Study of association between schizophrenia and functional polymorphisms of genes encoding Bcl-2 family proteins

Kristina Pirumyan, Anna Boyajyan

Department of Molecular and Applied Biology, Institute of Molecular Biology, National Academy of Sciences of the Republic of Armenia (NAS RA), Yerevan, Armenia

Email address
aboyajyan@sci.am (A. Boyajyan)

Citation

Abstract
It is proposed that both pre- and postnatal as well as genetically determined abnormalities of the apoptotic processes are among factors responsible for the development of schizophrenia. The ultimate vulnerability of cells to diverse apoptotic stimuli is determined by the relative ratio of various pro-apoptotic and anti-apoptotic members of the B-cell lymphoma 2 (Bcl-2) family proteins among which Bcl-2 proper is a major anti-apoptotic protein, and Bcl-2-associated X protein (Bax) is pro-apoptotic death promoter. Postmortem studies demonstrated the increased expression level of Bax encoding gene and high Bax/Bcl-2 proteins ratio in schizophrenia. However, it is yet unclear whether these alterations are genetically determined or caused by other factors. In the present study we, for the first time, evaluated the association of single nucleotide polymorphisms (SNPs) rs1057369 (A>G) and rs956572 (G>A), rs1801018 (A>G) of Bax and Bcl-2 encoding genes, BAX and BCL2, respectively, with schizophrenia in Armenian population using polymerase chain reaction with sequence-specific primers. BAX rs1057369 SNP was found negatively associated with this disorder; the presence of BAX rs1057369*G minor allele, especially in homozygous form, was associated with decreased risk of developing schizophrenia, whereas no association between schizophrenia and BCL2 rs956572 and rs1801018 polymorphisms was revealed. Based on the obtained results we concluded that the rs1057369*G minor allele of BAX may have a protective effect relative to schizophrenia, and that this effect is most pronounced in individuals with GG homozygous genotype.

1. Introduction

Schizophrenia is one of the most severe and disabling among mental disorders affecting up to 1% of the world population (1) and is characterized by cognitive impairments (2) linked to behavioral changes (3). It is considered a multifactorial disease, likely to be caused by alterations in genome, both acquired (originated during prenatal development) and inherent, combined with postnatal environmental factors (4, 5).

Apoptosis is a mechanism of cell death that operates in normal neurodevelopment and is increasingly recognized for its pathological features in
diverse neuropathological conditions including mental disorders (6, 7). Activation of apoptosis can lead to elimination of neurons and glia in the central nervous system (8, 9). Evidence of progressive clinical deterioration and subtle neurostructural changes following the onset of psychosis, shorter dendrites, reduced neutrophil count, reduction in neuronal and glial cell number, especially synaptic elements (10, 11), together with brain imaging data of schizophrenia-affected subjects (12) indicate that apoptosis may significantly contribute to the pathophysiology of schizophrenia. Moreover, alterations of the apoptotic processes in schizophrenia are detected at the levels of the brain (13, 14) and peripheral blood (15-17). Furthermore, one of the contemporary hypotheses of etiology and pathogenesis of schizophrenia (“neurodevelopmental hypothesis”) proposes that in schizophrenia apoptotic processes and their genetic regulation are altered starting from the early stages of neural development (18). It is proposed that both pre- and postnatal as well as genetically determined abnormalities of the apoptotic processes are among factors responsible for the development of schizophrenia (10) and that non-lethal apoptosis may contribute to schizophrenia-associated defects of synaptic plasticity (19), neurodegenerative alterations (20), and immune system dysfunctions, particularly, autoimmune and inflammatory reactions (16, 21, 22) directly linked to apoptosis (23, 24). All together these interrelated anomalies have a significant impact on cognitive function (25-29) and might be responsible for cognitive impairments in schizophrenia.

A family of B-cell lymphoma 2 (Bcl-2) proteins constitutes one of the most biologically relevant classes of apoptosis regulators acting at the effector stage of apoptosis, with some members functioning as suppressors of apoptosis and others as promoters of apoptosis. The ultimate vulnerability of cells to diverse apoptotic stimuli is determined by the relative ratio of various pro-apoptotic and anti-apoptotic members of the Bcl-2 family (30, 31). Bcl-2 proper and Bcl-2-associated X protein are members of the Bcl-2 family proteins (32). Bcl-2 proper is an integral, membrane-associated protein with anti-apoptotic and antioxidative effects (32, 33). Bcl-2 is a major anti-apoptotic protein that inhibits apoptotic and necrotic cell death induced by a diverse set of adverse conditions (34). Bcl-2 also plays critical roles in neuronal morphogenesis and synaptic plasticity (35, 36), and reduced Bcl-2 function is hypothesized to contribute to the impairment of cellular plasticity and resilience in patients with mood disorders (34).

Bcl-2-associated X protein (Bax) is pro-apoptotic member of the family of Bcl-2-related proteins, which has an extensive amino acid homology with Bcl-2 (37, 38). Whether the cell will live or die may depend on the level of either protein; while Bcl-2 prevents death, Bax is a death promoter (30-32).
Collection of Blood Samples and Extraction of Genomic DNA

About 5 ml of peripheral blood was collected from each study subject by venipuncture and transferred to EDTA-containing tubes. Genomic DNA samples were isolated from fresh blood according to a standard phenol-chloroform method (44) and stored at -30°C until use.

Genotyping of BAX Rs1057369 and BCL2 Rs956572, Rs1801018 SNPs

All DNA samples were genotyped for BAX rs1057369 (A>G; chromosome 19: 49464866; intron variant) and BCL2 rs956572 (G>A; chromosome 18: 60820571, intron variant), rs1801018 (A>G; chromosome 18: 60985879, synonymous codon) SNPs using PCR-SSP as described earlier (45). All primers for PCR-SSP were designed using the genomic sequences in the GenBank database (http://www.ncbi.nlm.nih.gov, Gene IDs: 581 and 596 for BAX and BCL2, respectively). The primer sequences for three mentioned SNPs were as follows: 1) rs956572: allele A reverse 5’-AGAGGGAAGCATGACTGAATT, allele G reverse 5’-AGAGGGGTCATGACTGAATT, constant forward 5’-CAGATCTGTGCTTTGAACCTCA, 2) rs1801018: allele A reverse 5’-ATCTCCGGTTATCGTACCCT, allele G reverse 5’-ATCTCCGGTTATCGTACCCCC, constant forward 5’-GATCCCCAGGAATTTGGAATA, 3) rs1057369: allele A reverse 5’-ATCTTCTCCAGATGTTGAGT, allele G reverse 5’-ATCTTCTCCAGATGTTGAGG, constant forward 5’-TTACAGGGGTGTGACCACATG.

The presence/absence of allele-specific amplicons in the PCR products was visualized in 2% agarose gel stained with ethidium bromide fluorescent dye using DNA molecular weight markers as a reference. To check the reproducibility of results, randomly selected DNA samples of study subjects (10% of total) were genotyped twice.

Statistical Analysis

Distributions of genotypes for all investigated SNPs were checked for correspondence to the Hardy-Weinberg (H-W) equilibrium. In order to find potential relevance of the selected SNPs to schizophrenia, their genotype and allele frequencies and minor allele carriage rates in patients and healthy control subjects were compared. The calculations of genotype and allele frequencies were based on the observed number of genotypes. The significance of differences in genotype and allele frequencies and minor allele carriage between patients and healthy control subjects was determined using Pearson’s Chi-square test. The odds ratio (OR), 95% confidence interval (CI), and Pearson’s p-value were calculated. P-values were adjusted by Bonferroni multiple correction approach (46), and those less than 0.05 were considered statistically significant. Statistical powers of the present study were calculated according to the protocol described elsewhere (47). The data was evaluated using “SPSS-13.0” (SPSS Inc, USA) software.

3. Results

The distribution of genotypes for the selected SNPs in both study groups complied with H-W equilibrium - no significant differences were detected between the observed and expected genotype frequencies (p>0.05). The genotyping success rate was 100%, and rescreening of randomly selected DNA samples of study subjects (10% of total) gave 100% identical results. Minor allele frequency in healthy control subjects for all studied polymorphisms was close to that reported for Central European Population (HapMap, NIEHS). Distribution of BAX rs1057369 and BCL2 rs956572, rs1801018 variants in study groups is shown in table 1

No significant difference was observed between the patients and controls regarding the genotype and allele frequencies and minor allele carriage of the BCL2 rs956572 and rs1801018 SNPs (p>0.05). Thus, the results obtained indicated that there is no significant difference in minor allele frequency of BCL2 rs956572 and rs1801018 SNPs between patients and controls (rs956572*A patients vs. controls: 66% vs. 64%, p=0.348, OR=1.34, 95% CI: 0.851-2.000; rs1801018*G patients vs. controls: 46% vs. 51%, p=0.372, OR=0.84, 95% CI: 0.679-1.048, respectively). The same applies to the carriers of the BCL2 rs956572*A and rs1801018*G minor alleles (rs956572*A patients vs. controls: 66% vs. 64%, p=0.132, OR=1.105, 95% CI: 0.88-1.525; rs1801018*G patients vs. controls: 74% vs. 79%, p=0.348, OR=0.93-1.525, 95% CI: 0.551-0.851). Statistical powers of this part of the study, indicating the differences in the carriage of the BCL2 rs956572*A and rs1801018*G minor alleles between patients and controls, were 7.52% and 15.11%, respectively. By contrast, significant difference in the genotype and allele frequencies, and minor allele carriage rates of the BAX rs1057369 SNP between patients and controls was found (p<0.05), and the negative association between schizophrenia and rs1057369 SNP was detected. Thus, minor allele frequency of BAX rs1057369 SNP in patients was lower than in controls (rs1057369*G patients vs. controls: 45% vs. 55%, p=0.00185, OR=0.68, 95% CI: 0.551-0.851). The same tendency was detected when comparing a number of the rs1057369*G minor allele carriers in patients and controls (rs956572*G patients vs. controls: 73% vs. 82%, p=0.026, OR=0.6, 95% CI: 0.415-0.871).

The obtained results demonstrated that BAX rs1057369*G minor allele is overrepresented in controls as compared to patients. Moreover, as it is shown in Table 1, a number of homozygous carriers of minor allele (GG) among controls was significantly higher than among patients (18% vs 29%, p<0.05). Statistical power of this part of the study, indicating the difference in the carriage of
the BAX rs1057369*G minor allele between patients and controls, was 38.99%.

Table 1. Genotype and allele frequencies and minor allele carriage of BCL2 rs956572, rs1801018 and BAX rs1057369 SNPs in patients with schizophrenia (SCH) and controls.

<table>
<thead>
<tr>
<th>BCL2 rs956572 (G&gt;A)</th>
<th>SCH (n=330)</th>
<th>Controls (n=326)</th>
<th>(P_{\text{nom}})</th>
<th>(P_{\text{corrected}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>110(0.34)</td>
<td>116(0.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>176(0.53)</td>
<td>150(0.46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>44(0.13)</td>
<td>60(0.18)</td>
<td>0.075</td>
<td>0.225</td>
</tr>
<tr>
<td>G</td>
<td>396(0.60)</td>
<td>382(0.59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>264(0.40)</td>
<td>270(0.41)</td>
<td>0.603</td>
<td>1.809</td>
</tr>
<tr>
<td>Carriage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>220(0.66)</td>
<td>210(0.64)</td>
<td>0.544</td>
<td>1.632</td>
</tr>
<tr>
<td>BCL2 rs1801018 (A&gt;G)</td>
<td></td>
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<td></td>
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<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>86(0.26)</td>
<td>68(0.21)</td>
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<td></td>
</tr>
<tr>
<td>AG</td>
<td>182(0.55)</td>
<td>186(0.57)</td>
<td></td>
<td></td>
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<tr>
<td>GG</td>
<td>62(0.19)</td>
<td>72(0.22)</td>
<td>0.295</td>
<td>0.885</td>
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<tr>
<td>Alleles</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>A</td>
<td>354(0.54)</td>
<td>322(0.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>306(0.46)</td>
<td>330(0.51)</td>
<td>0.124</td>
<td>0.372</td>
</tr>
<tr>
<td>Carriage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>244(0.74)</td>
<td>258(0.79)</td>
<td>0.116</td>
<td>0.348</td>
</tr>
</tbody>
</table>

Data is presented as absolute number (proportion).

4. Discussion

In the present study, for the first time, the potential association between schizophrenia disorder and functional polymorphisms of Bcl-2 family proteins encoding genes, BAX rs1057369 and BCL2 rs956572, rs1801018, was assessed.

BAX rs1057369 SNP was found negatively associated with schizophrenia: the presence of BAX rs1057369*G minor allele, especially in homozygous form, was associated with decreased risk of developing schizophrenia. It has to be mentioned that no data on association of this polymorphism with any diseased condition has been reported before.

Opposite to BAX rs1057369 SNP, BCL2 rs1801018 and rs956572 polymorphisms have been intensively studied in different diseased and other conditions. BCL2 rs1801018 SNP was mostly found to be associated with oncological disorders (48-51) as well as with poorer clinical outcomes and mortality in patients with traumatic brain injury (52).

BCL2 rs956572 polymorphism was shown to associate with the risk for developing bipolar disorder and was nominated as modulator of the expression of Bcl-2 protein and cellular vulnerability to apoptosis (53). Preclinical studies show that this SNP exerts functional effects on Bcl-2 expression, as the A homozygous genotype is associated with significantly lower Bcl-2 mRNA expression, 50% lower Bcl-2 protein levels, and greater cellular sensitivity to stress-induced apoptosis (53). This SNP was reported to affect gray matter volume in areas known to play key roles in the neurobiology of reward processes and emotion regulation and in the pathophysiology of mood disorders (54). Also, it was demonstrated that rs956572 SNP may modulate cognitive function and regional gray matter volume in non-demented elderly men, and affect language performance through its effect on the right middle temporal gyrus (55). In addition, it was shown that rs956572 associates with increased anterior cingulate cortical glutamate (56) and disrupted intracellular calcium homeostasis in bipolar I disorder (57) and that abnormal BCL2 gene expression in the AA genotype of rs956572 contributes to dysfunctional Ca(2+) homeostasis through inositol 1,4,5-trisphosphate receptor-dependent mechanism (58).

Regarding BCL2 rs956572 and rs1801018 polymorphisms, this study revealed no association between schizophrenia and these SNPs. Further studies with implication of other BAX and BCL2 SNPs will finally reveal whether alterations in BAX and BCL2 genes may contribute to changes in to Bax/Bcl-2 expression levels (39, 40), and, in general, to defects in apoptosis in schizophrenia (10).

5. Limitation of Study

The limitation of this study is a relatively small sample size of study subjects. Also, because all subjects involved in the present study belonged to Armenian population, further investigations in other populations are necessary to replicate the present findings.
6. Conclusion

In conclusion, our results, for the first time, revealed significant negative association between schizophrenia and rs1057369 polymorphism of BAX gene encoding pro-apoptotic Bax protein. According to these results the rs1057369*G minor allele of BAX may have a protective effect relative to schizophrenia at least in Armenian population. In addition, it was demonstrated that this effect is most pronounced in individuals with GG homozygous genotype. Our study also indicated no significant association between schizophrenia and rs956572, and rs1801018 polymorphisms of BCL2 gene encoding anti-apoptotic bel-2 proper protein.

Acknowledgement

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Declaration of Interest

The authors report no conflicts of interest.

Authors Contribution

Conception and design: AB; genotyping and statistical analysis: KP; interpretation of data and drafting the manuscript: AB+KP; finalizing the manuscript for submission: AB.

References


