Bioinformatics and Functional Analysis of Serine Racemase and D-Amino Acid Oxidase in Schizophrenia Patients: Postprint

Authors: Ping Junjiao (1); Gao Yongshuang (1); Wu Yong (1); Du Baoguo (1); Jiang Tingyun (1); Qian Gang (2)

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Abstract

Objective D-serine serves as an important neuroglial transmitter, and its synthesis and metabolism depend on serine racemase (SR) and D-amino acid oxidase (DAO). The SR and DAO genes are closely associated with schizophrenia. This study aims to elucidate the relationship between the structure and function of SR and DAO in schizophrenia patients.

Methods The SR and DAO genes were cloned from the blood of schizophrenia patients and subjected to bioinformatics analysis.

Results Sequence analysis revealed that the SR and DAO genes encode polypeptides of 340 and 347 amino acids, respectively, with homology to healthy humans, apes, and monkeys exceeding 96%. The predicted relative molecular masses of SR and DAO proteins are 36.57 kDa and 39.47 kDa, respectively, with theoretical isoelectric points of 6.11 and 6.36. Subcellular localization analysis demonstrated that SR protein is primarily localized in cellular mitochondria, while DAO protein is mainly localized in mitochondria and peroxisomes, suggesting that these two proteins primarily function in synthesis and metabolism within cells. Structural and functional analysis identified that SR protein possesses one domain, whereas DAO protein contains two domains and a linker region.

Conclusion It is hypothesized that SR and DAO play important roles in processes such as protein synthesis and metabolism in eukaryotic cells.

Full Text

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Ping Junjiao[1], Gao Yongshuang[1], Wu Yong[1], Du Baoguo[1], Jiang Tingyun[1], Qian Gang[2] [1]Zhongshan Third People' s Hospital, Zhongshan 528400, China; [2]Zunyi Medical College, Zunyi 563000, China

Abstract

Objective: D-serine serves as an important neuroglial cell transmitter, and its synthesis and metabolism depend on serine racemase (SR) and D-amino acid oxidase (DAO). Both SR and DAO genes are closely associated with schizophrenia. This study aims to elucidate the relationship between structure and function of SR and DAO in schizophrenia patients.

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Results: Sequence analysis revealed that the SR and DAO genes encode polypeptides of 340 and 347 amino acids, respectively, sharing over 96% homology with healthy humans, apes, and monkeys. The predicted relative molecular masses of SR and DAO proteins were 36.57 kDa and 39.47 kDa, with theoretical isoelectric points of 6.11 and 6.36, respectively. Subcellular localization analysis indicated that SR protein is primarily localized in mitochondrial compartments, while DAO protein is mainly distributed in mitochondria and peroxisomes, suggesting that these two proteins primarily function in synthesis and metabolism within cells. Structure and function analysis identified one structural domain in SR protein and two structural domains plus a linker region in DAO protein.

Conclusion: SR and DAO are inferred to play important functional roles in protein synthesis and metabolism processes in eukaryotic cells.

Keywords: schizophrenia; serine racemase; D-amino acid oxidase; structure prediction; sequence analysis

Introduction

Genetic studies have demonstrated that the pathogenesis of schizophrenia results from the accumulation of multiple pathogenic genes. With the development of molecular biology, increasing evidence has confirmed that schizophrenia patients exhibit genetic abnormalities, including genes related to dopamine D2 and D3 receptors, reelin, GluR6, MTHFR, NRG1, and bdnf. D-serine was

discovered in mammals, and subsequent research revealed regionally high concentrations of D-serine in the central nervous systems of mammals, including humans. Studies have shown that D-serine possesses stronger activation efficacy than glycine. Serine racemase (SR) specifically catalyzes the conversion of L-serine to D-serine, while DAO, present in both the central nervous system and peripheral tissues of mammals, promotes the oxidative deamination of neutral D-serine and plays a crucial role in D-serine metabolism. Compared with normal individuals, schizophrenia patients, as well as those with major depression and bipolar disorder, show 39% and 21% reductions in SR in the frontal cortex and hippocampus, respectively. While DAO remains unchanged in the frontal cortex, DAO protein is significantly increased in the hippocampus. Additionally, D-serine levels in cerebrospinal fluid are reduced in schizophrenia patients.

Miranda et al. first isolated the SR gene from human blood, and subsequent studies have identified associations between SR gene variants and schizophrenia. Research has also demonstrated a close relationship between the DAO gene and schizophrenia. Polymorphisms in the DAO gene have been correlated with schizophrenia in the Xi' an population. A case-control study of 340 normal individuals and 340 schizophrenia patients in the Japanese population revealed an association between DAO haplotypes and schizophrenia. Similarly, studies in the Irish population have found the DAO gene to be closely related to schizophrenia. While numerous reports have addressed D-serine synthesis and metabolism, as well as its distribution, storage, and release mechanisms in the central nervous system, gene-level studies remain fundamental. Current research on SR and DAO in China has primarily remained at the gene sequence level, with rare reports on protein three-dimensional structural properties. The lack of detailed three-dimensional structural information hinders further investigation of protein function and catalytic mechanisms. Homology modeling and molecular dynamics simulation methods have proven effective for theoretical simulation of biological macromolecules and can guide experimental observations. Therefore, this study cloned SR and DAO genes and employed bioinformatics analysis to examine the structural and functional properties of SR and DAO proteins, aiming to deepen understanding of their roles in schizophrenia and provide a molecular-level theoretical basis for antipsychotic drug development.

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1. Materials and Methods

1.1 Study Subjects

Schizophrenia patients visiting the outpatient clinic or admitted to the inpatient department of Zhongshan Third People' s Hospital, with ancestral roots in the Zhongshan region, were enrolled in this study. Inclusion criteria were: age 18–59 years; either sex; educational level of primary school or above; sufficient visual and auditory capacity to complete required examinations; diagnosis of schizophrenia according to DSM-IV criteria; disease duration less than 5 years;

total score 60 on the PANSS positive and negative symptom scale; passing general physical, neurological, and psychiatric examinations. Exclusion criteria included: current severe organic disease; substance abuse; heavy smoking or alcohol consumption; pregnancy or lactation; exclusion of organic mental disorders, epilepsy-related mental disorders, depression, bipolar disorder, atopic dermatitis, stroke, Alzheimer' s disease, and cancer patients. All participants voluntarily enrolled and provided informed consent. All subjects were diagnosed by two attending physicians or above from our hospital, and those with uncertain diagnoses were excluded.

1.2 Experimental Methods

1.2.1 Blood Sample Collection: Five milliliters of fasting peripheral venous blood were collected from schizophrenia patients in the morning, anticoagulated with 100 L of 0.5 mol/L EDTA (pH 8.0), and stored at -20 °C for later use.

1.2.2 Genomic DNA Extraction: Thawed blood samples were used to extract genomic DNA using the OMEGA D3392-01 whole genome DNA extraction kit. Extracted DNA quality was assessed using UV spectrophotometry and 1% agarose gel electrophoresis.

1.2.3 Primer Design and Target Gene Fragment Amplification: SR and DAO gene sequences were identified by searching the NCBI human gene database, and primers were designed using Primer 5.0 software. The total reaction system was 20 L, containing 0.2 L Taq DNA polymerase, 2.0 L 10× PCR Buffer, 0.2 L 25 mmol/L dNTP, 0.5 L each of forward and reverse primer (10 mol/L), 1.0 L template DNA, and double-distilled water to reach 20 L. PCR amplification conditions were: initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR reactions were performed on an ABI9700 PCR instrument.

1.2.4 PCR Product Sequencing: PCR amplification products were recovered using the OMEGA D2500-01 kit, quantified, and sequenced. The sequencing reaction system consisted of 2 L Mix (Bigdye3.1, 5× sequencing buffer, H2O), 2 L purified PCR product, and 1 L primer (5 mmol/L). Sequencing reactions were performed on an ABI9700 PCR amplifier with cycling conditions of 2 min at 96 °C followed by 30 cycles of 95 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. After sequencing reactions, PCR products were purified using the OMEGA 1320-01 kit and sequenced on an ABI3730xl genetic analyzer. Obtained sequences were subjected to Blast alignment to confirm they were target sequences.

1.2.5 Bioinformatics Analysis: NCBI online BLAST tools (www.ncbi.nih.gov) were used to compare sequences against GenBank' s non-redundant nucleotide and protein databases. NCBI ORF finder (http://www.ncbi.nim.nih.gov/) was used to predict open reading frames. Expasy' s ProtParam program was employed to analyze amino acid composition, theoretical relative molecular mass,

and isoelectric point of SR and DAO proteins. ProtScale program was used for protein hydrophobicity analysis. GOR4 online tool was applied for secondary structure analysis of SR and DAO. Signal peptide prediction was performed using http://www.cbs.dtu.dk/services/SignalP/. Subcellular localization was predicted using Harvard University' s online tool. Protein structural domains were predicted using SMART server and InterProScan software. CBS online Profun tool was used to predict functional classification of SR and DAO proteins in schizophrenia patients. Three-dimensional structures of SR and DAO were predicted using the homology modeling server SWISS-MODEL.

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2. Results

2.1 SR and DAO Amino Acid Sequence Analysis

Full-length DNA sequences of SR and DAO genes were obtained through cloning and sequencing from schizophrenia patients. After removing vector, intron, and redundant sequences, ORF finder analysis revealed that SR and DAO gene sequences were 1023 bp and 1044 bp in length, respectively, each containing a complete open reading frame. SR encoded a polypeptide of 340 amino acid residues, while DAO encoded a polypeptide of 347 amino acid residues. NCBI Blast alignment showed that the amino acid sequences of both polypeptides shared high homology with apes, monkeys, marmosets, cattle, horses, and cats, with homology exceeding 85%.

2.2 Physicochemical Property Analysis of SR and DAO

ProtParam online software from Expasy was used to analyze the 340-amino-acid and 347-amino-acid sequences encoded by SR and DAO genes in schizophrenia patients. The predicted results showed that the relative molecular masses of SR and DAO proteins were 36.57 kDa and 39.47 kDa, respectively, with theoretical isoelectric points (pI) of 6.11 and 6.36. Atomic compositions were C H N O S for SR and C H N O S for DAO.

SR protein contained 34 negatively charged residues (Asp+Glu) and 31 positively charged residues (Arg+Lys), with an instability index of 32.55, indicating stable protein status. DAO protein contained 38 negatively charged residues (Asp+Glu) and 34 positively charged residues (Arg+Lys), with an instability index of 31.68, also indicating stable protein status.

Hydrophobicity analysis of SR protein revealed a maximum hydrophobicity value of 4.200, indicating the strongest hydrophobicity at that position, with 35% of amino acids showing hydrophobic peaks greater than 0. The maximum hydrophilic peak was -3.800, and the overall protein exhibited high hydrophilicity with a hydrophilicity assessment of -8.99 [Figure 1: see original paper]A.

DAO protein hydrophobicity analysis showed a maximum hydrophobicity value of 4.500, with 35% of amino acids having hydrophobic peaks greater than 0. The

maximum hydrophilic peak was -4.500, also demonstrating high hydrophilicity with an assessment of -17.29 [Figure 1: see original paper]B.

2.3 Secondary Structure Prediction

GOR4 method was used to analyze the secondary structure of SR and DAO proteins in schizophrenia patients. As shown in [Figure 2: see original paper], SR protein secondary structure consisted of 32.06% helices, 17.65% extended strands, and 50.29% random coils, with helices and random coils being the main components of the protein' s secondary structure.

DAO protein secondary structure comprised 20.17% helices, 22.19% extended strands, and 57.64% random coils, indicating that extended strands and random coils were the primary structural components.

2.4 Subcellular Localization and Signal Peptide Prediction

Online tool PSORT was used to predict subcellular localization of SR and DAO proteins in schizophrenia patients. Results showed that SR and DAO proteins were primarily localized in peroxisomes and mitochondria of cells. Signal peptide prediction using http://www.cbs.dtu.dk/service/SignalP/ revealed that SR protein lacked a signal peptide, while DAO protein had the highest composite cleavage site score of 0.749 at amino acid positions 16 and 17. This suggests that SR has no transmembrane structure or signal peptide, whereas DAO contains a signal peptide sequence.

2.5 Domain, Tertiary Structure Analysis and Function Prediction

InterPro online software was used for preliminary protein domain prediction. Results showed that SR protein contained only one domain involved in cellular amino acid metabolic processes, while DAO protein contained two domains and a linker region, corresponding to D-amino acid metabolic processes, oxidation-reduction processes, and an FAD-binding region.

Functional classification of SR and DAO proteins in schizophrenia patients was analyzed using CBS online Profun software. As shown in and , SR protein showed probabilities of 10.174 for amino acid biosynthesis, 5.397 for fatty acid metabolism, 3.438 for transformation, 3.203 for coenzyme biosynthesis, 2.866 for energy metabolism, and 1.520 for intermediate metabolism. Predicted values for enzyme protein and membrane protein were 2.248 and 0.499, respectively, indicating that SR is an enzyme protein. Among enzyme categories, isomerase and lyase showed the highest probabilities at 1.711 and 1.549.

DAO protein exhibited probabilities of 8.432 for amino acid biosynthesis, 5.466 for cell membrane, 5.407 for intermediate metabolism, 3.789 for fatty acid metabolism, and 3.525 for coenzyme biosynthesis. Enzyme protein and membrane protein prediction values were 2.787 and 0.283, respectively, confirming

DAO as an enzyme protein. Among enzyme categories, lyase and oxidoreductase showed the highest probabilities at 1.640 and 0.964.

Three-dimensional structures of SR and DAO proteins in schizophrenia patients were predicted using SWISS-MODEL homology modeling server. As shown in [Figure 3: see original paper], SR protein contained one structural domain, while DAO protein contained two structural domains and a linker region. Random coils constituted the main structural component of both SR and DAO proteins, occupying a large proportion of the structure as evident in [Figure 3: see original paper].

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3. Discussion

Schizophrenia is a polygenic genetic disease whose occurrence and development may involve multiple genes and factors, with different susceptibility loci potentially existing in different pedigrees. Numerous studies have demonstrated that D-serine possesses stronger activation efficacy than glycine. Since NMDA receptor dysfunction is considered a pathophysiological factor in schizophrenia, research has increasingly focused on D-serine. Studies have found that abnormal D-serine levels or enzyme genes involved in D-serine synthesis and metabolism are associated with schizophrenia.

D-serine synthesis and metabolism depend on SR and DAO. In this study, cloning of SR and DAO genes from schizophrenia patients with family history revealed 100% homology with healthy individuals, indicating that abnormalities in these two genes are not direct causative factors of schizophrenia. In 2014, the Schizophrenia Working Group of the Psychiatric Genomics Consortium analyzed the genomes of 150,000 volunteers using advanced techniques and identified 108 genetic regions associated with schizophrenia in the human genome, none of which contained SR and DAO genes, consistent with our findings.

Subcellular localization analysis showed that SR and DAO proteins in schizophrenia patients are primarily localized in mitochondria and peroxisomes. Mitochondria are sites of energy conversion, tricarboxylic acid cycle, and oxidative phosphorylation, which aligns with the functional characteristics of SR and DAO as racemase and oxidase. Domain prediction revealed that SR protein contains one highly conserved domain, while DAO protein contains two highly conserved domains and a linker region. Multiple amino acid sequence alignment identified two highly conserved sequences in SR and DAO proteins, showing high homology across different species and suggesting functional consistency.

In protein secondary structure prediction, random coils accounted for large proportions in both SR and DAO proteins (50.29% and 57.64%, respectively). Random coils are significantly influenced by side-chain interactions and often constitute the central loops of enzyme active sites and other protein-specific functional

regions, playing crucial roles. We speculate that these regions are important sites where SR and DAO proteins exert their functions as serine racemase and oxidase.

Schizophrenia is a severe mental illness with tremendous harmfulness to individuals, families, and society. Genes are intrinsic factors influencing disease occurrence, and numerous studies have investigated schizophrenia susceptibility genes. However, research on SR and DAO amino acid mutation sites and protein structure-function properties in schizophrenia is rarely reported. Therefore, investigating structural protein properties at the gene level is particularly important for clarifying the nature and mutation sites of SR and DAO in schizophrenia.

This study employed bioinformatics software to analyze SR and DAO and construct three-dimensional structures, deepening our understanding of their functions in schizophrenia and providing a molecular-level theoretical basis for antipsychotic drug development.

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Note: Figure translations are in progress. See original paper for figures.