Schizophrenia Research 124 (2010) 208–215

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Non-synonymous variants in the *AMACR* gene are associated with schizophrenia

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ARTICLE INFO

Article history:
Received 29 June 2010
Received in revised form 24 August 2010
Accepted 31 August 2010
Available online 26 September 2010

Keywords:
Schizophrenia
5p13
AMACR
Genetic association
Case-control study

ABSTRACT

Background: The AMACR gene is located in the schizophrenia susceptibility locus on chromosome 5p13, previously identified in a large Puerto Rican pedigree of Spanish origin. The AMACR-encoded protein is an enzyme involved in the metabolism of branched-chain fatty and bile acids. The enzyme deficiency causes structural and functional brain changes, and disturbances in fatty acid and oxidative phosphorylation pathways observed in individuals with schizophrenia. Therefore, AMACR is both a positional and functional candidate gene for susceptibility to schizophrenia.

Methods: The study had a two-step design: we performed mutation analysis of the coding and flanking regions of AMACR in affected members of the pedigree, and tested the detected sequence variants for association with schizophrenia in a Puerto Rican case-control sample ($n=383$) of Spanish descent.

Results and conclusion: We identified three missense variants segregating with the disorder in the family, rs2278008, rs2287939 and rs10941112. Two of them, rs2278008 and rs2287939, demonstrated significant differences in genotype ($P=4\times10–4$, $P=4\times10–4$) and allele ($P=1\times10–4$, $P=9.5\times10–5$) frequencies in unrelated male patients compare to controls, with the odds ratios (OR) 2.24 (95% CI: 1.48–3.40) and 2.25 (95% CI: 1.49–3.38), respectively. The G–C–G haplotype of rs2278008-rs2287939-rs10941112 revealed the most significant association with schizophrenia ($P=4.25\times10–6$, OR = 2.96; 95% CI: 1.85–4.76) in male subjects. There were no statistically significant differences in genotype, allele, and haplotype frequencies between female schizophrenia subjects and controls. Our results suggest that *AMACR* may play a significant role in susceptibility to schizophrenia in male patients.

1. Introduction

Alpha-Methylacyl-CoA Racemase (*AMACR*) is an enzyme involved in peroxisomal and mitochondrial oxidative metabolism of branched-chain fatty acids and bile acids. Deficiency of this enzyme results in the accumulation of pristanic acid and C-27 bile acid intermediates in tissues and body fluids to toxic levels (Ferdinandusse et al., 2000a). In the neural cell cultures derived from rat brain the accumulation of pristanic acid caused depolarization of mitochondrial membranes, elevation of concentrations of intracellular Ca2+ most of all in the myelin-producing oligodendrocytes, and induced cell death in neurons, astrocytes, and oligodendrocytes (Rönicke et al., 2009). In the rat hepatoma cell lines the C-27 bile acid intermediates induced apoptosis, disrupted the integrity of

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doi:10.1016/j.schres.2010.08.040

plasma membranes, and impaired mitochondrial oxidative phosphorylation (Sokol et al., 2001). The accumulation of bile acid intermediates contributed to liver disease (Ferdinandusse et al., 2009). The mechanism of toxicity of pristanic acid and C-27 bile acid intermediates in humans is poorly understood. However, accumulation of pristanic acid in humans influences functions of brain cells and leads to alterations of brain structure, encephalopathy, progressive demyelinating neuropathy, pigmentary retinopathy, and xanthomas with sterol storage in the nervous system and vessels (Nakashima et al., 1994; Cuebas et al., 2002; Mukherji et al., 2003; Schwarz et al., 2008).

Mitochondria are a main cellular target of pristanic acid. The brain contains a large number of mitochondria due a high energy demand, and the accumulation of pristanic acid disturbs the aerobic metabolism by decreasing the mitochondrial membrane potential and enhancing the generation of cellular reactive oxygen species (ROS) in neurons, astrocytes and oligodendrocytes (Boekema and Braun, 2007; Rönicke et al., 2009). Disturbances in fatty acid and oxidative phosphorylation pathways have been observed in several brain regions of individuals with schizophrenia, including the prefrontal cortex of post-mortem schizophrenia brains (Prince et al., 1999; Hakak et al., 2001; Maurer et al., 2001; Middleton et al., 2002; Prabakaran et al., 2004). The additional symptoms of the *AMACR* deficiency include seizures, memory deterioration, confusion, irrational behavior, and depression (Thompson et al., 2007). Moreover, the *AMACR* deficiency and schizophrenia share several phenotypically similar symptoms, including white matter alterations (Clarke et al., 2004; Höistad et al., 2009), impairments in working memory and other cognitive functions that are the prime features of schizophrenia (Kremen et al., 2010; Jahshan et al., 2010), sensory deficits (Javitt, 2009) and pigmentary retinopathy (McDonald et al., 1998). Alterations in the metabolism of pristanic and bile acids were also detected in diabetes, obesity, and hyperlipidemia — diseases which may be at increased risk in patients with schizophrenia independent of medication and other secondary effects (Bremer, 2001; Ryan et al., 2003; Bellivier, 2005; Leucht et al., 2007; Yeap et al., 2008). These observations suggest that the *AMACR* gene could be considered a functional candidate gene for susceptibility to schizophrenia.

The *AMACR* gene is located on chromosome 5p13. Although several studies obtained either positive LOD scores or detected structural changes at 5p13 region in patients with schizophrenia, no schizophrenia-related genes at this locus have been identified yet (Silverman et al., 1996; Crowe and Vieland, 1999; Ekelund et al., 2000; Garver et al., 2001; Paunio et al., 2001; Cooper-Casey et al., 2005; Suarez et al., 2006; Holliday et al., 2008; Walsh et al., 2008). In an earlier study we identified a small region on 5p13 linked to schizophrenia in Family 17, a single Puerto Rican pedigree of Spanish origin (Bespalova et al., 2005). The region of about 3 Mb is flanked by markers D5S1993 and D5S631 and contains several annotated positional candidates genes for susceptibility to schizophrenia, including *AMACR*.

Mutations in the *AMACR* gene have been identified in patients with sensory motor neuropathy (Ferdinandusse et al., 2000b), and the association of sequence variants in the coding region of *AMACR* has been observed in patients with hereditary prostate cancer and colorectal adenoma (Zheng et al., 2002; Daugherty et al., 2007a). To investigate the involvement of the *AMACR* gene in schizophrenia, we have performed a two-step design study. We first screened the coding, 3′-non-coding and flanking regions of the gene for mutations in schizophrenia-affected members of the Puerto Rican family, and identified sequence variants shared by affected members of the pedigree. Then, we investigated frequencies of the shared variants in the Puerto Rican ethnically matched case-control sample.

2. Materials and methods

2.1. Study subjects

As more fully described in earlier studies (Silverman et al., 1996; Bespalova et al., 2005), Family 17's proband and his first degree relatives were ascertained in New York City but were native to the Cordillera Central, the central mountain region of Puerto Rico, where most of the other branches of the family still resided. The central mountain region was settled primarily by people of Spanish descent (Vazquez Calzada, 1981). All affected members of the family met DSM-III-R or

Table 1
Observed haplotypes on chromosome 5p13 in the Family 17 branch with key recombinants.

MARKERS	HAPLOTYPES													
	101	102	201	202	203	204	205	206	207	208	209	210	211	212
D5S416	6**9**	2 6	**9** 6	**9** 2	**9** 2	6 2	6 6	6 2	6 2	**9** 6	6 2	**9** 6	**9** 6	6 2
D5S419	1**5**	5 9	**5** 9	**5** 5	**5** 5	1 5	1 9	1 5	1 5	**5** 9	1 5	**5** 9	1 9	1 5
D5S1993	1**5**	2 5	1 5	**5** 2	**5** 2	1 2	1 5	1 2	1 2	**5** 5	1 2	**5** 5	1 5	1 2
D5S477	2**1**	4 3	2 3	**1** 4	**1** 4	2 4	2 3	2 4	2 4	**1** 3	2 4	**1** 3	2 3	**1** 4
D5S1350	7**5**	3 5	7 5	**5** 3	**5** 3	7 3	7 5	7 3	7 3	**5** 5	7 3	**5** 5	7 5	**5** 3
D5S1470	7**1**	3 2	7 2	**1** 3	**1** 3	7 3	7 2	7 3	7 3	**1** 2	7 3	**1** 2	7 2	**1** 3
D5S583	1**9**	3 1	1 1	**9** 3	**9** 3	1 3	1 1	1 3	1 3	**9** 1	1 3	**9** 1	1 1	**9** 3
D5S2062	6**5**	5 7	6 7	**5** 5	**5** 5	**5** 5	6 7	6 5	6 5	**5** 7	6 5	**5** 7	6 7	**5** 5
D5S1506	1**1**	1 9	1 1	**1** 1	**1** 1	**1** 1	1 9	1 1	1 1	**1** 9	1 1	**1** 9	1 9	**1** 1
D5S631	5**4**	3 3	5 3	**4** 3	**4** 3	**4** 3	5 3	5 3	5 3	**4** 3	5 3	5 3	5 3	**4** 3
D5S426	7**8**	2 1	7 1	**8** 2	**8** 2	**8** 2	7 1	7 2	7 2	**8** 1	7 2	7 1	7 1	8 2
D5S395	8**8**	1 3	8 3	**8** 1	**8** 1	**8** 1	8 3	8 1	8 1	**8** 3	8 1	8 3	8 3	8 1

Three schizophrenia-affected (203, 208, 210), two SPD-affected (202, 212), and unaffected (201, 204, 205, 206, 207, 209, 211) siblings and their unaffected parents (101, mother; 102, father) are presented. The individual ID numbers are as previously reported (Silverman et al., 1996; Bespalova et al., 2005). The maternal disease-associated ("at-risk") haplotype is in bold. The MLR (framed) is defined by recombination breakpoints in individuals 210 and 212. The markers are ordered from telomere to centromere.