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Transthyretin: No association between serum levels or gene variants and schizophrenia

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Abstract

It has been proposed that schizophrenia results from an environmental insult in genetically predisposed individuals. Environmental factors capable of modulating transcriptional activity and their carriers could link the genetic and environmental components of schizophrenia. Among these is transthyretin (TTR), a major carrier of thyroid hormones and retinol-binding protein (RBP). Retinoids and thyroid hormones regulate the expression of several genes, both during development and in the adult brain. Decreased TTR levels have been reported in the cerebrospinal fluid of patients with depression and Alzheimer's disease, and the absence of TTR influences behavior in mice. DNA variants capable of altering TTR ability to carry its ligands, either due to reduced transcription of the gene or to structural modifications of the protein, may influence development of the central nervous system and behavior. In the present study we searched for variants in the regulatory and coding regions of the *TTR* gene, and measured circulating levels of TTR and RBP. We found a novel single nucleotide polymorphism (SNP), ss46566417, 18 bp upstream of exon 4. Neither this SNP nor the previously described rs1800458 were found associated with schizophrenia. In addition, serum TTR and RBP levels did not differ between mentally healthy and schizophrenic individuals. In conclusion, our data does not support an involvement of the *TTR* gene in the pathophysiology of schizophrenia.

Keywords: Transthyretin; Retinol-binding protein; Schizophrenia; Thyroid hormones; Retinoids; Association studies

1. Introduction

Schizophrenia is a complex neurodevelopment disorder (Beckmann, 1999). Epidemiological studies indicate an increased risk for developing schizophrenia in relatives of probands with the disease (Gottesman, 1991), suggesting a genetic predisposition. However, monozygotic twins are frequently phenotypically discordant, which implies that environmental factors must play a role in the disease etiology (Tsuang et al., 2001).

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Thyroid hormones and retinoids are among the environmental factors proposed to be altered in schizophrenia (Goodman, 1998; Palha and Goodman, 2005). It is widely recognized that thyroid hormones and retinoids are essential for the normal development of the central nervous system, and that lack of adequate levels during pregnancy leads to several neurological defects (Lane and Bailey, 2005; Luo et al., 2004; Morreale de Escobar, 2003; Morreale de Escobar et al., 2004; Wietrzych et al., 2005). Thyroid hormones and retinoids act as modulators of the expression of several genes through the binding of retinoic acid and triiodothronine to the corresponding nuclear receptors. Interestingly, evidence of altered retinoid and thyroid hormone metabolisms in schizophrenic patients is arising from studies in postmortem brains, in which RARα and myelin-basic protein, genes whose expression is regulated by thyroid hormones and retinoids, were found to have altered expression (Hakak et al., 2001; Rioux and Arnold, 2005).

By influencing ligand availability, carriers of thyroid hormones and retinoids indirectly regulate the transcription of several genes. Among the modulators of thyroid hormone and retinoid availability is transthyretin (TTR), a major carrier of thyroxine and retinol [through association with (RBP)], both in serum and in cerebrospinal fluid (CSF) (Palha, 2002). TTR has been implicated in behavior: decreased TTR CSF levels were found in patients with depression (Sullivan et al., 1999) and with Alzheimer's disease (Serot et al., 1997). Whether this decrease is the result or a consequence of the disease or whether it is caused by medication is still unclear. Supporting that both processes might be implicated in behavior are the observations that TTR-null mice (Palha 2002) present increased motor activity in behavior tests that address anxiety-like and depression-like behaviors (Sousa et al., 2004), and that clozapine treatment induces TTR expression in the brain (Chen and Chen, 2005).

The *TTR* gene is a single copy gene, on 18q12.1, composed of four exons (Tsuzuki et al., 1985) that encodes a 14 kDa subunit which assembles as a tetramer (Blake et al., 1978). Whereas plasma TTR originates primarily from the liver, CSF TTR is mainly produced and secreted from the choroid plexus, where it represents about 20% of the total protein synthesis (Aldred et al., 1995). The observation that homologues of the TTR protein can be found in a wide range of species (Eneqvist et al., 2003) and that its synthesis starts early during embryonic development (Larsen and DeLallo, 1989), suggests a relevant role for TTR in development.

These observations, prompted us to investigate the *TTR* gene as a candidate gene in schizophrenia. In the present study we searched for variants in the regulatory and coding regions of the *TTR* gene, and association between the polymorphisms detected and schizophrenia were analyzed in three different samples. We also investigated serum TTR and RBP levels in schizophrenic and in mentally healthy individuals.

2. Materials and methods

2.1. Samples

Two case–control samples were used: one from Portugal-mainland and a second from Brazil. A total of 244 unrelated schizophrenic patients (175 males and 69 females) and 210 controls (131 males and 79 females) were recruited from the north and center of Portugal-mainland. The Brazilian sample consisted of 69 cases and 85 controls, all unrelated males living in the area of Porto Alegre. A third, independent sample composed of 73 patients (47 males and 26 females) and their parents from the Azorean Islands (Portugal), was used for family-based association analysis. All subjects were of European ancestry. All participants gave informed consent for genetic studies and ethic committees of the institutions involved approved the study.

All patients from Portugal-mainland and Azorean Islands, as well as 45% of Portugal-mainland controls and 67% of the Azorean parents were evaluated using the Diagnostic Instrument for Genetic Studies (DIGS) (Nurnberger et al., 1994), a semi-structured interview that assesses the criteria for schizophrenia and other psychiatric diseases. The diagnosis was made based on the Diagnostic and Statistical Manual of Mental Disorders, review of the third edition (DSM-IIIR, 1985) (American Psychiatric Association, 1987). All Brazilian patients were classified using the Operational Checklist for Psychotic Disorders (OPCRIT) (McGuffin and Farmer, 2001). No psychological assessment interviews have been conducted in the remaining Portuguese mainland controls (students and tissue donors) or Azorean parents, or in any of the Brazilian controls (blood donors).

2.2. Polymorphism screening

Using single strand conformational polymorphism (SSCP) analysis, a sub-sample of 60 patients from the Portuguese mainland was used for screening variants in all four exons and adjacent splicing sites, as well as in the promoter and the 3′ untranslated regions. For this analysis the PCR products were subjected to electrophoresis on a non-denaturing polyacrylamide gel under two temperature conditions: 4 °C and 25 °C. After completion of the electrophoresis, band patterns were visualized with silver staining, using standard protocols. PCR products from subjects displaying altered band patterns in the SSCP analyses were sequenced in both directions. The sequencing reactions were performed using the BigDye Sequencing Kit 3.1 and run on the 3700 sequencer, both from Applied Biosystems (Foster City, CA).

The TTR sequence was obtained from the GenBank Data Libraries (Accession No. M11844) and the Primer3 program (Rozen and Skaletsky, 2000) was used for primer design. Primer sequences are available upon request.