Secretagogin (SCGN) Plasma Levels and their Association with Cognitive and Social Behavior in Children with Autism Spectrum Disorder (ASD)

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ABSTRACT

Objective: To investigate the secretagogin (SCGN) plasma levels in children with autism spectrum disorder (ASD) compared to age and gender-matched healthy control, and its association with cognitive and social behaviors by using childhood autism rating scale (CARS) and social responsiveness scale (SRS).

Study Design: Case-control study.

Place and Duration of Study: Autism Research and Treatment Center, Al-Amodi Autism Research Chair, Department of Physiology, Faculty of Medicine, King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia, from October 2015 to May 2016.

Methodology: SCGN levels were determined in the plasma of thirty-seven (37) autistic children using enzyme-linked immunosorbent assay (ELISA), categorized as mild-moderate and severe as indicated by their CARS scores and compared with thirty (30) age and gender-matched control samples. Correlation between SCGN levels and different cognitive and social behavior scales (CARS and SRS) was determined by Spearman’s correlation coefficient (r).

Results: The results indicated that autistic children (n=37) had significantly (p= 0.005) lower plasma level of SCGN (45.7 (26.2) ng/ml [median (IQR)]) than those of healthy controls (n=30, 70.8 (48.6) ng/ml [median (IQR)]). Children with severe (n=28, 76%) as well as mild to moderate autism (n=9, 24%) also exhibited significantly lower SCGN levels (47.5 (27) ng/ml [median (IQR)], p =0.014) and (45.7 (16.6) ng/ml [median (IQR)], p = 0.02), respectively than healthy controls (n=30, 70.8 (48.6) ng/ml [median (IQR)]). However, there was no significant difference between the SCGN levels of children with mild to moderate and severe autism (p = 0.66). Spearman’s correlation coefficient (r) was used to determine the relationships between SCGN levels and different variables (CARS, SRS). However, the results showed no significant correlation between SCGN and these scales. (CARS, r=-0.03, p=0.86; SRS, r=0.21, p=0.20).

Conclusion: The low SCGN plasma levels in children with ASD probably indicate that SCGN might be implicated in the pathogenesis of autism. However, these data should be treated with caution until further investigations are performed using larger sample sizes to determine whether the decrease in plasma SCGN levels is a mere consequence of autism or it plays a pathogenic role in the disease.


INTRODUCTION

Autism is a neurodevelopmental disorder characterized by abnormal reciprocal social interactions, communication deficits and repetitive behaviors with restricted interests. Although the etiology and pathogenesis of the disorder are still unclear, increasing evidence suggests that genetic, environmental and immune system deregulation may be implicated. The discovery of valid biomarkers for autism will enable earlier and more targeted methods for diagnosis and intervention. While there is increasing promise of finding biomarkers, the widely accepted biomarkers for autism are still lacking.

Secretagogin is a member of the EF-hand (E-helix-loop-F-helix-hand) super family of calcium-binding proteins, that in humans is encoded by the SCGN gene. SCGN is highly expressed in numerous brain regions. It is strong candidate as a biomarker for neuroendocrine tumor, stroke, and eventually psychiatric conditions. Moreover, SCGN has been hypothesized to exert a neuroprotective role in neurodegenerative diseases like Alzheimer’s disease. However, detail of molecular pathways and its regulation is not clear. This protein plays an important role in the release of the stress hormone corticotropin-releasing hormone (CRH) and which only then enables stress processes in the brain.

Human and mouse brain has been examined to some extent for SCGN expression in a previous study. The human brain reveals maximum expression of SCGN in the cerebellum. Recent clinical studies on SCGN revealed its potential implication as a novel blood biomarker for the diagnosis of stroke and distinct tumors of endocrine origin such as adenocarcinomas of the
stomach, pancreas, prostate, colorectum, kidney, and lung small cell carcinoma. In addition, SCGN is released into serum after neural damage in cerebral ischemic diseases, suggesting that SCGN can be used as a marker for brain trauma.

However, numerous studies have so far indicated a neuroprotective effect of Ca²⁺ binding proteins. SCGN is capable of binding four Ca²⁺ ions at physiological intracellular Ca²⁺ levels with a binding affinity of Ca²⁺, similar to other calcium binding proteins. Calcium (Ca²⁺) is a universal second messenger, which plays a crucial role in signal transduction in many fundamental physiological processes. In the central nervous system (CNS), calcium signaling is utilized by neurons to control membrane excitability, neurotransmitter release, gene expression, cellular growth, differentiation, and cell death. Different variants of high transient and local Ca²⁺ concentrations in neurons play an important role in information processing. Calcium also plays an important role in the secretory pathway of neurons regulating processes. A large body of evidence has indicated that unbalanced Ca²⁺ homeostasis contributes to the development of neurological and neurodegenerative diseases. The finding could lead to SCGN based treatments for stress in cases of mental disorders such as depression, post-traumatic stress disorder, and chronic stress caused by pain.

This study was aimed to examine the SCGN protein levels in the plasma of autistic children, compare it with healthy controls and determine the correlation between SCGN and autism severity scales (CARS, SRS) in an attempt to clarify the role of SCGN in the etiology of autism.

**METHODOLOGY**

Children with ASD were recruited from the Autism Research and Treatment Center, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia for this study with and age-matched healthy children served as controls. The diagnosis of ASD was made by a pediatric neuropsychiatrist and a pediatrician, based on the criteria of ASD as defined in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Patients with associated neurological diseases were excluded. Written consents were obtained from the parents of each subject, according to the guidelines of the Ethics Committee of King Khalid Hospital, King Saud University, Riyadh, Saudi Arabia and appropriate institutional review board approval was obtained. All procedures that followed were in accordance with the Helsinki Declaration for all human or animal experimental investigations. This study was conducted at the Autism Research and Treatment Center, King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia from October 2015 to May 2016.

The CARS score sheet was filled as a scale for autism severity. A total score of at least 30, strongly suggests the presence of autism. Children who scored between 30 and 36 were labeled with mild to moderate autism while those with scores between 37 and 60 were labelled with severe autism.

SRS score of 76 or higher was considered severe; and score between 60 and 75 was taken as mild to moderate range of social impairment.

After an overnight fasting, blood samples (3 ml) were collected from each subject (autism and control) in test tubes containing EDTA. Blood samples were immediately centrifuged at 4°C at 3,000 rpm for 20 minutes to collect plasma samples, which were stored in a freezer at - 80°C until the time of analytical assays. All samples were assayed in duplicate and in a double-blind manner. The assay reproducibility generally ranged from 5 to 10% error.

The concentration of SCGN was measured in plasma of control and autistic subjects using a commercially available sandwich enzyme immunoassay (ELISA) kit from Cusabio Biotech Co. Ltd. (Wuhan, China). All biochemical analyses were performed in duplicate, and the median (IQR) values were reported. No significant cross-reactivity or interference was observed.

All results were analyzed using a commercially available software package, IBM, SPSS statistics version 21. CARS SRS, Age and SCGN protein values are presented as median (IQR). Because of non-normally distributed continuous variables, the Mann-Whitney U-test was used for comparisons of SCGN protein levels between the autistic and control groups. The null hypothesis was that there would be no difference in the data distributions for SCGN protein levels between participants diagnosed with ASD and control children. Spearman's correlation coefficient (r) was used to determine the relationships between SCGN levels and different variables (Age, CARS and SRS). For all statistical tests employed in the present study, a two-tailed p-value ≤ 0.05 was considered statistically significant.

**RESULTS**

Thirty-seven children with ASD (median age = 7, IQR = 2 years) were inducted as case and 30 age-matched children (median = 7, IQR = 4,5 years) served as controls. The general characteristics of the study participants and the results of the SCGN levels were summarized in Table I. Levels of SCGN were compared between children with different severity of autism (mild-moderate or severe) and age-matched control subjects. Changes in behavioral characteristics in subject with autism were assessed by the CARS and SRS.

Table I indicates that autistic children (n=37) had significantly lower (p= 0.005) plasma level of SCGN than...
healthy controls (n = 30). The SCGN plasma of children (n = 28, 76%) with severe autism (CARS > 36.5) and children (n = 09, 24%) with mild to moderate autism (CARS < 36.5) were significantly lower plasma values (p = 0.014, and p = 0.02, respectively) than normal controls. However, there was no significant difference between the SCGN plasma levels of children with mild to moderate and severe autism (p = 0.66).

All participants of study also completed the CARS and SRS. The median (IQR) values of CARS scores of all autistic, severe and mild to moderate autism were 44 (6), 44 (6) and 33 (4), respectively, which strongly suggest the severity of autism. The median (IQR) values of total SRS scores of all the autistic, severe and mild to moderate autism categories were found to be 77 (26.6), 77 (26.6) and 77 (12.5), respectively which were not significantly different to each other.

Spearman’s correlation coefficient (r) was calculated to determine the relationships between SCGN levels and different scales (CARS, SRS) as shown in Figures 1 and 2. However, resulting graphs showed no correlation between SCGN and these variable scales (CARS, r = -0.03, p = 0.86; SRS, r = 0.21, p = 0.20).

### DISCUSSION

The purpose of this study was to examine the SCGN protein levels in the plasma of autistic children and compare with normal children. It was also aimed to explore relation between SCGN and autism severity scales (CARS, SRS) in an attempt to clarify the role of SCGN in individuals with ASD. CARS assesses the child on a scale from one to four in each of 15 dimensions or symptoms, (including the ability to relate to people; emotional response; imitation; body use; object use; listening response; fear or nervousness; verbal communication; non-verbal communication; activity level; level and reliability of intellectual response; adaptation to change; visual response; taste, smell and touch response; and general impressions. The SRS is a validated test of interpersonal behavior, communication, and stereotypical traits in autism. It consists of five subscales: social awareness, social cognition, social communication, social motivation, and autistic mannerism. Total SRS raw scores range from 0 to 195, corresponding to significant social impairment as observed in individuals with ASD.

To the authors’ knowledge, this is the first study to investigate the SCGN levels in patients with autism. The present study found that plasma levels were significantly lowered in autistic patients compared with controls; which generally supports the hypothesis that SCGN are implicated in the pathophysiology of ASD. The mechanism underlying SCGN protein involved in the mediation of autism like behaviors is poorly understood at the moment. However, these data should be treated with caution until further investigations are performed, with larger samples, to determine whether the lower plasma levels of SCGN are a mere consequence of autism or have a pathogenic role in the disease. Thus, while there is clearly a variety of SCGN in individuals with autism, these two facts imply two things: First, the presence of these SCGN protein may be a secondary marker of the disease rather than directly pathogenic. Second, individual children with a similar clinical

### Table 1: Plasma levels of SCGN protein in autistic children and their relation to the severity of autism. Values are given in median (IQR).

<table>
<thead>
<tr>
<th>Age, years</th>
<th>SCGN (ng/ml)</th>
<th>p-value</th>
<th>CARS score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with autism (n = 37)</td>
<td>7 (2)</td>
<td>45.7 (26.2)</td>
<td>–</td>
</tr>
<tr>
<td>Patients with mild to moderate autism (n = 09)</td>
<td>7 (4.5)</td>
<td>45.7 (16.6)</td>
<td>0.020*</td>
</tr>
<tr>
<td>Patients with severe autism (n = 28)</td>
<td>7 (2)</td>
<td>47.5 (27)</td>
<td>0.014**</td>
</tr>
<tr>
<td>Healthy children (n = 30)</td>
<td>7 (4.5)</td>
<td>70.8 (48.6)</td>
<td>0.005^^</td>
</tr>
</tbody>
</table>

*Comparing control subjects with mild to moderate autistic children; **Comparing control subjects with severe autistic children. ^^Comparing mild to moderate with severe autistic children.
phenotype have very different responses to the SCGN protein.

We could not trace data in the literature concerning the role of SCGN protein family in single group of autistic children to compare our results. Although initial results reported in this study look promising; and on the basis of the preliminary results, there seems to be suggestive evidence in support of SCGN contributions to the pathophysiology of autism.

There is increasing evidence that SCGN may play a role in CNS activity.23 There are possibilities that SCGN is involved in common basic cellular processes that also affect the nervous system or it may affect particular neurodevelopmental processes directly, and play an important role in the development of ASD. In both cases, SCGN would not only be biomarkers for ASD symptoms, but it also would be therapeutic target for this disorder.

Social impairments and cognitive deficits are core features of autism. In autistic patients, difficulties in understanding the facial expressions of others, attention deficits, and communication problems are very common symptoms.24 CARS and SRS are autism severity scales that have been designed to measure cognitive deficits and social impairments, respectively. Few studies have drawn correlations between changes in plasma levels of different proteins and severity scores in children with autism.22,25 However, the present study did not find any significant correlation between SCGN plasma levels and CARS or SRS scales. The way in which SCGN did not associate with social behaviors is still unknown and requires further research. These results revealed that SCGN was an independent predictor of the CARS and the SRS scores. These findings provide a preliminary, direct evidence of altered levels of SCGN protein in subjects with ASD, compared to normal controls; which may contribute to the early pathogenesis of ASD, offer valuable biomarker, and point to novel therapeutic interventions. However, these data should be treated with caution until further investigations are performed, with a larger subject population, to determine whether the decrease of SCGN levels is a mere consequence of autism or has a pathogenic role in the disease. Future studies are recommended for the molecular properties and function of SCGN in individual with ASD.

The limitations of this study are the small sample size and its case-control design. In future, studies with a larger subject population are needed.

CONCLUSION

ASD is a neurodevelopment disorder with a number of etiologies and different mechanisms that leads to abnormal development. The identification of autism biomarkers in patients with different degrees of clinical presentation (i.e., mild, moderate and severe) will give greater insight into the pathogenesis of this disease and will enable effective early diagnostic strategies and treatments for this disorder.

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REFERENCES


