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Research Report

Functional characterization of SNPs in *CHRNA3/B4* intergenic region associated with drug behaviors

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ABSTRACT

The cluster of human neuronal nicotinic receptor genes (*CHRNA5/A3/B4*) (15q25.1) has been associated with a variety of smoking and drug-related behaviors, as well as risk for lung cancer. *CHRNA3/B4* intergenic single nucleotide polymorphisms (SNPs) rs1948 and rs8023462 have been associated with early initiation of alcohol and tobacco use, and rs6495309 has been associated with nicotine dependence and risk for lung cancer. An *in vitro* luciferase expression assay was used to determine whether these SNPs and surrounding sequences contribute to differences in gene expression using cell lines either expressing proteins characteristic of neuronal tissue or derived from lung cancers. Electrophoretic mobility shift assays (EMSAs) were performed to investigate whether nuclear proteins from these cell lines bind SNP alleles differentially. Results from expression assays were dependent on cell culture type and haplotype. EMSAs indicated that rs8023462 and rs6495309 bind nuclear proteins in an allele-specific way. Additionally, GATA transcription factors appeared to bind rs8023462 only when the minor/risk allele was present. Much work has been done to describe the rat *Chrnb4/a3* intergenic region, but few studies have examined the human intergenic region effects on expression; therefore, these studies greatly aid human genetic research as it relates to observed nicotine phenotypes, lung cancer risk and potential underlying genetic mechanisms. Data from these experiments support the hypothesis that SNPs associated with human addiction-related phenotypes and lung cancer risk can affect gene expression, and are potential therapeutic targets. Additionally, this is the first evidence that rs8023462 interacts with GATA transcription factors to influence gene expression.

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1. Introduction

Nicotine dependence (ND) is the leading cause of preventable death in the world (Albuquerque et al., 2009; CDC, 2010) and is the major risk factor for chronic obstructive pulmonary disease (COPD) and lung cancer (Young et al., 2008). Genome Wide Association Studies (GWAS) have implicated the nicotinic acetylcholine receptor (nAChR) cluster of genes (*CHRNA5/CHRNA3/CHRNB4*) in risk for ND (Berrettini et al., 2008; Bierut et al., 2008; Saccone et al., 2007, 2009; Thorgeirsson et al., 2008; Weiss et al., 2008), COPD and lung cancer (Amos et al., 2008; Hung et al., 2008; Liu et al., 2008; Thorgeirsson et al., 2008; Wang et al., 2009c). Recent meta-analyses sustain these associations (Bierut et al., 2010; Consortium, 2010; Thorgeirsson et al., 2010; Timofeeva et al., 2012). The most well studied single nucleotide polymorphism (SNP) within the cluster has been rs16969968, a missense variant that results in a change from aspartic acid (D) to asparagine (N) in the nAChR α5 subunit and decreased response to agonist when the risk (398N) allele is present (Berrettini and Doyle, 2012; Bierut et al., 2007, 2008; Saccone et al., 2007). Yet, there are other known genetic influences on ND and lung cancer risk within this region, and variants in the cluster have a complex linkage disequilibrium (LD) structure, complicating identification of other functional polymorphism(s) (Pergadia et al., 2009).

Additional molecular experiments are necessary to understand other functional SNPs within the cluster (Berrettini and Doyle, 2012). At least one locus associated with increased risk for ND and lung cancer (locus 3 tagged by rs588765), is also associated with significantly increased α5 mRNA levels in frontal cortex of individuals harboring the risk allele (Wang et al., 2009c). However, many *CHRNA3* promoter or intergenic variants investigated thus far (See Fig. 1 for extent of investigation based on three previous studies) have not been shown to directly impact transcription (Doyle et al., 2011; Fornasari et al., 1997). A notable exception is the intergenic SNP rs6495309, which has been shown to influence luciferase expression in lung cancer cell lines (Wu et al., 2009). The Oct-1 transcription factor (TF) has been shown to

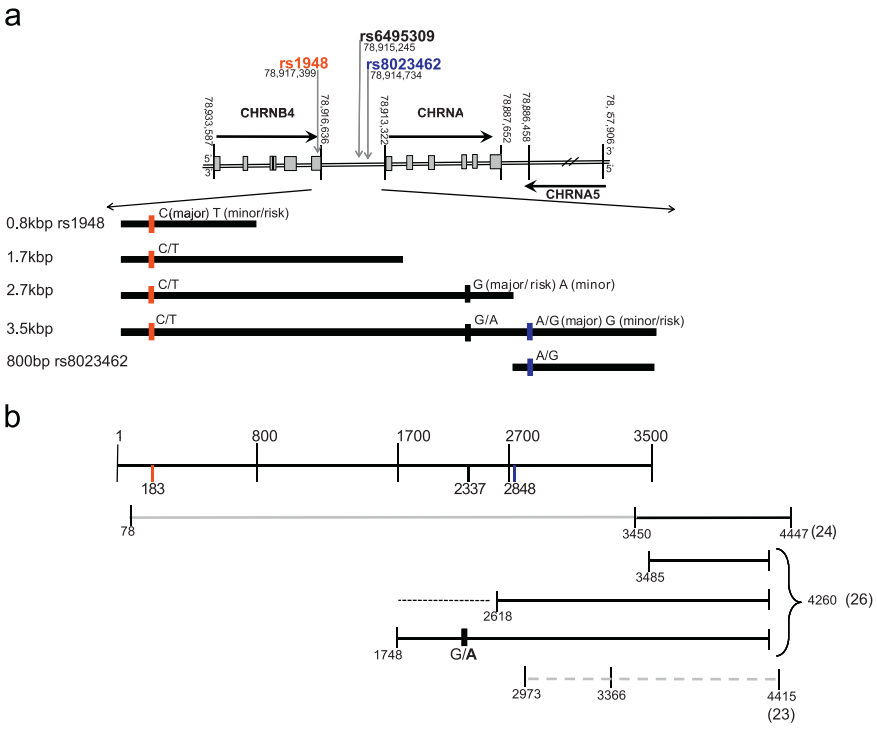

Fig. 1 – Constructs (a): the *CHRNA5/A3/B4* cluster with the reverse strand (coding strand for *CHRNA3* and *CHRNB4*) of human chromosome 15 is shown on top, 5′ to 3′. Positions indicated from the Feb 2009 GRCh37/hg19 NCBI assembly database delineate borders of each gene and identify each SNP position. Gray boxes indicate exons. *CHRNA5* is not to scale (indicated by hash marks) and exons are not identified. Bold arrows indicate direction of transcription. The five different length constructs, inserted upstream of the SV40 promoter in the promoter–reporter pGL3 luciferase expression vector (not shown), as well as each possible allele for each SNP, is shown below the cluster diagram. Previous human expression studies (b): top line, indicating the 3.5 kbp construct used here, describes nucleotide base pairs from relative position 1–3500. Numbers across the top denote nucleotide bps in the 3.5 kbp construct, bottom numbers indicate relative SNP locations. Constructs shown below this metric are from 3 independent studies using luciferase reporter assays and human sequences upstream of *CHRNA3* and inclusive of the promoter. Solid, dark lines indicate a significant effect of the sequence when placed upstream of the luciferase reporter (Fornasari et al., 1997; Wu et al., 2009). Gray solid line indicates no effect of fragment (no promoter used in construct) (Fornasari et al., 1997). Dotted black line indicates possible repressive region, and bold A at rs6495309 indicates repressed expression compared to G (Wu et al., 2009). Dashed gray line indicates no effect (inclusive of *CHRNA3* promoter, numerous variants investigated) on expression (Doyle et al., 2011).