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Decreased Na+/K+ ATPase α1 (ATP1A1) gene expression in major depression patients' peripheral blood

Research Article

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Abstract: Major depression affects the central nervous system and thereafter the autonomic nervous system, immune system, and endocrine system. Na+/K+ ATPase, as a major mediator of cellular transmembrane ionic gradients, plays an important role in nervous signal transduction. Three types of Na+/K+ ATPase α subunit isoforms (ATP1A1, ATP1A2, and ATP1A3) are found in brain but vary in the type of cell and level of expression. It has been confirmed that reduced expression of ATP1A2 and ATP1A3 are related to depressive disorder. However, there is no reported correlation between ATP1A1 and major depression. This study investigated the potential correlation between ATP1A1 gene expression level and major depression. The expression levels of ATP1A1 gene in the peripheral circulation of both depressive patients and healthy human controls were quantified by using reverse transcripted quantitative polymerase chain reaction. Statistical analysis showed a significant decrease of ATP1A1 expression level in major depression patients when compared to that of healthy controls ($P<0.01$). The differences of gene nucleotide sequences and protein structures among ATP1A1, ATP1A2, and ATP1A3 were also illustrated. This study demonstrates, for the first time, that ATP1A1 gene expression level is significantly associated with major depression and suggests that ATP1A1 could be a significant molecular marker for diagnosis.

Keywords: *Na+/K+ ATPase • Major depressive disorder • Gene expression • ATP1A1 • Molecular marker*

1. Introduction

Na+/K+ ATPase is a transmembrane protein with its primary physiological role in the maintenance of cellular transmembrane sodium and potassium ion gradients necessary for the control of resting membrane potential, cell volume, intracellular pH, calcium concentration, and the transportation of other solutes [1]. A functional Na+/K+ ATPase is composed of two subunits, α and β, with multiple isoforms and distribution patterns in different tissues [2]. The main function of the α subunit is to hydrolyze ATP coupled with Na^+ and K^+ transport. There are three types of α subunit isoforms defined as α1, α2, and α3 that are each encoded by the genes ATP1A1, ATP1A2, and ATP1A3, respectively [3]. The central nervous system expresses α1, α2, and α3 subunit isoforms, with the most highly expressed subunit in the cerebrum and cerebellum being α1 [1]. Previous studies demonstrated that inhibition of Na+/K+ ATPase activity decreased the uptake of norepinephrine [4], serotonin, and dopamine [5], and, therefore, altered neuronal firing [6].

Major depressive disorder, also known as major depression, is a common mental disorder, which affects about 10-20% of the population [7] with a suicidal outcome in approximately 15% of patients [8]. It is known that major depression affects the central nervous system and thereafter affects the autonomic nervous system, immune system, and neuroendocrine system

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(hypothalamus-pituitary-adrenal axis), which involves the abnormalities of serotonin transporter, calcium signaling, and neurotrophic factors, *etc*. [9]. Because of the dysfunction of multiple systems, the gene expression levels in peripheral blood leukocytes will be affected and changed accordingly. Genome-wide gene expression analysis by using either DNA microarray [10] or whole genome cRNA microarray [11] found that more than 100 genes were related to major depressive disorder. Other studies also showed that the decreased expression level of ATP1A3 gene that codes for the α3 subunit isoform might play an important role in major depressive disorder [12,13], while the ATP1A2 gene that codes for the α2 subunit isoform has a significant association with bipolar disorder [14] and anxiety-related behavior [15]. However, there is no report about the potential gene expression level change of ATP1A1 that codes for the most abundant α1 subunit isoform in the cerebrum in major depressive disorder patients. This study investigated ATP1A1 gene expression levels in both normal and clinically confirmed major depressive disorder patients' peripheral blood leukocytes.

2. Experimental Procedures

2.1 Subject selection

All participants were from the Chinese Han ethnic group. A total of 30 healthy subjects (19 males, 11 females, age 22 to 62) were recruited for generation of the normal gene expression baseline. In total 30 drug-free outpatients (11 males, 19 females, age 29 to 77) were involved in this study. All subjects were recruited from the clinic of Beijing Friendship Hospital, Capital Medical University (Beijing, China). All research procedures were reviewed and approved by Institutional Research Ethics Committee of Capital Medical University and a written informed consent statement was obtained from each blood donor. All subjects were clinically screened to eliminate the potential diseases of other systems. The patients were diagnosed with major depressive disorder according to the criteria of Diagnostic and Statistical Manual of Mental Disorders-fourth edition (DSM-IV) (American Psychiatric Association).

2.2 Peripheral blood collection and total RNA extraction

Following fasting, 2 ml of peripheral venous blood was collected from patients and healthy controls. Whole blood total RNAs were isolated using PureLink® RNA Mini Kit (Life Technologies, Carlsbad, CA, USA) according to manufacturer's instructions. All isolated total RNAs were subjected to BioSpec-nano Spectrophotometer (Shimadzu, Kyoto, Japan) for quantization at OD_{260} and quality assessment at OD_{260}/OD_{280} and OD_{260}/OD_{230} respectively. In addition, 1% agarose gel electrophoresis was also applied for total RNA quality control.

2.3 Reverse transcription and real-time PCR

High quality total RNAs were reverse transcripted into cDNAs using PrimerScript™ RT Reagent Kit (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. Briefly, the 10 μl reaction system was composed of 500 ng total RNA, 25 pmol Oligo dT primer, 50 pmol Random 6 mers, 2 μl 5x PrimerScript buffer, 0.5 μl PrimerScript™ RT Enzyme Mix, and RNase free dH_2O. The reaction was performed on S1000™ Thermal Cycler (BIO-RAD, Hercules, CA, USA) by the program of 37°C for 15 min and 85°C for 5 s.

Beacon Designer™ software (PREMIER Biosoft, Palo Alto, CA, USA) was applied to design primers according to GenBank nucleotide sequence records of ATP1A1 gene (Accession number: NM_000701) and reference control gene, β-actin (Accession number: NM_001101) (Table 1). The primer sequences of ATP1A1 gene were subjected to nucleotide blast investigation against GenBank "Human genomic + transcript" databases to ensure the specificity (*i.e.* no sequence overlapping with other Na+/K+ ATPase α subunit isoforms). The real-time PCR was performed by using Applied Biosystems 7500 Fast Real-Time PCR System (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instruction. Briefly, the 25 μl reaction system was composed of 2 μl of diluted cDNA templeate, 12.5 μl SYBR® Green Real-Time PCR Master Mix (Life Technologies, Carlsbad, CA, USA), and 0.2 mmol L^{-1} of each primer. The reaction conditions were 95°C for 20 s followed by 40 cycles of 95°C for 3 s, 60°C for 30 s,

Gene name	GenBank accession number	Primer sequences F: forward R: reverse	Amplicon length (bp)	R^2	Slope of Linear equation
ATP1A1	NM_000701	F: GGTCCCAACGCCCTCACTC R:ACCACACCCAGGTACAGATTATCG	179	>0.99	<-3
β-actin	NM_001101	F: GGCGGCACCACCATGTACCCT R: AGGGGCCGGACTCGTCATACT	202	>0.99	<-3

Table 1. Real-time PCR primer sequences, amplicon size, and related factors of each target gene.

and then 95°C for 15 s, 60°C for 1 min, 95°C for 15 s, 60°C for 15 s. In addition, the amplified fragments were checked by running 1% agarose gel electrophoresis of the PCR products. A 10-fold dilution series of cDNA (10^{-3} to 10^{-10} times) from each target gene was applied as a standard to construct a relative standard curve and determine the PCR efficiency that would be used in converting quantification cycle into relative quantity. The optimal reaction cofactors, primer sequences, and amplicon size of each target gene have been described in Table 1.

2.4 Data analysis

The real-time PCR data was processed and analyzed by using 7500 Fast Real-Time PCR System Sequence Detection Software (version 1.4) (Life Technologies, Carlsbad, CA, USA). The standard curve of each target gene and their amplification (Rn *vs*. Cycle) and dissociation curves were derived. The equations of each target gene amplification were then calculated for optimal experimental condition evaluation. The final absolute copy number of each target gene was calculated by using the formulation of target gene absolute copy numbers = sample concentration/sample molecular weight×6×10^{14}, and then was converted to target gene relative copy numbers by using the formulation of target gene relative copy numbers = target gene absolute copy numbers/reference control gene absolute copy numbers. The data were statistically assessed by using Microsoft Excel T-test program.

2.5 Bioinformatics comparative analysis of Na+/K+ ATPase α subunit isoforms (α1, α2, α3)

The nucleotide sequences of all three types of Na+/K+ ATPase alpha subunit isoform genes (ATP1A1, ATP1A2, and ATP1A3) were compared using BLAST software (http://blast.ncbi.nlm.nih.gov/). The amino acid sequences of all three isoforms were also analyzed by using online bioinformatics tools that included TMpred for transmembrane structure prediction (http://www.ch.embnet.org/software/TMPRED_form.html) and COILS for coiled-coil domain prediction (http://www.ch.embnet.org/software/COILS_form.html).

3. Results

3.1 Real-time PCR reactions of target and reference control genes

Both ATP1A1 and β-actin genes showed satisfactory amplification and unique product in each gradient reaction with single sharp amplification peak and smooth amplification curve. Both genes' standard equations showed good linear relationship with R^2 greater than 0.99 and the slope less than -3 (Table 1), which indicated successful reactions for all target genes' amplification.

3.2 The absolute and relative copy numbers of each target gene

The absolute and relative copy numbers of each target gene of both healthy control and patient groups have been calculated. The comparative analysis of ATP1A1 absolute copy numbers in both healthy control group and major depression patients showed that there was more than 80% decrease of ATP1A1 expression levels in major depression patients compared to that in healthy controls, while the reference control gene, β-actin, showed no significant changes in either group (Figure 1). The results of the unequal variance two group t-test showed statistical differences, considering that the related P-values for ATP1A1 and β-actin genes were 0.0011 and 0.3029, respectively. This means that there was a very significant difference in the levels of ATP1A1 gene expression between healthy controls and major depression patients ($P<0.01$). However, the expression level of β-actin showed no significant difference between the two groups. Further, relative copy numbers of ATP1A1 gene also showed a significant difference between healthy control and patient groups ($P<0.01$) with remarkable case distribution patterns between both healthy control and major depression groups (Figure 2). However, the difference between ATP1A1 and β-actin gene expression levels of male and female subjects within the groups studied was not statistically significant.

3.3 Comparison of gene and protein structures of ATP1A1 to ATP1A2 and ATP1A3

The nucleotide sequence comparison of all three types of α subunit isoforms showed 79% of sequence

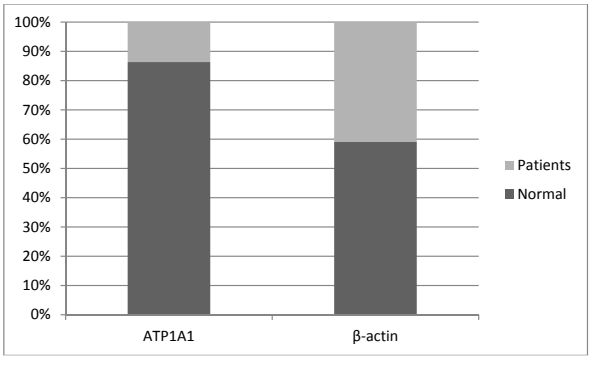

Figure 1. ATP1A1 gene expression absolute copy numbers in both normal and patients' groups.

identities between ATP1A1 and ATP1A3 genes, but no significant similarities were determined between the sequences of ATP1A1 and ATP1A2 genes. However, the comparison of amino acid sequences showed 87% identities between ATP1A1 and ATP1A2 or ATP1A1 and ATP1A3. All three isoforms showed strong 9 transmembrane helices and similar functional coiled-coil domains (Figure 3).

4. Discussion

It has been reported that major depressive disorder mainly affects the central nervous system and thereafter affects the autonomic nervous system, immune system, and endocrine system, which may cause a series of somatic symptoms in major depression patients [9]. ATP1A1, the highest expressed Na+/K+ ATPase α subunit isoform in cerebrum and cerebellum, as a major mediator of cellular transmembrane sodium and potassium ionic gradients, plays an important role in central nervous system nervous signal transduction [1]. The results of this study show that there is a significant decrease of ATP1A1 gene expression level in major depressive disorder patient's peripheral blood comparing to that of normal healthy controls ($P<0.01$), which might reflect a similar ATP1A1 gene expression level change in the central nervous system [9]. A significant reduction of ATP1A1 gene expression can affect the functions of central nervous system in multiple aspects. The reduction of Na+/K+ ATPase activity affects directly neurotransmitter signaling, neural activity, mood, and whole body behavior through reduction in norepinephrine, dopamine, and serotonin uptake and increasing acetylcholine release [4,5,16].

According to previous animal experimental results, ATP1A1 regulates sodium and potassium homeostasis in pyramidal cells, which contributes to the control of resting membrane potential of pyramidal cells [17]. The reduction of ATP1A1 gene expression level may cause the amount and functional deficiencies of its coded Na+/K+ ATPase α1 subunit isoform on cell surface, and may impact the transmembrane ionic gradients of the cell, and then reduce the capability of neural signal transduction.

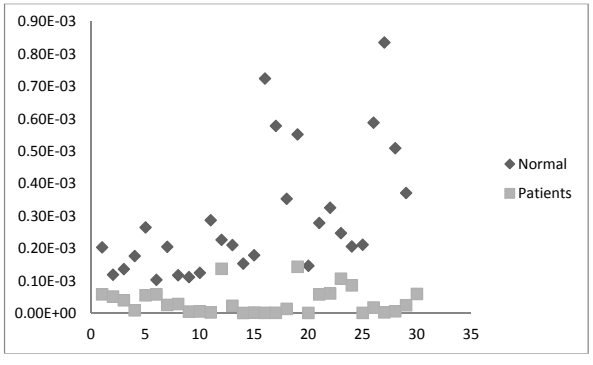

Figure 2. Case distribution patterns of ATP1A1 gene expression relative copy numbers in both normal and patients' groups.

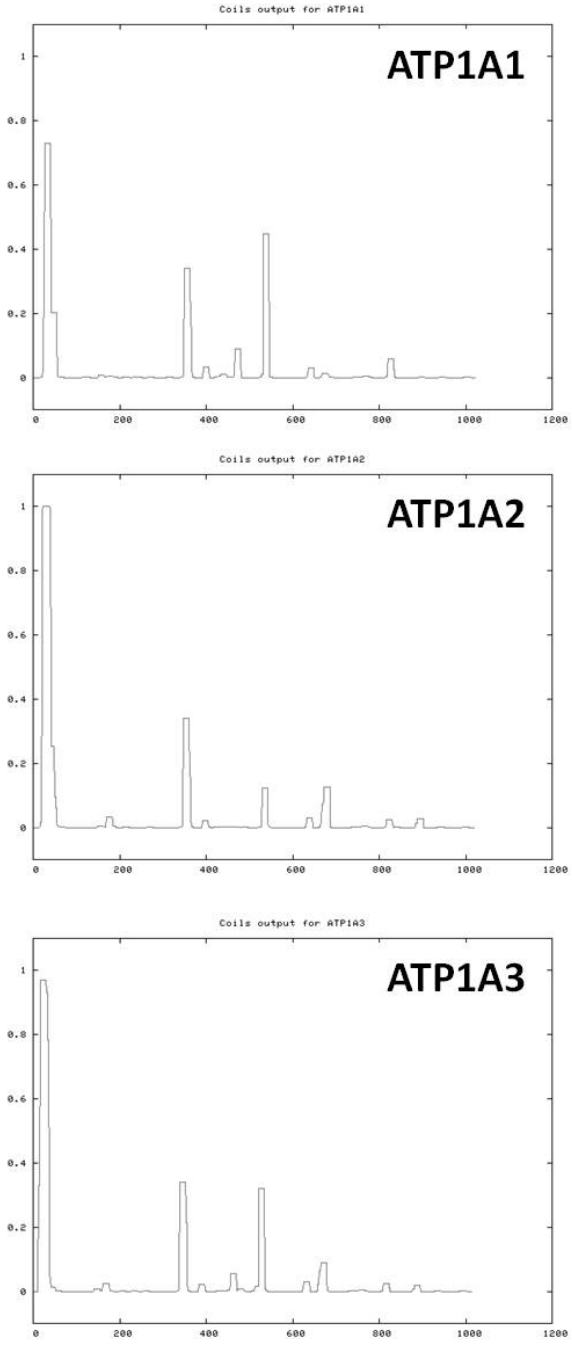

Figure 3. Coiled-coil domain prediction of ATP1A1, ATP1A2, and ATP1A3.

Earlier neuropathological studies reported the reduction of density and size of pyramidal neurons in the dorsolateral prefrontal cortex in major depressive disorder patients [18-20]. Findings of this study also suggested that the neuropathological phenomenon might be related to the decreased expression of ATP1A1. Na+/K+ ATPase is the key component of cell membrane to regulate cellular homeostasis [17]. The decreased expression of ATP1A1 will cause a reduction in the amount of/or functional deficiency of the α1 subunit isoform on the cell membrane, which may induce the imbalance of ionic osmolarity across the cell membrane that may further cause cell damage and trigger apoptosis. Further, previous studies have also confirmed that ATP1A1 regulates the normal function-formation of a cohesive neuroepithelium, restriction of neuroepithlial permeability and the production of cerebrospinal fluid [21]. The impacts on these processes could also harm the normal function of the central nervous system.

Three types of Na+/K+ ATPase α subunit isoforms are found in the brain but vary with cell types and in expression levels [15]. Studies have also shown that α3 subunit coding gene (ATP1A3) is involved in both unipolar and bipolar depressions and was correlated with the mood changes in depressive disorders [12,13,22], while α2 subunit coding gene (ATP1A2) was significantly associated with bipolar disorder [14] and anxiety-related behavior [15]. However, there is no report about the correlation of α1 subunit coding gene (ATP1A1) with major depressive disorder. The present results showed that among these three isoforms' genes, only ATP1A3 has demonstrated the nucleotide sequence similarity of 79% and 82% to both ATP1A1 and ATP1A2, respectively. There is no similarity found between ATP1A1 and ATP1A2 genes' nucleotide sequences. The virtual protein translation studies showed that the similarity of the protein amino acid sequences amongst all three types of α subunit isoforms were determined to be 87%. The coiled-coil domain analysis showed that there were two similar predicted domain structures in all three types of isoforms. One domain structure showed similar confidence amongst three types of isoforms and another domain demonstrated a higher confidence in ATP1A2 and ATP1A3 isoforms (>95%) than it in ATP1A1 (<75%) (Figure 3). By comparing all predicted coiled-coil domains amongst three types of isoforms, the results demonstrated that besides those two similar domains that might contribute to the conservation of their Na+/K+ ATPase basic function, there were some potential structure differences, especially between ATP1A1 and other two isoforms. The potential protein structure differences amongst those three types of isoforms may be related to their different brain distribution sites, expression levels, and their correlations to different type of depressive disorders.

In conclusion, this study demonstrated for the first time that ATP1A1 gene expression level is associated with major depressive disorder. By comparing all three types of brain expressed Na+/K+ ATPase α subunit isoforms' open reading frame sequences, amino acid sequences, and potential protein structures, the study provides evidence and tools illustrating the involvement of different Na+/K+ ATPase α subunit isoforms in different types of depressive disorders. The present results could lead to the development of a molecular diagnostic method for major depressive disorder based upon the application of ATP1A1 gene as a molecular marker. It could also inspire further related research.

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