

Association of *AVPR1A* gene microsatellites with Autism Spectrum Disorder in south Indian population

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Abstract: Pharmacological studies have shown the therapeutic potential of Arginine Vasopressin Receptor (*AVPR1A*) for Autism Spectrum Disorders (ASDs) with socialization and communication symptoms. Present study investigated the association of promoter microsatellites (RS1 and RS3) polymorphisms of *AVPR1A* gene with ASDs in south Indian ethnicity. We recruited 225 subjects that constitutes of 118 ASD subjects diagnosed based on DSM-IV criteria and 107 controls, aged between 2 and 25 years of south Indian ethnicity. Case control association with RS1 allele length- 318bp, ($P = 0.009$); allele length- 330bp ($P = 0.02$); overall association with RS1 ($P = 0.02$) and RS3 allele length- 333bp ($P = 0.002$), 335bp ($P = 0.045$) over all association ($P = 0.002$) with ASDs was found to be significant. Our results reveal that, RS1 and RS3 microsatellites of *AVPR1A* gene contribute to the susceptibility of ASD in south Indian ethnicity.

Keywords— Autism Spectrum Disorder (ASD), *AVPR1A*, Allele, Microsatellites.

I. INTRODUCTION

Autism spectrum disorders (ASDs), are complex neurodevelopmental disorder which severely affects an individual's social life with communication difficulties, restricted and repetitive behaviors (NIH-National Institute of Mental Health, 2018). In the light of increased incidence of ASD's in the recent decades, the focus of research has now shifted towards ASD. However, the cause of ASD still remains unclear, partly because of its etiological heterogeneity. It is only recently that the effects of vasopressin on neural signals underlying social cognitive processes are being characterized. Oxytocin(OT) and Arginine-Vasopressin(AVP) are molecularly similar neuropeptides that are evolutionarily conserved and have important roles in mammalian social behaviour and cognition [1]. *AVPR1A* is one of the most extensively studied receptor

that mediates social behaviours like aggression, social recognition and paternal behaviour [2] with major effects on memory [3]. Interestingly, the studies in human subjects have revealed a significant association between the autism and heritable genetic defects in *AVPR1A* [4].

Human *AVPR1A* gene is located at 12q14–15 with two coding exons separated by 2.2-kb intron [5] *AVPR1A*, a G protein receptor is expressed in the septal nuclei, thalamus and basal nuclei of amygdala in the brain. These neuropeptides are transported through neurosecretory axons to the posterior hypothalamus to function as neurohypophyseal peptides [6] Various studies have established the possible associations between polymorphisms in the promoter region of the *AVPR1A* gene and ASDs. The human *AVPR1A* promoter region contains two microsatellite repeats, RS1 and RS3, in the 5' flanking region. Of these,

RS3 is a complex repeat located 3625bp upstream of the transcription start site, and RS1 is a (GATA)_n repeat located 553bp upstream of the start site. Different family based studies have shown the significant transmission of RS1 and RS3 microsatellites [7][8][4]. Recently, RS1, RS3, and intronic microsatellite (AVR) showcased the transmission of alleles in Finnish population [9] Shorter repeat alleles of RS1 and RS3 decreased the relative promoter activity in the human neuroblastoma cell line SH-SY5Y[10]

II. RELATED WORK

Research conducted on the behaviour of the Prairie vole a commonly-used animal model to study genes involved in affiliative social behaviours provided preliminary clues on the mammalian genetics and neural mechanism of social behaviour. It strongly suggests that regulatory defects of this system might contribute to neurodevelopmental disorders such as ASD [11] Though the expression of *AVPR1A* is species specific, studies conducted on Prairie voles have revealed that differential expression of proximal 5'-flanking region might result in behaviour changes[12] Furthermore, humanized *AVPR1A* mice displayed enhanced reciprocal social interactions [13] A group of scientists generated three mouse lines in which the mouse *avpr1a* 5'-flanking region was replaced with corresponding sequence from Prairie voles and showed significant contributions of the microsatellite using genome editing in mice [14] As mentioned above, animal model experiments provided prominent supports on the role of *AVPR1A* gene and promoter microsatellites in the development of ASDs.

Intranasal administration AVP antagonists have probed the effects of *AVPR1A* signalling on social behaviour. Genetic association studies have inspected the association of variation in the *AVPR1A* gene and social traits [12] Supporting this evidence, the *AVPR1A* antagonist, balovaptan, has the potential to be the first pharmacotherapy to help improve core socialization and communication symptoms of ASD. It was granted the 'breakthrough therapy' designation by the Food and Drug Administration and is proceeding to phase II testing supported by Roche, Basel (2018). These findings, together with the known role of *AVPR1A* in social skill formation, suggest a link between vasopressin dysregulation and development of autism. However the reports of RS1 and RS3 microsatellites association were only available from different ethnicity but none based on Indian ethnicity. Hence we focused our study to identify RS1 and RS3 association with ASDs in the south Indian population.

III. METHODOLOGY

1. Selection of subjects

We recruited 225 subjects, which constituted of 118 ASD subjects (90 Male and 28 females) and 107 controls (56 males and 51 females), between the ages of 2 to 25 years

(Mean age = 7 ± 10 , standard deviation = 4.82 ± 3.33) from south Indian ethnicity as self-reported. The ASD subjects diagnosed by the multidisciplinary team of neurologist, clinical psychologist and speech-language pathologist were by DSM-IV criteria. Fragile X syndrome and other syndromic forms of ASD are excluded from this study. All participants/guardians received a full explanation of the study protocols and objectives before obtaining their informed and written consent. Ethical clearance for this study was obtained from Mahatma Gandhi National Institute of Research and Social Action (MGNIRSA-affiliated research centre, University of Mysore) and also from ICCONS (Institute of Communicative and Cognitive Neuroscience) Ethical Committee.

2. Microsatellite selection and Genotyping

Selected markers (RS1 and RS3) identified as pharmacological importance with ASD and also exhibited importance for replication in different population (Table.1). PCR (Polymerase chain reaction) primers were designed based on previous studies [8] Approximately 5 ml of peripheral blood sample was collected in BD K₂vacutainer® (BD, NJ, USA) containing EDTA as anticoagulant. DNA was extracted by standard phenol chloroform and salting out method. PCR conditions used were denaturation step 95°C–5min, 95°C–30sec, annealing with 55°C–30sec, 72°C–40sec for 30 cycles then Extension 72°C–10min. Microsatellite peaks were measured on the Applied Biosystems 3730xl and sized with Genemapper 4.0 (Applied Biosystems™, Foster City, CA, USA).

3. Statistical Analysis

Case control association test for RS1 and RS3 microsatellites were performed using the UNPHASED 3.1.7[15] Power was calculated using online sample size estimator (<http://osse.bii.a-star.edu.sg/calculation2.php>).

IV. RESULTS AND DISCUSSION

AVPR1A microsatellites RS1 and RS3 allelic length shows significant association with ASDs. (Table.2 and Table.3) RS1 allele length- 318bp ($P = 0.009$); allele length- 330bp ($P = 0.02$); and overall association with RS1 ($P = 0.02$) exhibited a significant association with ASDs. In case of RS3 allele length- 333bp ($P = 0.002$), 335bp ($P = 0.045$) and over all association ($P = 0.002$) also showed the same pattern. Allele frequency of RS1-318bp was in cases-0.077 and in control-0.023 but the frequency in RS1-330bp was in cases 0.043 and in control 0.009. However, in the case of RS3 allelic frequency and count for 333bp in cases 0.24 controls 0.13. Similarly in 335bp in cases -0.017 and in controls is 0.051 (Fig.1 and Fig.2). Power estimated for RS1 is 46.50% based on MAF (Minor Allele Frequency) for Cases – 7.8%, Control – 2.3% and significant level 5%. For RS3 power to detect association was 56.00% based on MAF

in Cases 24.1 % and control 13.1% and significant level as 5%.

Discussion

The present case-control study was designed to identify the association status of popular microsatellites of commonly studied behaviour related gene *AVPR1A*. It is more strongly implicated in neuropsychiatric phenotypes, specifically ASD. Earlier studies have shown the presence of microsatellite repeats upstream of the gene *AVPR1A*. In humans, the promoter region of the *AVPR1A* includes a number of highly polymorphic microsatellites like RS1 and RS3. Long repeats of *AVPR1A* RS3 promoter region predicts increased expression in post-mortem hippocampal specimens [16] The mechanism by which the microsatellite allele length actually affects transcription efficacy is not yet known. The current study explores the influence of microsatellite (RS1 and RS3) length variability with occurrence of ASD in south Indian population.

However, this study has limited power and there were no reported case control study on RS1 and RS3 markers with ASD. However, we have adapted a statistical methodology from a study on choral singers and non-musicians [17]. The literature reported so far has produced inconsistent association with different alleles and markers implicated in *AVPR1A* gene. In non-clinical studies, it has been identified that RS1 and RS3 alleles of a specific length were highly associated with the social phenotype under investigation. In a study of personality traits, the RS1 320bp target allele mediated the association with harm avoidance and novelty seeking. The carriers also showed significantly lower left amygdala activity relative to the non-carriers[4] For RS3, the presence of a target allele (327–334bp) has been associated with less altruistic behaviour [18].

Latest studies suggest that targeting these elements in arginine vasopressin pathways are possible candidate for drug discovery as well as a source of potential biomarkers. This could help in early diagnosis of social disorders, especially ASDs [19]. Both RS3 and RS1 length variants were found to be associated with restrictive, repetitive behaviours- core feature of ASDs- but not overall diagnosis [20]. Along with all supporting evidence, the present study concludes that association of RS1 and RS3 allelic length variations may be important in the development of ASD.

V. CONCLUSION AND FUTURE SCOPE

Evidence from both human and animal studies implicated *AVPR1A* receptor in mediating and modulating key social behaviours that are challenging for children with ASD. No pharmaceutical treatments are currently exists for the better behavioural improvement in ASD. Present study supported the *AVPR1A* microsatellites (RS1 and RS3) and its association with ASD in south Indian population. However,

Inconsistency in allelic length in different studies may be due to different ethnicity, statistical methodologies and mostly because of the lack of specificity of RS1 and RS3 primers used. Application of this methodology using the same primer sets in similar population may provide better clarity in allelic length in association to RS1 and RS3 markers with ASDs. Thus present findings recommends a replication study for the same markers in a larger case-control sample set after quantifying for various behavioural parameters along with expressive and responsive language levels within the same population which is necessary to prove functional association of *AVPR1A* microsatellites with ASDs.

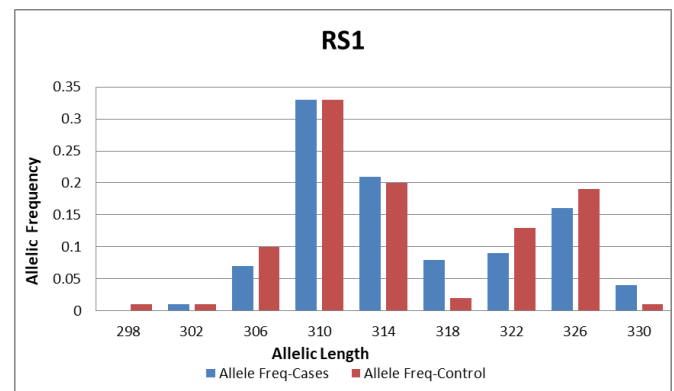


Fig.1: Allelic frequency of RS1 microsatellites in ASD and control subjects.

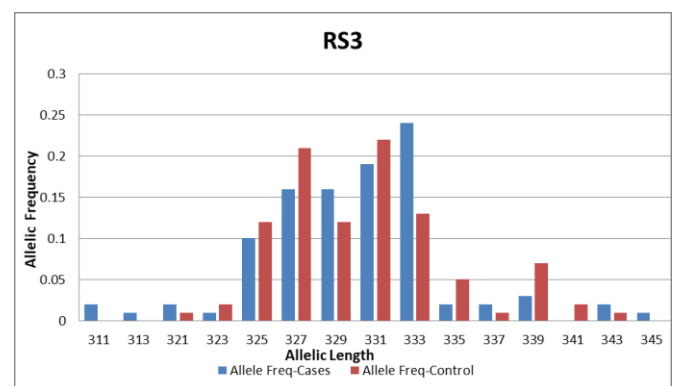


Fig.2: Allelic frequency of RS3 microsatellite in ASD and control subjects.

CONFLICT OF INTEREST

For this research paper there is no conflict of interest as confirmed by the authors.

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