SNAP-25: A NOVEL CANDIDATE GENE IN PSYCHIATRIC GENETICS

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SNAP-25: EGY ÚJ KANDIDÁNS GÉN A PSZICHIÁTRIAI GENETIKÁBAN

A SNAP-25 (szinaptoszóma-asszociált fehérje, 25 kDalton) a SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) egyik fontos alkotója. Maga a SNARE egy szinaptikus vezikulum-dokkoló komplex, mely az exocitózishoz, vagyis a neurotranszmitterek kibocsátásához szükséges. A SNAP-25 hiányos egér modellen kapott eredmények nagyban elősegítették azt, hogy a SNAP-25 gén a pszichiátriai genetika egyik jelentős kandidáns génjévé vált elsősorban a figyelemhiányos hiperaktivitás (ADHD) és a szkizofrénia vonatkozásában. A kandidáns génvizsgálatok során nagy számú SNP-t (single nucleotide polymorphisms, SNP) vizsgálnak. A miR SNP egy funkció szempontjából új típusú variáns, amely egy adott mikroRNS adott target mRNS-hez való kötődését befolyásolja. In silico vizsgálataink alapján a SNAP-25 gén 3' nem-átiródó régiójában (3' UTR) két miR SNP valószínűsíthető. Amennyiben ezen SNAP-25 miR SNP-k in vivo szerepét sikerül igazolni, jelentős szerepet kaphatnak a pszichiátriai genetikai kutatásokban.

KULCSSZAVAK: SNAP-25, mikorRNS, pszichiátriai genetika, ADHD, depresszió

SUMMARY

SNAP-25 (synaptosomal-associated protein of 25 kDa) is an integral part of SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor), a docking complex for synaptic vesicle exocytosis and neurotransmitter release. Results with SNAP-25 deficient mouse models highly accelerated association studies of SNAP-25 as a candidate gene for psychiatric disorders, such as Attention Deficit Hyperactivity Disorder (ADHD) and Schizophrenia. Candidate gene studies implicate a large number of single nucleotide polymorphisms (SNPs). Among the numerous SNPs, the miR SNPs are novel functional variants affecting the binding of specific microRNA to their target mRNA. According to our in silico studies there are two putative miR SNPs in the 3'untranslated region (3'UTR) of the SNAP-25 gene. If the putative miR SNPs are shown to have a function in vivo their implication in further psychogenetic association studies will have a higher impact.

KEYWORDS: SNAP-25, microRNA, psychiatric genetics, candidate gene study, ADHD, depression

THE ROLE OF SNAP-25 IN NEUROTRANSMISSION

The process of membrane trafficking is a key element of neurotransmission including the synthesis, translocation, docking and the final exocytosis of synaptic vesicles. Fusion of the vesicles with the presynaptic membrane is mediated by the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, involving synaptosomal-associated protein of 25 kDa (SNAP-25), syntaxin-1 (Stx-1) and synaptobrevin (VAMP) (Hayashi, et al. 1994). As SNAP-25 and Stx-1 are associated to the presynaptic membrane, while VAMP is the integral protein of the synaptic vesicle (Figure 1), forming a stable heterotrimer of these three proteins results in docking of a vesicle for subsequent exocytosis. SNAP-25 is known to play a central role in the regulation of vesicle targeting and was shown to interact with voltage gated calcium channels, the triggering signals of exocytosis (Catterall, 1999, Tafoya, et al. 2008). The protein has two isoforms, SNAP-25a and SNAP-25b formed by alternative splicing. SNAP-25b is the dominant form in adults while SNAP-

Figure 1. Schematic model of the four alpha helix heterotrimer SNARE formation

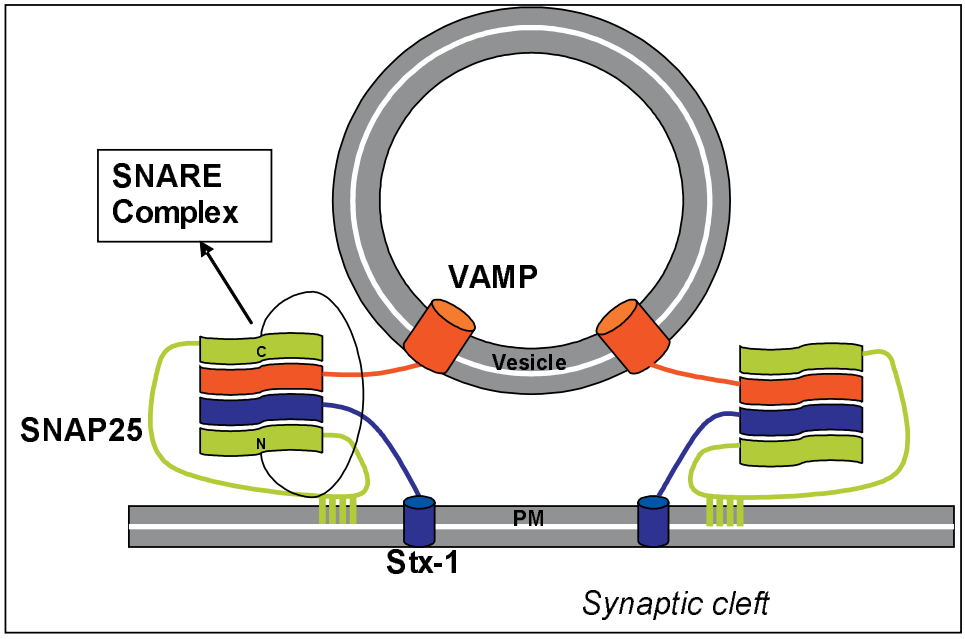
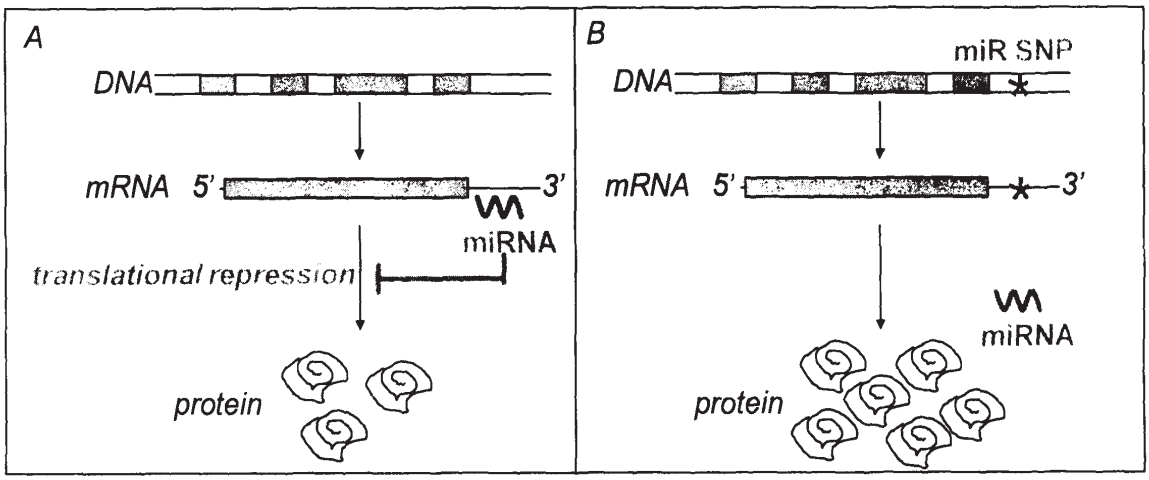


Figure 2. miR SNP disrupts the function of miRNA



A: microRNAs (miRNA) are translational repressors of gene expression when binding to their target sequence on the mRNA.
B: If an SNP is located in the target sequence of a miRNA (miR SNP), the binding and the translational repression is disrupted.

25a is mainly expressed in embryonic development (Bark et al. 2004).

As the heterotrimer formation is a crucial event in targeting the vesicle, the SNARE proteins and their interaction has been extensively studied. SNAP-25 is the largest component of the heterotrimer, containing two alpha-helices conjoined in a coiled formation. The central part of the SNAP-25 protein is anchored to the presynaptic membrane through a palmitoyl side chain of the cysteine-rich membrane interacting region (Pobbati, et al. 2006). The central anchoring permits a free interaction of the SNAP-25 protein with its SNARE complex counterparts. Syntaxin-1 and Synaptobrevin are transmembrane proteins bound to their particular membranes through their C-terminal peptides and interact with SNAP-25 through their SNARE motifs. Ultimately, a „four helix bundle" is formed and pulls the vesicle and plasma membranes together (Figure 1)(Zhang, et al. 2002).

SNAP-25 DEFICIENT ANIMAL MODELS

Over the past few years, a significant amount of research has been devoted to elucidate the functions of the SNAP-25 protein to its genetic level.

Two distinct mice models have been developed to demonstrate consequences of SNAP-25 deficiency: the "coloboma mutant mouse" and the "blind-drunk" one. The coloboma mutant mouse has a heterozygous deletion on chromosome 2 encompassing numerous genes including SNAP-25 (Hess, et al. 1992). Heterozygous deletion of the SNAP-25 gene results in 50% decrease of the protein expression and, as a consequence, a deficit in dopamine release. This is a proposed mouse model of Attention Deficit Hyperactivity Disorder (ADHD) as this mouse exhibits locomotor hyperactivity and learning deficiencies. When the mouse was rescued with a functional SNAP-25 transgene, the hyperactivity phenotype faded and the dopaminergic neurotransmission was restored (Wilson, 2000).

In a more recent study, the "blind-drunk mouse" was identified as a consequence of a missense mutation leading to an isoleucine-threonine substitution in a conserved region of SNAP-25b. As a result, the mutant SNAP-25b exhibits a 2-fold increase in its binding affinity to Syntaxin-1 compared to its wildtype form. This abnormal immobilization leads to the impairment in exocytotic vesicle recycling. The phenotypes of the "blind-drunk mouse" were characterized by ataxia and sensorimotor gating, both of which are main behavioral component implicated in schizophrenia (Jeans, et al. 2007).

ASSOCIATION STUDIES BETWEEN SNAP-25 GENE AND PSYCHIATRIC DISEASES

Based on the results with animal models of SNAP-25 (see above), genetic polymorphisms of this gene were shown to participate in association analyses with various psychiatric diseases, such as ADHD and schizophrenia. Haplotypes of SNAP-25 3'UTR region were found to associate to ADHD by a transmission disequilibrium test of 97 probands and their parents suggesting that the SNAP-25 gene plays a role in the development of ADHD (Barr, et al. 2000). This association between ADHD and SNAP-25 gene has been successfully replicated in other studies (for a review see (Faraone, et al. 2005)). In a recent study, 61 SNPs across the SNAP-25 gene have been assayed and six of them were found to associate with ADHD and co-morbid major depressive disorder (Kim, et al. 2007).

Recent post-mortem studies revealed a decreased level of SNAP-25 protein in the hippocampus of patients with schizophrenia and bipolar disorder (Scherk, et al. 2008). These symptomatic findings coincide with those of the blind-drunk mouse and suggest a possible role of SNAP-25 variation in schizophrenia. Furthermore, the putative ADHD low risk allele of SNAP-25 was shown to associated with schizophrenia (Carroll, et al. 2009). In contrast, a Japanese study quoted no susceptible relationship between members of SNARE complex and schizophrenia (Kawashima, et al. 2008). Therefore additional investigations are needed to demonstrate the possible role of SNAP-25 genetic variants in schizophrenia and mood disorders.

miRNA: A NON-CODING RNA FOR REGULATION OF GENE EXPRESSION

Micro RNAs (miRNAs), the putative translational regulators of eukaryotic gene expression, belong to the small non-coding RNAs. More than a thousand specific miRNA were predicted (Berezikov, et al. 2005) and nearly 700 have been identified in humans (Griffiths-Jones, et al. 2008) since their discovery in C. elegans at 1993 (Lee, et al. 1993, Wightman, et al. 1993). These small RNAs are coded in intronic or in non-coding part of the genome. The 1-4000 bp primary microRNA is processed in the nucleus by the Drosha complex. The formed 70-80 bp stem-loop precursor miRNA is transported into the cytosol by Exportin-5. The Dicer enzyme complex then converts it into a 20-23 bp miRNA (Kim, et al. 2006) and initiates the formation of the RNA-induced silencing complex (RISC). The mature, single-stranded miRNAs are complementary to the 3' untranslated region (3' UTR) of one or more target mRNAs (Filipowicz, et al. 2008), thus promoting the mRNA degradation and/or inhibiting directly the target protein synthesis. For mRNA degradation a perfect complementarity is crucial which is characteristic for plants. In mammals the partial complementarity is more characteristic therefore we can predict that miRNAs affect mostly the process of translation (Filipowicz, et al. 2008). The seed sequence in miRNA responsible for effective mRNA

binding involves 7 nucleotides starting at the 2nd nucleotide from the 5' end (Grimson, et al. 2007, Lewis, et al. 2005). If there is an SNP either in the seed sequence or in the mRNA target, labeled as miR SNP, the binding of the miRNA complex is disrupted. This may alter the expression at the coded protein level, leading to phenotypic changes, such as diseases (Sethupathy et al. 2008). Studies on the frequencies of SNPs in miRNAs region and in their targets demonstrated that polymorphisms are rare in miRNAs. Among 474 miRNA coding regions, only 65 SNPs were found (SNP density: 1.3 per kb), and only three of them were located in the seed region (Saunders, et al. 2007). Opposite to the highly conserved miRNAs, nearly 400 SNPs were found in about 29,000 target sites demonstrating that SNPs at the target site are rather frequent (SNP density: 2.7 per kb) (Saunders, et al. 2007).

FUNCTIONAL STUDIES OF miRNA BINDING

Computational analysis is only the first step in revealing the putative function of a specific miRNA. As a next step, experimental evidences should be provided to demonstrate that (1) the miRNA and its target mRNA are both expressed in the same tissue, (2) they bind to each other *in vivo*, and (3) the binding down regulates the translation in a cellular system. There are several techniques for analyzing the miRNA expression profile in tissues; as primer extension analysis, northern blotting, dot blotting, and the recently developed microarrays (Kim, et al. 2006). On the other hand, reporter gene systems are the appropriate tools for the study of translational inhibition. Here the 3'UTR region of the target gene including the miRNA binding site is ligated to the 3' end of a reporter gene, such as luciferase. Subsequently, both the construct and the miRNA is transfected into a cell culture to demonstrate the translational repression, if any, by the quantitative assay of the synthesized reporter protein.

Martin and coworkers (Martin, et al. 2007) applied the aforementioned system to examine the effect of a miR SNP in the angiotensin II type 1 receptor (AT1R) gene. They found that the polymorphism attenuated miR-155 binding with significant changes in the expression level of the reporter. In addition, both miR-155 and its target gene were shown to be expressed in the same cell type by in situ hybridization. Finally, the effect of the miR-155 on AT1R expression was also measured *in vivo* by transfection of miR-155 inhibitor into human primary vascular smooth muscle cells (Martin, et al. 2007).

miR SNP AS RISK FACTORS IN VARIOUS DISEASES

Although genetic variations in miRNA binding sequences are considered to have an important impact in health and diseases, only a few examples have been revealed recently demonstrating a specific miR SNPs as genetic risk factors of a common disease. Most results were obtained in relation to various cancer types (Chin, et al. 2008, Yu, et al. 2007), but there are also examples for the functional role of miR SNPs in cardiovascular (Sethupathy, et al. 2007), autoimmune (Tan, et al. 2007) and neuropsychiatric diseases. For example the rs13212041 AG polymorphism in serotonin receptor 1B (HTR1B) gene, altering the target site of miR-96, was shown to associate with aggressive behavior (Jensen, et al. 2008, Sethupathy, et al. 2008). Additionally, Abselon et al. found an association between a frameshift mutation in SLITRK1 gene and Tourette's syndrome, where the mutation (var321-SLITRK1) strengthens a miR-189 target site (Abelson, et al. 2005).

There are several, available online databases (eg. http://www.patrocles.org/, http://compbio.utmem.edu/miRSNP/) for searching sequences of miRNAs and their targets, as well as SNPs located at these positions. We performed an *in silico* search for putative miR SNP sites in the 3' UTR of SNAP--25 gene. According to the data in PolymiRTS Database, the rs1051312 CT is considered to alter putative miRNA binding sites for the SNAP-25 gene. It creates the target sequence of mi-R 510 microRNA. In addition, there is another SNP (rs3746544 TG) adjacent to rs1051312 CT lying in the same target sequence. Both SNPs disrupt the target sequence of miR-641 microRNA. Although these SNPs have been studied earlier among other SNPs of the SNAP-25 gene (Barr, et al. 2000), their functions in

translational regulation were not considered. Therefore investigations are in progress in our laboratory to obtain molecular evidences for the *in vivo* function of these putative miR SNPs in the SNAP-25 gene to provide a theoretical background for further association analyses between the SNAP-25 polymorphisms and psychiatric diseases.

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