**In silico analysis of dysbindin (DTNBPI) gene intronic variants and their plausible role in splicing**

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**ABSTRACT**

The dysbindin gene (DTNBPI) has shown to be associated with schizophrenia (SCZ) in several populations. Genetic variations in the DTNBPI gene have been implicated as risk factors in the pathogenesis of SCZ. Association analysis of the genotypic and allelic frequencies has not show any statistically significant association across population. Further, haplotypic combinations of the SNPs showed weak association in the worldwide case-control sample. In the present study, In silico analysis of the intronic SNPs using exonic splicing enhancer sequences (ESE) suggested that the SNPs were clustered near segments associated with anomalous gene splicing. Some of the SNPs were found to abrogate, create or change the regulatory specificity of predicted exonic splicing enhancer sequences. Thus it could be hypothesized that the SNPs may be involved in controlling splicing regulation. Expression studies involving these polymorphisms will shed light in further understanding the role of these SNPs in SCZ.

**Keywords:** Glycoprotein, lysosome, Linkage disequilibrium (LD), Schizophrenia(SCZ), exonic splicing enhancer sequences (ESE)

**INTRODUCTION**

The dysbindin gene (DTNBPI) is located in chromosome 6p22.3, one of the replicated regions of positive linkage for SCZ [1]. Dysbindin-1 protein coded by the DTNBPI gene is a coiled-coil protein, with molecular weight, 40–50 kDa protein that binds both α- and β-dystrobrevin, which are components of the dystrophin glycoprotein complex. The dystrophin complex is located in postsynaptic densities [2]. Further, dysbindin, along with its binding partner Muted is an essential component of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). The molecular components of which are proposed to be involved in the regulation of vesicular trafficking and dendrite branching [3]. Biophysical findings suggest that dysbindin functions to regulate exocytosis and vesicle biogenesis in endocrine cells and neurons [4] suggesting a possible mechanism in the pathogenesis of schizophrenia at the synaptic level.

In particular, dysbindin protein has been shown to participate in diverse functions in the central nervous system (CNS). The protein has been found to play a role in the play a role in the glutamate neural transmission in the brain [5]. Furthermore, cell culture studies have shown positive correlations between dysbindin-1 mRNA and expression of synaptic markers suggesting its role in glutamate neurotransmission [6]. Small interfering RNA (siRNA) mediated knockdown of endogenous dysbindin protein suggests that dysbindin might influence exocytotic glutamate release via upregulation of the molecules in pre-synaptic machinery.
Expression studies have demonstrated reduced dysbindin-1 mRNA in the hippocampal formation of patients with SCZ. DTNBP1 interactome and associated network analysis implicates that the DTNBP1 protein is involved in the biogenesis of lysosome-related organelles complex 1 and interacts with other proteins viz., retinoic acid, beta-estradiol, calmodulin and tumour necrosis factor [7] pointing to a diverse role for the protein the CNS.

A strong genetic association between DTNBP1 single nucleotide polymorphisms SNPs and SCZ has been replicated through many studies, however, equivocally [8]. Previous reports have shown that diverse high-risk SNPs and haplotypes could influence dysbindin-1 mRNA expression. Genotype phenotype correlation of a defined SCZ risk haplotype tags has been reported by several researchers, how ever the risk haplotype has differed across populations. Bray et al., 2005 reported that the 'protective' haplotypes index high DTNBP1 gene expression. With the above given evidence for DTNBP1 in SCZ and lack of association of variants, In silico sequence analysis of the studied SNP variants for their putative role in splicing regulation was undertaken.

MATERIALS AND METHODS

**In silico analysis**

To identify ESEs that are recognized by individual splicing regulatory proteins (SF2/ASF, SC35, SRp40 and SRp55), we used the ESE finder program version 2.0 (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home) [9]. Putative ESE motifs that agree with the consensus sequences for specific SR proteins have high values in the ESE finder program, and a decrease in the score relative to a threshold value is indicative of a decrease or loss of enhancement of transcript splicing [10].

**RESULTS**

Prior evidence of alternative splicing at candidate genes for psychoses has been suggested [11]. In silico analysis (figure-2) of using ESE finder program revealed that the sequences flanking SNP sequence (rs2619539) were associated with the four strong candidate ESE sequences. The results of ERE analysis are summarized in Figure-1. The strength and specificity of the SR35 site at the locus was also altered. The nucleotide change caused the obliteration of a site for a SR protein SF2/ASF and introduced a SRp40 site. The A-G base conversion (rs2005976) had several effects on the ESE cluster. A cluster of overlapping candidate ESE sequences was observed flanking sequences. It resulted in the elimination of ESE sequence for SF2/ASF, and SF2/ASF (IgM-BRCA1). A modest increase in the predicted quantitative strength of the SF2/ASF splice site and introduces a third very strong overlapping site specific for SRp40.

The strength of two remaining SR35 sites were also altered. The nucleotide change due to SNPs rs1011313 and rs3213207 did not result in any ESE sequence change.

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Fig. 1: Effects of nucleotide changes of DTNBP1 SNPs on ESE sequences
A- ESE finder identified four corresponding strong candidate ESEs at the sequences flanking (rs2619539) for SRp40, SF2/ASF (IgM-BRCA1), SF2/ASF, SC35. The G-C substitution (arrow head) obliterates a SF2/ASF site and introduces a SRp40 site, further, it changes the specificity of the SC35.

B- ESE finder identified four corresponding strong candidate ESEs at the sequences flanking (rs1011313) for SRp40, SF2/ASF (IgM-BRCA1), SF2/ASF, SC35. The C-T substitution did not alter any splicing sequence.

C- ESE finder identified four corresponding strong candidate ESEs at the sequences flanking (rs2005976) for SRp40, SF2/ASF (IgM-BRCA1), SF2/ASF, SC35. The A-G substitution obliterates a SF2/ASF further, it changes the specificity of the SF2/ASF (IgM-BRCA1) and SC35 sequences.

D- ESE finder identified four corresponding strong candidate ESEs at the sequences flanking (rs3213207) for SRp40, SF2/ASF (IgM-BRCA1), SF2/ASF, SC35. The A-G substitution did not alter any splicing sequence.

DISCUSSION
The current study is an attempt to use Bioinformatics tools viz., ESEs to study exonic cis-elements is splicing and gene regulation. The ERE tool work with classical splicing signals (50 splicing site, 30 splicing site and branch site) by binding specific serine/arginine-rich splicing regulatory proteins (SR proteins) to promote accurate splicing [12]. Preferential splicing can be regulated by the presence of ESEs within the sequence.

Several reasons could be attributed to this negative association at the DTNBPI. The difference in MAF across studied populations or ethnicities might also be one reason for non-replication across studies [13]. The data bank of International HapMap project (http://www.hapmap.org/index.html.en/), [14] points at variations in the MAFs of the SNPs across different populations. The LD analysis of this locus using the hapmap data indicates that there is high genetic heterogeneity in the
DTNBP1 gene (data not shown). The SNPs reported in the DTNBP1 gene to date that showed significant results are intronic and none are located in canonical splice sites to demonstrate a direct correlation between variants and gene expression.

A recent study demonstrated that the non-coding SNPs participate in alternative splicing of dysbindin-1, the result leading to impairment of the functional interaction between dysbindin-1 and associated proteins [15]. These observations brings us to the question? What is the role of SNPs and how they affect expression in SCZ. Mutations (Missense, silent) in the coding/non-coding regions of a gene disrupt sequences recognized by splicing regulators such ESE, ESS or the recently identified composite exonic regulatory elements (CERES) can be considered an additional mutation mechanism leading to disease in humans [16].

In silico ESE analysis revealed that two intronic SNPs studied would affect preferential splicing. The A-G base conversion due to rs2005976 had several effects on the intronic ESE cluster. It resulted in the elimination of ESE sequence for SR proteins SF2/ASF, and SF2/ASF (IgM-BRCA1). The conversion also slightly increased the predicted quantitative strength of the SF2/ASF splice site and introduces a third very strong overlapping site specific for SRp40. The SNP sequence (rs2619539) altered the strength and specificity of the SR35 protein, further; it caused the obliteration of a site for a SR protein SF2/ASF and introduced a SRp40 site.

In summary SNPs were clustered near segments associated with anomalous gene splicing. Some of the SNPs were found to abrogate, create or change the regulatory specificity of predicted exonic splicing enhancer sequences. Heterologous expression analysis of the functional splice variants and allele expression imbalance studies could shed further light on the putative functional role of these non-synonymous SNPs in splicing and gene expression.

REFERENCES
9. ESEfinder: A web resource to identify exonic splicing enhancers.