood was used for

CRP level detection and the second portion was used for DNA isolation and PCR exam later.

2.3. Variable measures

2.3.1. Circulating CRP

Plasma CRP was assayed by using the Abbott Architect c16000 chemistry analyzer (Diamond Diagnostics, MA, USA) as previously described [23]. In this procedure, polystyrene beads are coated with monoclonal antibodies against CRP, which can aggregate CRP antigen and thereby increase the intensity of detecting light. The light intensity is related to the amount of CRP concentrated on the beads. The assay has a detection range of 0.175–1100 mg/L. Logarithmic transformation was used to normalize CRP values distribution.

2.3.2. Depressive symptoms

Depressive symptoms were measured by using Hamilton Rating Scale for Depression (HAM-D-17). The patient is rated by a clinician among 17 dimensions with a score on a 3 or 5 point scale. A score of 0-7 is considered to be normal. Scores of 18 or higher indicate moderate or severe depression.

2.3.3. DNA isolation and SNPs analysis.

DNA was isolated from frozen whole blood samples as previously described [22,24]. The CRP gene is located on chromosome 1 (1q21–23), which has two exons and encodes a protein with 204 amino acid length. In the present study, we have employed five common CRP gene polymorphisms: rs3093059, rs1417938, rs1800947, rs1130864 and rs1205 (Figure 1). We selected these markers by using Tagger

algorithm and the HapMap genotype data in China [6]. Subjects were genotyped by using high resolution melting method (LightCycler 480) followed by software analyze (LightCycler 480 software release 1.5.1.62 SP1).

2.4. Statistical analyses

All data statistical analyses were performed by using SPSS v15.0 (SPSS, Inc., Chicago, IL). χ^2 -Tests was used to compare CRP genotypes distribution within those predicted SNPs, Bonferroni correction was used in post-hoc test to correct for multiple testing (one genotype vs two other genotypes), with a new level of significance as 0.017[27]. The association between CRP SNPs and circulating CRP concentration was investigated and evaluated by using one-way ANOVA with tukey post hoc. The Data were expressed as Mean±SEM.

3 Results

3.1 Population characteristics

Table 1 shows demographic, biochemical and inflammatory values in fDEP and non-fDEP groups. No statistically significant differences ($p < 0.05$) was found in all the parameters we have collected here.

3.2 Circulating CRP levels and depression

In our research, we have found fDEP patients have a significant higher circulating CRP level than non-fDEP patients. In non-fDEP group, the circulating CRP level was 1.144±1.452 mg/L, while in fDEP patients, this number was increased to 1.744±1.635 mg/L ($p=0.035$) (Figure 2).

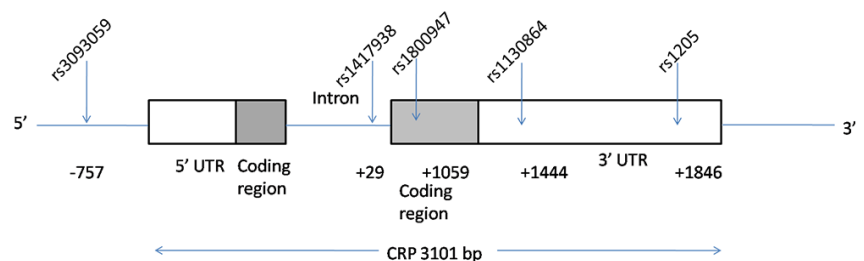


Figure 1: The C-reactive protein (CRP) gene region (chromosome 1q21–q23) and approximate locations of the tag single-nucleotide polymorphism (SNP) locations (dbSNP) relative to the transcription start site.

3.3 CRP polymorphisms and fDEP.

Here we involved 5 well established CRP SNPs (rs3093059, rs1417938, rs1800947, rs1130864 and rs1205, Figure

1) polymorphism and found A allele in rs1417938 and C allele in rs1205 SNP were overrepresented in fDEP patients. On the other hand, rs3093059, rs1800947 and

rs1130864 did not differ between fDEP and non-fDEP patients (table 2).

3.4 CRP polymorphism and circulation CRP level.

The serum CRP level among genotypes of rs3093059 and rs1130864 polymorphisms was comparable. However, we found compare with its major allele, both CC genotype in rs1205 and AA genotype in rs1417938 SNP showed a significant higher serum CRP level (table 3).

4. Discussion

In our study, we examined multiple demographic, biochemical and inflammatory values between fDEP patients and non-fDEP controls, and as shown in Table 1. Most of those factors were not significantly changed between these two groups. However, as shown in Fig 2, we have found an increase in serum CRP level in fDEP patients. Furthermore, we found that two of five variants of the CRP gene were modest but significantly associated with depression patients who had a positive depression family history. These associations were not influenced by other potential mediating factors, such as age, gender and physical or mental health. Furthermore, we reported the circulation CRP level in depression patients with family history was significantly higher than those patients without family history.

To date, inconsistent results have been reported from studies for CRP variants and depression. Two studies have found no associations between depressive and affective symptoms [11-12]. In contrast, Luciano et al [13] examined the relationship between depressive mood state and CRP SNPs in two elderly cohorts while adjusting for age and gender. They reported a modest significant association between rs1800947 and depression in one of the cohorts. Although they found no association with depression and rs1205, Almeida et al [14] reported an association between rs1205 and depression in 3700 men aged 70 years and over. Another study also indicated certain CRP gene haplotype was positively related with depression and high serum CRP level [15].

Considering the difference between reports may come from huge individual patients

Table 1. Demographic features, biochemical and inflammatory variables of the study groups

Variable	non-fDEP groups (n=100)	fDEP groups (n=100)	p value
Gender (male/female)	59/41	56/44	0,571
Age (years)	47.85± 7.23	45.09± 8.12	0,801
BMI (kg/m2)	26.64± 4.22	25.31± 5.01	0,839
Chol (mmol/L)	5.31±0.55	5.18±0.89	0,901
Tg (mmol/L)	1.71±0.79	1.85±0.98	0,912
LDL-C (mmol/L)	3.01±0.98	2.98±0.85	0,981
HDL-C (mmol/L)	1.21±0.76	1.18±0.89	0,977
Gly (mmol/L)	4.71±1.22	4.36±0.87	0,785
Cortisol (nmol/L)	454.4± 26.76	461.32±41.21	0,888
Uric acid (umol/L)	276.64± 17.99	257.68± 17.98	0,458
Homocysteine (umol/L)	14.53± 1.36	18.12±2.11	0,155
Ceruloplasmin (mg/L)	298.4 ± 31.22	269.84±19.68	0,441
HAMD-17 (score)	24.46± 1.77	24.06 ± 1.46	0,862

abbreviation: Chol:cholesterol; Tg: triglyceride; LDL-C: Low-density lipoprotein-Cholesterol; HDL-C: High-density lipoproteins-Cholesterol; Gly-fasting glycose

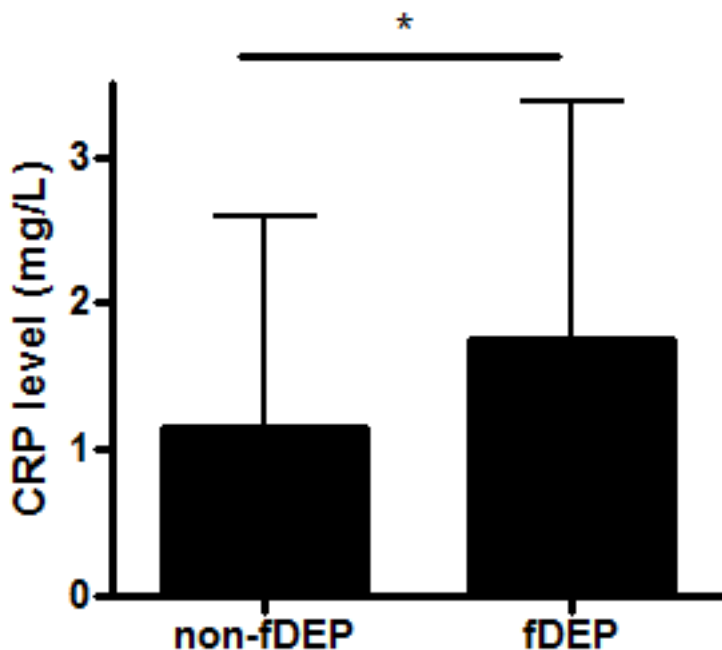


Figure 2: Circulating CRP level in f-DEP and non-DEP groups. Mean ±SEM, * p<0.05.

variations, the current viewpoint in literatures is certain CRP SNPs could regulate serum CRP level [16-19,25]. For example, CC genotype in rs1205 could up regulate serum CRP level [20], while T allele of rs1417938 could down regulate serum CRP level [21]. In the present study, we also found CC genotype in rs1205 and AA genotype in rs1417938 showed a significant higher serum CRP level. The detailed mechanisms for those regulations are still unknown and might also include epigenetic modifications of the gene, which have not been investigated in this study.

Because both depression and CRP polymorphism are around 40% inheritable, we thus analyzed our data and tried to find out whether CRP polymorphism might be related to depression in an inherited manner. Our results here have showed that A allele in rs1417938 and C allele in rs1205 SNP were potentially related with fDEP patients. Those data indicated certain CRP polymorphisms were positive with depression family history. Considering inherited factors would usually show dominant effects than other sporadic factors in diseases, our data further support CRP polymorphism may relate with depression occurrence.

As many previously reports have indicated CRP gene polymorphisms may regulate serum CRP level, we thus also investigated serum CRP level. An interesting result in comparing the major alleles, is that both A allele in rs1417938 and C allele in rs1205 could up regulate serum CRP level, which is consistent with previous reports [16-19]. Finally, we also found serum CRP level in fDEP patients was significantly higher than non-fDEP, which fits the above data regard to high-CRP expression related polymorphisms distribution between non-fDEP and fDEP groups. Although we have to acknowledge that CRP polymorphisms may have functions other than regulating serum CRP, our data still strongly suggests that higher level of serum CRP level might be one reason for depression occurrence.

Previous literature have found serum CRP level might be gender determined or related. Furthermore, a large population scale survey has found serum CRP level were significantly higher in female than male [26]. To investigate whether gender was involved in our study as

Table 2. Frequency of SNPs in different groups

SNP and genotype		non-fDEP (n=100)	fDEP (n=100)	P	post-hoc (genotype vs all others)
		%	%		
rs3093059	TT	88	86	0,902	
	TC	10	12		
	CC	2	2		
rs1417938	TT	56	37	0,005	
	TA	43	48		
	AA	3	13		0,016
rs1800947	GG	84	89	0,32	
	GC	4	5		
	CC	12	6		
rs1130864	CC	45	50	0,172	
	CT	45	45		
	TT	10	5		
rs1205	TT	48	39	0,013	
	CT	46	41		
	CC	6	20		0,003

Table 3. Associations between CRP SNPs and serum CRP levels

SNP and genotype		N	Mean serum CRP (mg/L)	SE	P
rs3093059	TT	174	1,39	0,31	0,976
	TC	22	1,58	0,52	
	CC	4	1,51	0,89	
rs1417938	TT	93	1,01	0,13	0,031
	TA	91	1,36	0,18	
	AA	16	1,98	0,19	
rs1800947	GG	173	1,45	0,35	0,61
	GC	9	0,89	0,17	
	CC	18	0,48	0,28	
rs1130864	CC	95	1,13	0,31	0,753
	CT	90	1,44	0,29	
	TT	15	1,28	0,18	
rs1205	TT	87	0,49	0,26	0,024
	CT	87	0,97	0,21	
	CC	26	1,87	0,54	

a correlated variation, we analyzed our data carefully but failed to find a gender dependent CRP difference. This inconsistency with previous studies may come from: 1. our project recruited fewer patients than previously reported, which might be insufficient to show the gender influence on serum CRP; 2. participants in previous studies included both depressed and non-depressed people; however in our study, all participants were patients with depression.

According to our hypothesis, participants in our project may all maintain a relative higher CRP level than healthy controls, which might overcome gender influence. All above reasons might lead to failure to show a gender dependent serum CRP changes.

In summary, by researching inherited CRP SNPs and depression, our data have showed certain high-CRP expression SNPs appeared in fDEP patients with higher frequency, which

strongly proved those CRP polymorphisms were positively related with depression. Furthermore, our results also showed fDEP patients had higher serum CRP level than non-fDEP, which indicated higher serum CRP level was positively related with depression. Our results could supply novel information for depression occurrence and treatment.

References

- [1] Devaraj S, Valleggi S, Siegel D, Jialal I, Role of C-reactive protein in contributing to increased cardiovascular risk in metabolic syndrome. *Curr Atheroscler Rep.* 2010, 12:110-118.
- [2] The polarity protein Par3 regulates APP trafficking and processing through the endocytic adaptor protein Numb M Sun, SZ Asghar, H Zhang. *Neurobiology of disease.* 2016. 93:1-11
- [3] McDade TW, Borja JB, Kuzawa CW, Perez TL, Adair LS. C-reactive protein response to influenza vaccination as a model of mild inflammatory stimulation in the Philippines. *Vaccine.* 2015, 33 :2004-2008
- [4] Siedlinski M, Klanderma B, Sandhaus RA, Barker AF, Brantly ML, Eden E, McElvaney NG, Rennard SI, Stocks JM, Stoller JK, Strange C, Turino GM, Campbell EJ, Demeo DL. Association of cigarette smoking and CRP levels with DNA methylation in α -1 antitrypsin deficiency. *Epigenetics.* 2012, 7: 720-728
- [5] Lucassen PJ, Oomen CA, Naninck EF, Fitzsimons CP, van Dam AM, Czeh B, Korosi A. Regulation of Adult Neurogenesis and Plasticity by (Early) Stress, Glucocorticoids, and Inflammation. *Cold Spring Harb Perspect Biol.* 2015, 7:a021303
- [6] Sun, M., Bernard, L. P., DiBona, V. L., Wu, Q., Zhang, H. Calcium Phosphate Transfection of Primary Hippocampal Neurons. *J. Vis. Exp.* (81), e50808, doi:10.3791/50808 (2013).
- [7] Pankow, J.S., Folsom, A.R., Cushman, M., Borecki, I.B., Hopkins, P.N., Eckfeldt, J.H., Tracy, R.P. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. *Atherosclerosis*, 2001, 154: 681–689.
- [8] Yang, Kun, Chuanfeng Zhang, Lei Sun, Dong Li, and Xin Hong. Cigarette smoke condensate could promote human bronchial epithelial BEAS-2B cell migration through shifting neprilysin trafficking. *Journal of Cancer Research and Therapeutics.* 2016.
- [9] Su S, Miller AH, Snieder H, Bremner JD, Ritchie J, Maisano C Jones L, Murrah NV, Goldberg J, Vaccarino V. Common genetic contributions to depressive symptoms and inflammatory markers in middle-aged men: the Twins Heart Study. *Psychosom Med* 2009, 71: 152–158
- [10] Altemus M, Sarvaiya N, Neill Epperson C. Sex differences in anxiety and depression clinical perspectives. *Front Neuroendocrinol* 2014, 35: 320–330
- [11] Bremner, M.A., Beekman, A.T., Deeg, D.J., Penninx, B.W., Dik, M.G., Hack, C.E., Hoogendijk, W.J., 2008. Inflammatory markers in late-life depression: results from a population-based study. *J. Affect. Disord.* 106, 249–255.
- [12] Gaysina D, Pierce M, Richards M, Hotopf M, Kuh D, Hardy R. Association between adolescent emotional problems and metabolic syndrome: the modifying effect of C-reactive protein gene (CRP) polymorphisms. *Brain Behav Immun*, 2011, 25: 750–758
- [13] Luciano M, Houlihan LM, Harris SE, Gow AJ, Hayward C, Starr JM, Deary IJ. Association of existing and new candidate genes for anxiety, depression and personality traits in older people. *Behav Genet* 2010, 40: 518–532
- [14] Almeida OP, Norman PE, Allcock R, van Bockxmeer F, Hankey GJ, Jamrozik K, Flicker L. Polymorphisms of the CRP gene inhibit inflammatory response and increase susceptibility to depression: the Health in Men Study. *Int J Epidemiol.* 2009, 38: 1049–1059
- [15] Halder I, Marsland AL, Cheong J, Muldoon MF, Ferrell RE, Manuck SB. Polymorphisms in the CRP gene moderate an association between depressive symptoms and circulating levels of C-reactive protein. *Brain Behav Immun.* 2010, 24:160-167.
- [16] Fan AZ, Yesupriya A, Chang MH, House M, Fang J, Ned R, Hayes D, Dowling NF, Mokdad AH. Gene polymorphisms in association with emerging cardiovascular risk markers in adult women. *BMC Med Genet.* 2010, 11: 6
- [17] M Sun, T Zhou, L Zhou, Q Chen, Y Yu, H Yang, K Zhong, X Zhang, F Xu. Formononetin protects neurons against hypoxia-induced cytotoxicity through upregulation of ADAM10 and sAPP α , *Journal of Alzheimer's Disease* 28 (4), 795-808
- [18] Valkanova V, Ebmeier KP, Allan CL. CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. *J Affect Disord.* 2013, 150:736-744.
- [19] H Wang, M Sun, H Yang, X Tian, Y Tong, T Zhou, T Zhang, Y Fu, X Guo. Hypoxia-inducible factor-1 mediates up-regulation of neprilysin by histone deacetylase-1 under hypoxia condition in neuroblastoma cells, *Journal of neurochemistry* 131 (1), 4-11
- [20] Ancelin ML, Farré A, Carrière I, Ritchie K, Chaudieu I, Ryan J. C-reactive protein gene variants: independent association with late-life depression and circulating protein levels. *Transl Psychiatry.* 2015, 5:e499 .

- [21] Davey Smith G, Ebrahim S, Lewis S, Hansell AL, Palmer LJ, Burton PR. Genetic epidemiology and public health: hope, hype, and future prospects. *Lancet*. 2005, 366: 1484–1498
- [22] Y Yu, L Zhou, M Sun, T Zhou, K Zhong, H Wang, Y Liu, X Liu, Xylocoside G reduces amyloid- β induced neurotoxicity by inhibiting NF- κ B signaling pathway in neuronal cells. *Journal of Alzheimer's Disease* 30 (2), 263-275
- [23] Komurcu-Bayrak E, Erginel-Unaltuna N, Onat A, Ozsait B, Eklund C, Hurme M, Mononen N, Laaksonen R, Hergenc G, Lehtimäki T. Association of C-reactive protein (CRP) gene allelic variants with serum CRP levels and hypertension in Turkish adults. *Atherosclerosis*. 2009, 206: 474–479
- [24] J Yu, M Sun, Z Chen, J Lu, Y Liu, L Zhou, X Xu, D Fan, D Chui, Magnesium modulates amyloid- β protein precursor trafficking and processing. *Journal of Alzheimer's Disease* 20 (4), 1091-1106
- [25] Lacom A, Bas A, Tudela P, Giménez M, Mòdol JM, Pérez M, Ausina V, Dominguez J, Prat-Aymerich C. Correlation of inflammatory and cardiovascular biomarkers with pneumonia severity scores. *Enferm Infecc Microbiol Clin*. 2014, 32 :140-146
- [26] Liu Y, Al-Sayegh H, Jabra R, et al. Association between C-reactive protein and depression: modulated by gender and mediated by body weight. *Psychiatry Res (Ireland)*, 2014, 219: 103-108
- [27] Latta, S.C., C.A. Howell, M.D. Dettling, and R.L. Cormier. Use of data on avian demographics and site persistence during overwintering to assess quality of restored riparian habitat. *Conservation Biology* 2012, 26: 482-492