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ORIGINAL PAPER

Right Anterior Theta Hypersynchrony as a Quantitative Measure Associated with Autistic Traits and K-Cl Cotransporter KCC2 Polymorphism

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Abstract
Our aim was to use theta coherence as a quantitative trait to investigate the relation of the polymorphisms in *NKCC1* (rs3087889) and *KCC2* (rs9074) channel protein genes to autistic traits (AQ) in neurotypicals. Coherence values for candidate connection regions were calculated from eyes-closed resting EEGs in two independent groups. Hypersynchrony within the right anterior region was related to AQ in both groups ($p < 0.05$), and variability in this hypersynchrony was related to the rs9074 polymorphism in the total group ($p < 0.05$). In conclusion, theta hypersynchrony within the right anterior region during eyes-closed rest can be considered a quantitative measure for autistic traits. Replicating our findings in two independent populations with different backgrounds strengthens the validity of the current study.

Keywords Autistic traits · Coherence · Excitation/inhibition imbalance · Quantitative traits · KCC2 · Theta oscillations

Introduction

Most studies of psychiatric disorders compare cases to controls to identify risk-associated variation. Although this approach has been effective, it is not sufficient alone because elements of psychiatric phenotypes are not restricted to patients, and substantial variability also exists in the healthy population (Baron-Cohen et al. 2001; Bralten et al. 2018; Lundström et al. 2012; Robinson et al. 2016). These elements are frequently referred to as traits. Traits are components of a larger *phenotype that differ between individuals in a species and show a relatively high level of stability across time and situations* (Plomin et al. 2009). Quantitative traits often follow a Gaussian dispersion at the population level. According to the liability-threshold model, liability (or risk) is distributed normally, and a disorder occurs only when a certain threshold is exceeded (Kopnik et al. 2017). Thus, common disorders can be considered extremes of quantitative traits.

Autism spectrum disorders (ASD)—neurodevelopmental conditions characterized by difficulties in social communication and repetitive behaviors—are among the disorders known to have an association with traits in the general population (Robinson et al. 2016). People with ASD exhibit a varying severity of core symptoms (American Psychiatric Association 2013). That is, ASD represents a spectrum among the affected. Similarly, autistic traits have a Gaussian distribution in the general population (Bourgeron 2015), and there is evidence concerning behavioral, genetic and physiological continuity of traits (Barttfeld et al. 2013; Bralten et al. 2018; Constantino and Todd 2003; Dennis et al. 2011; Jones et al. 2014). Considering ASD as a continuous variable based on key traits may help us to identify the genetic and physiological basis of this neurodevelopmental disorder.

Another promising approach for identifying mechanisms of neuropsychological disorders is to use quantitative biological measures instead of clinical symptoms. Quantitative traits, which are closer to the molecular effects of risk genes, can also be helpful in untangling genetic contributions of disorders as they are more directly related to the biological

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effects of susceptibility genes (Rasetti and Weinberger 2011). Therefore, we sought to use brain activity, measured via electroencephalography (EEG), as a quantitative trait.

Connectivity atypicalities are commonly reported in ASD and therefore present a potential quantitative trait. The most common findings are decreased long-range connectivity and increased local connectivity (Belmonte 2004; Just et al. 2004; Monk et al. 2009; Ouyang et al. 2017; Travers et al. 2012; Vissers et al. 2012), alterations of the right posterior-temporal region activity especially during social stimuli experiments (Abu-Akel et al. 2017; Donaldson et al. 2018; Kana et al. 2014; Lombardo et al. 2011; Pantelis et al. 2015; Pelphrey et al. 2011) and frontal connectivity changes (Redcay and Courchesne 2008; Wang et al. 2006). Among oscillations, theta band oscillations have an important role in the distribution of information over long distances and control of local information processing by theta-gamma coupling (Tort et al. 2010, 2013). Therefore, theta oscillation changes in the brain have a very wide impact on neural function. As they are involved both in synaptic plasticity and information regulation, changes in theta oscillations are observed in all brain atypicalities (e.g., epilepsy, schizophrenia, Alzheimer's Disease, etc.; Karakaş 2020; Pevzner et al. 2016). Moreover, studies show theta synchronization changes in ASD (Coben et al. 2008; Murias et al. 2007; O'Reilly et al. 2017; Sperdin et al. 2018). Needless to say, changes in connectivity are not confined to a single oscillatory band; for example connectivity alterations for ASD in the alpha band have been replicated several times (Dickinson et al. 2020; Haartsen et al. 2019; Seymour et al. 2019). Here, we focus on theta oscillations as a general indicator of functional changes in brain networks.

There is evidence that links gamma-Aminobutyric acid-A ($GABA_A$) receptors to the mechanism of theta production (Gołębiewski et al. 1996; Pevzner et al. 2016; Takács et al. 2018). Septal GABAergic cells act as pacemakers of theta generation in CA1 pyramidal cells through disinhibiting hippocampal interneurons (Freund and Antal 1988; Wang 2002; Ylinen et al. 1995). Any change in interneuronal cell function or number could contribute to changes seen in the theta band. In accordance with this, oscillation atypicalities in ASD are thought to originate from an excitation/inhibition (E/I) imbalance where a shift occurs toward the former (Collins et al. 2006; Zikopoulos and Barbas 2013). Evidence of this imbalance is primarily seen in findings related to GABA (Delong 2007; Gogolla et al. 2009; Kana et al. 2007; Ma et al. 2005; Orekhova et al. 2008; Piton et al. 2013; van Kooten et al. 2005), the only inhibitory neurotransmitter in the brain. All of these data suggest that disturbances in GABA-A signaling and theta oscillations might contribute to the pathophysiology of ASD within a common pathway. GABA hyperpolarizes the membrane potential by activating Cl^- permeable GABA-A receptor channels. In the early stages of development, GABA is weakly inhibitory in immature neurons, but in the late prenatal period, it gains a strong inhibitory effect (Ben-Ari et al. 1989). The transition from weak to strong inhibition is mediated by the internal Cl^- level in the GABAergic neuron (Ben-Ari et al. 1989; Tyzio et al. 2006). In the weak inhibition state, there is an increased expression of the Cl^- importer channel sodium/potassium/chloride cotransporter-1 (NKCC1), while in the strong inhibition state, the expression of Cl^- exporter channel potassium chloride cotransporter 2 (KCC2) is increased (Lu et al. 1999; Rivera et al. 1999). Linking those transporters to neuropathology, studies found improvement in cognitive tests and repetitive movements in a subset of individuals with ASD after administration of bumetanide, an NKCC1 antagonist (Du et al. 2015; Lemonnier et al. 2012; Sprengers et al. 2020).

To investigate the possible role of K-Cl cation chloride cotransporters on autistic traits, we chose to investigate single nucleotide polymorphisms (SNPs) which might have an impact both on the transporters and autistic traits. The minor allele of rs3087889, which is located in the 3′ untranslated region of *NKCC1*, was associated with lower expression of an alternative transcript of the gene in the brain (Morita et al. 2014). This SNP was also associated with schizophrenia risk and abnormal cognition in the same study. Regarding *KCC2*, individuals with ASD may display an increase in rare variants that may result in CpG methylation in the 3′ region of the gene, but this has not yet been validated (Merner et al. 2015). Also patients with Rett syndrome have a decreased KCC2 protein level and KCC2/NKCC1 ratio in their cerebrospinal fluid (Duarte et al. 2013).

EEG coherence is a measure of phase consistency of signals between different brain locations and reflects functional cortical connectivity (Nunez and Srinivasan 2006). High coherence reflects synchronized neural oscillations (integration), whereas low coherence suggests independent neuronal populations (segregation). Although coherence may be considered a poor measure of oscillatory connectivity because of volume conduction effects (Vinck et al. 2011), it has the advantage of considering both phase synchronization and amplitude which enables favoring observations that have a higher signal-to-noise ratio and therefore have a higher quality phase estimate (Bastos and Schoffelen 2016). Indeed, there is a growing debate about whether phase-relations or amplitude relations govern large scale brain networks (Foster et al. 2016).

We measured EEG coherence in theta frequency band (4–8 Hz) as a quantitative phenotype of autistic traits. The candidate quantitative phenotypes were theta coherence values for right anterior region, left anterior region, right posterior region, left posterior region, right frontal to parietal connections, left frontal to parietal connections, and global connections. We then examined the association of SNPs

rs9074 (*KCC2*) and rs3087889 (*NKCC1*) with the coherence value that was significantly associated with autistic traits, and also the direct association between these SNPs and autistic traits. We chose rs9074 because it is located in the 3′ untranslated region of the gene, which has the potential to carry gene expression regulatory sites, and rs3087889 because its minor allele is associated with lower expression of the gene.

We hypothesized that lower theta synchronization will be related to higher autistic traits and the minor allele of the SNPs will be related to this lower theta coherence. To evaluate the consistency of the findings, we included data from two independent samples from different backgrounds and using different collection methods to replicate and validate our results.

Methods

Study Design and Measurement Tools

We included two populations in the study. The first population belongs to Ankara University, Turkey (AU Group) and the second population to University of Virginia, United States (UVA Group).

In the AU Group, during their visit to the AU-BAUM Brain Research Center, participants completed the Turkish language version of the Autism Spectrum Quotient (AQ) (Baron-Cohen et al. 2001; Köse et al. 2010) and the Handedness Scale (Chapman and Chapman 1987; Nalçaci et al. 2002). Then, EEG was recorded from each participant during resting state with eyes closed. Two 2.5-min recording were obtained with a short break in between. Finally, 5 ml of venous blood was collected in EDTA containing tubes (BD Vacutainer K2 EDTA Blood Collection Tubes, BD Biosciences, San Jose, CA) from 60 individuals. Five people declined to provide a blood sample.

In the UVA Group, during their visit to the UVA Social Neuroscience Lab in the Department of Psychology, participants completed the AQ and provided a self-report of handedness. EEG was recorded from each participant for five minutes during resting state with eyes closed. On a separate visit occurring within 1 week, 8 ml of venous blood was collected in mononuclear cell separation tubes (BD Vacutainer CPT with sodium citrate, BD Biosciences, San Jose, CA).

The AQ is a 50-item self-report measure of preferences and tendencies in daily life. The maximum score is 50 points, and higher scores indicate higher levels of autistic traits. The scale mainly quantifies attention switching, social skills, attention to detail, communication, and imagination.

Participants

AU Group: Sixty-five young adult participants (32 females and 33 males, mean age = 23.17 years, *SD* = 3.33 years) were recruited for the current study. Inclusion criteria were right-handedness (mean score = 13.91, *SD* = 1.31, range [13, 17]) and ages 18 to 30 years. Excluded from the study were people with a history of a neurological, psychiatric or developmental disorder and those that used any neuropsychiatric medicine within the last six months. All participants provided written informed consent. The study was approved by the ethical committee of Ankara University School of Medicine.

UVA Group: Ninety-seven young adult participants (59 females and 38 males, mean age = 18.90 years, *SD* = 1.36 years) were recruited from the University of Virginia's Psychology Department Participant Pool and received partial course credit for their participation. Inclusion and exclusion criteria were the same with the previous group. All participants provided written informed consent. The study was approved by the University of Virginia Institutional Review Board for Health Sciences Research.

Electrophysiological Data Collection and Processing

Data Collection

AU Group: The EEG data were recorded using BrainVision (Brain Products GmbH, Germany) with 30 Ag–AgCl active electrodes mounted on an elastic cap using the extended 10–20 system. During recording, all electrodes were referenced to the FCz electrode. Eye movements were monitored using vertical and horizontal electrooculography (EOG) electrodes attached to the external canthi and the supraorbital regions of the right eye. Both EEG and EOG signals were digitally amplified and sampled at 1000 Hz. All electrode impedances were kept below 10 kΩ.

UVA Group: The EEG data were collected with BioSemi ActiveTwo System (Cortech Solutions, Wilmington, NC) with 32 Ag–AgCl active electrodes mounted on an elastic cap using the 10–20 system. During recording, all electrodes were referenced to the CMS/DRL. Eye movements were monitored using vertical and horizontal EOG electrodes and mastoid activity is recorded with external electrodes. Both EEG and EOG signals were digitally amplified and sampled at 2048 Hz. All electrode offsets were kept below 20 kΩ.

Data Processing

Both of the groups' data were analyzed using BrainVision Analyzer 2.1 software (Brain Products GmbH, Germany). Initially, the raw EEG data were passed through a 0.5–70 Hz band pass filter. Because of the difference in line power

frequency between countries, a notch filter with frequency of 50 Hz for AU Group and frequency notch filter of 60 Hz for UVA Group is used. The channels were re-referenced offline to the digital average of mastoid electrodes. Eye blinks and movements were corrected using an independent component analysis (Makeig et al. 2004). The data were divided into non-overlapping segments of 2048 ms for AU Group and 2000 ms for the UVA Group as there was a difference in sampling frequency. The first five segments of each recording were removed considering that the participants might not have yet transitioned into a resting state. The remaining 136 segments for the AU Group and 145 segments for UVA Group were visually examined for muscle artifacts, and those containing artifacts were removed. The mean number of segments after the removal of artifacts was 126.00 ($SD = 9.141$, range [101, 136]) for AU Group and 122.71 ($SD = 14.08$, range [92, 144]) for the UVA Group. Fast Fourier transforms were computed using a Hanning window with 10% taper length and 0.5 Hz resolution. The mean voltage value (μV) was computed for each channel.

Coherence Analysis

Coherence was calculated using BrainVision Analyzer with the magnitude-squared coherence method. Magnitude-squared coherence is estimated on the basis of the magnitude of the cross-spectrum and the auto-spectra of the input signals. It yields values between zero (signals are uncorrelated) and one (signals are linearly dependent). If the phase difference between the two signals at a given frequency is constant or of low variability across segments, the magnitude-squared coherence will be larger. On the contrary, if the variability of phase difference across segments is large, then coherence trends to zero.

The connections between F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1 and O2 were analyzed. As one of the main limitations on the interpretation of coherence results is interference of volume conduction (Srinivasan et al. 2007), to lower the interference, we did not include connections that are closely placed. In a study by Srinivasan et al. (2007), half sensitivity area, that is the surface area of the cortex that contains sources that could contribute at least half the signal contributed by the strongest source location for each electrode, was calculated. According to this study, the EEG half-sensitivity areas ranged from 8 to 35 cm^2 (median = 13 cm^2) where the largest area would be approximately 6×6 cm. Therefore, we restricted our coherence analysis with electrodes that are spaced more than 6 cm. The distance between electrodes was calculated on a 56 cm cap as this is the smallest size that was used in the study.

The electrodes were grouped into seven different connection types according to the hypothesized connection alterations in ASD: right anterior region (R-Ant; F4-T8, C4-T8), left anterior region (L-Ant; F3-T7, C3-T7), right posterior region (R-Post; C4-P8, P4-T8), left posterior region (L-Post; C3-P7, P3-T7), right frontal to parietal connections (R-FronPar; F4-P4, F4-P8, F8-P4, F8-P8), left frontal to parietal connections (L-FronPar; F3-P3, F3-P7, F7-P3, F7-P7), global connections (Global; full list can be seen on Table S1).

Coherence was calculated separately for each connection in accordance with the classic frequency band of theta (4–8 Hz). To ensure statistical normality, the coherence values obtained from each 0.5 Hz frequency bin were first z-transformed using Fisher's r to z procedure. Then, the z-scores were averaged within frequency band for each electrode. The resulting averages were back-transformed using the Fisher inverse function to obtain a coherence value for each electrode pair (Den Bakker et al. 2018). For every predefined region, the coherence values for connections in the corresponding group were averaged, and statistical analyses were performed on the coherence data.

Genotyping

AU Group: DNA was isolated from 500 μl samples of whole blood collected in EDTA tubes using a Purelink Genomic DNA mini kit (Invitrogen, USA) following the manufacturer's protocols. The isolation quality of the DNA samples was measured by spectrophotometry by Nanodrop 2000 (Thermo Scientific Inc., USA).

UVA Group: Blood samples were immediately spun at 1800 relative centrifugal force (rcf) for 30 min at 23 °C to separate the mononuclear cell fraction per product protocol. The mononuclear cells were then lysed, and DNA was extracted using reagents supplied in the Gentra Puregene Blood Kit (Qiagen, Germantown, MD). The isolation quality and quantity of the DNA samples was measured by spectrophotometry by Nanodrop 2000 (Thermo Scientific Inc., USA). DNA was stored at − 20 °C before further analysis.

Both groups' samples were genotyped for *KCC2* (rs9074, assay ID C-1414789-10) and *NKCC1* (rs3087889, assay ID C-3098965-10) by TaqMan® SNP genotyping assays (Applied Biosystems, USA). The DNA (2 ng) and SNP genotyping assays were combined in a 96-well plate on a Roche LightCycler 480 II (Roche Diagnostics GmbH, Germany) for the AU Group, and 7500 Fast Real Time PCR (Applied Biosystems, USA) for the UVA Group. In the AU Group, every plate included two no template control and minimum of three duplicate samples. For both SNPs, a total of ten samples were duplicated to confirm the results. The endpoint genotyping program of LightCycler 480 Software version 1.5 was used to identify the genotypes. For the UVA Group, every plate included positive controls for each genotype, no template control and duplicates of all samples.

Allelic discrimination was performed using Taqman Genotyper Software.

The distribution of genotypes was tested using χ^2 for the Hardy–Weinberg equilibrium (HWE). To determine the mode of inheritance (recessive, dominant or multiplicative) of the SNPs, the mean values of determined intermediate phenotype were tested against the genotypes using the Mann–Whitney U test.

Statistical Analysis

For all values, outliers with a z-score higher than ± 3.0 were excluded from the analysis. Differences between genders were analyzed with Student's T-test and correlation of autistic traits and coherence values with age by Spearman's correlation. We first computed a stepwise linear regression to determine which, if any, of the seven candidate coherence values (R-Ant, L-Ant, R-Post, L-Post, R-FronPar, L-FronPar, Global) were associated with autistic traits. For this analysis, we considered each population separately to assess the replicability of our results. Stepwise criteria for the probability of F was 0.050 to include the coherence value and 0.100 to remove the coherence value. We then tested for associations between the coherence value that emerged as a significant predictor of autistic traits and *KCC2* SNP rs9074 and *NKCC1* SNP rs3087889 using stepwise regression with the above-mentioned criteria. Finally, we tested for direct associations between these SNPs and autistic traits using stepwise regression with the above-mentioned criteria. We pooled the two sample populations together for these regressions using SNPs as predictors to increase the sample size necessary to identify genetic associations. All statistical analyses were performed using SPSS software version 22.0 (IBM Corp. Released 2011).

Results

Right Anterior Theta Coherence is Associated with Autistic Traits

AU Group

Autistic trait scores were calculated (mean score = 17.34, $SD = 5.55$, range [6; 32]) and compared as a function of gender [females ($M = 16.63$, $SD = 6.475$) vs. males ($M = 18.03$, $SD = 4.462$); t (63) = − 1.022, $p = 0.311$] and age [r_s (63) = 0.098, $p = 0.438$].

Males ($M = 0.391$, $SD = 0.046$) had significantly higher R-Post coherence values than females ($M = 0.364$, $SD = 0.030$) after Bonferroni correction for seven comparisons; t (63) = − 2.837, $p = 0.006$. There was no correlation between age and coherence values. Descriptive statistics of the coherence values are presented in Table 1.

Table 1 Descriptive statistics of resting state theta coherence values (N = 63–65)

Coherence	Min	Max	M	SD
R-Ant	0.357	0.668	0.483	0.071
L-Ant	0.362	0.672	0.480	0.078
R-Post	0.299	0.502	0.387	0.043
L-Post	0.302	0.458	0.373	0.033
R-AntPost	0.271	0.402	0.328	0.028
L-AntPost	0.280	0.396	0.329	0.027
Global Coherence	0.323	0.452	0.368	0.027

Table 2 Descriptive statistics of resting state theta coherence values (N = 95–97)

Coherence	Min	Max	M	SD
R-Ant	0.234	0.828	0.510	0.111
L-Ant	0.159	0.728	0.504	0.114
R-Post	0.094	0.770	0.412	0.127
L-Post	0.049	0.712	0.414	0.131
R-AntPost	0.034	0.493	0.221	0.092
L-AntPost	0.036	0.490	0.235	0.091
Global Coherence	0.089	0.460	0.299	0.069

All seven coherence values were entered into a stepwise linear regression to determine the measures that maximally predict autistic traits. A significant regression coefficient was found for the R-Ant coherence; F (1, 62) = 5.264, $p = 0.025$, adjusted $R^2 = 0.063$, standardized β = 0.280.

UVA Group

Autistic trait scores were calculated (mean score = 17.65, $SD = 6.52$, range [3; 34]) and compared as a function of gender [(females: $M = 17.33$, $SD = 6.334$) vs (males: $M = 18.13$, $SD = 6.866$); t (94) = − 0.588, $p = 0.558$] and age [r_s (95) = 0.138, $p = 0.179$]. Descriptive statistics of the coherence values are presented in Table 2.

The seven coherence values were entered into a stepwise linear regression to determine the measures that best predicted autistic traits. A significant regression coefficient was found for the R-Ant coherence; F (1, 92) = 4.030, $p = 0.048$, adjusted $R^2 = 0.032$, standardized β = 0.206.

Group Comparisons

Comparing between groups, the UVA Group ($M = 18.90$, $SD = 1.365$) was significantly younger than the AU Group ($M = 23.17$, $SD = 3.329$); t (160) = 11.313 $p < 0.001$. For

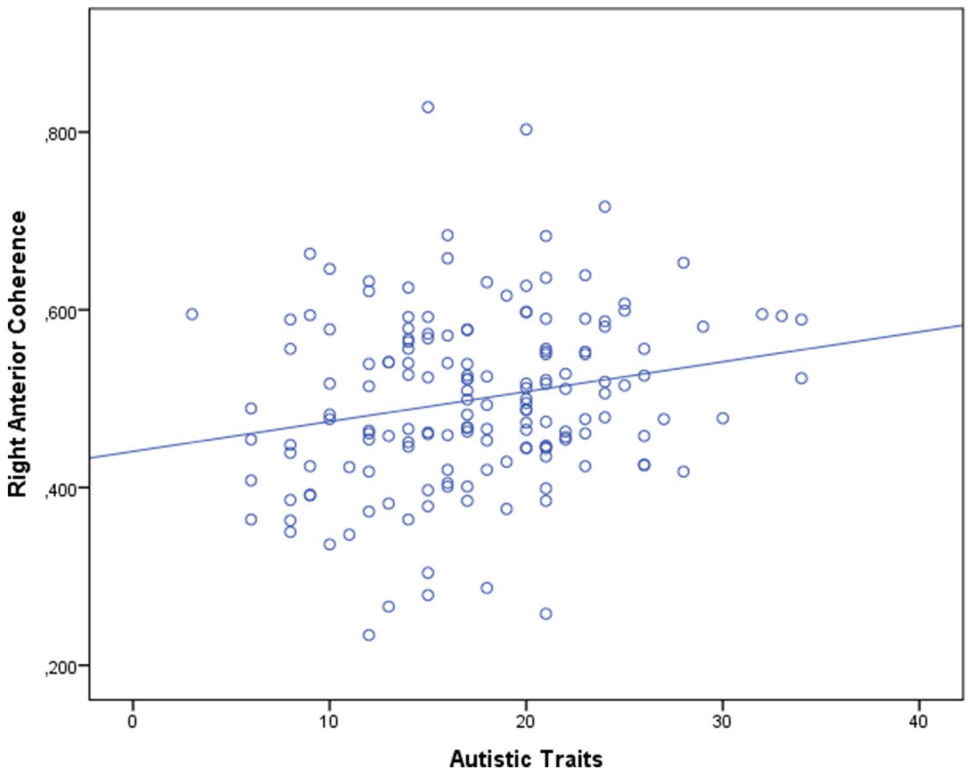

Fig. 1 Scatter plot of autistic traits to right anterior coherence values for the total group

autistic traits, there was no difference between the AU Group ($M = 17.34$, $SD = 5.546$) and the UVA Group ($M = 17.65$, $SD = 6.526$); $t\ (159) = -0.311$, $p = 0.756$. For comparison of coherence values between groups, a two-way MANCOVA indicated no significant effects of group or sex on coherence values after controlling for age; F (3, 154) = 1.547, p = 0.167. A scatter plot of autistic traits and R-Ant coherence values for the total group can be seen in Fig. 1.

KCC2 rs9074 Polymorphism is Associated with Right Anterior Theta Coherence

For the AU Group, the rs9074 genotypes were distributed as GG: 42.4%, GA: 47.5%, AA: 10.2% with allele frequencies in HWE [n = 59, $\chi 2\ (1) = 0.205$, p = 0.650]. For rs3087889, the genotypes were distributed as TT: 51.7%, AT: 37.9%, 10.3%. The allele frequencies were again in HWE [n = 58, $\chi 2\ (1) = 0.415$, p = 0.519].

For the UVA Group, the rs9074 genotypes were distributed as GG: 52.6%, GA: 41.2%, AA: 6.2% with allele frequencies in HWE [n = 97, $\chi 2\ (1) = 0.251$, p = 0.616]. For rs3087889, genotypes were distributed as TT: 56.7%, AT: 39.2%, 4.1%. The allele frequencies were in HWE [n = 97, $\chi 2\ (1) = 0.665$, p = 0.414].

To determine the genetic model for both SNPs, the mean values of the genotypes for R-Ant coherence were compared. Table 3 shows the results of the coherence values for the genotypes. For rs9074**,** as mean coherence values of GG and GA groups were similar, we selected the recessive model for minor allele and considered the AA genotype separately

Table 3 Descriptive statistics for R-Ant coherence values grouped by genotype

	AU Group					UVA Group				
	n	M	SD	Min	Max	n	M	SD	Min	Max
Rs9074										
GG	25	0.487	0.083	0.347	0.627	50	0.507	0.101	0.266	0.716
GA	28	0.474	0.064	0.385	0.658	40	0.500	0.119	0.234	0.828
AA	6	0.531	0.059	0.474	0.616	6	0.598	0.109	0.482	0.803
Rs3087889										
TT	30	0.505	0.078	0.376	0.658	55	0.504	0.110	0.234	0.803
TA	22	0.457	0.062	0.347	0.581	37	0.517	0.111	0.266	0.828
AA	6	0.494	0.069	0.420	0.592	4	0.523	0.136	0.350	0.663

from GG + GA. The Mann–Whitney U test indicated that for the AU Group, the R-Ant coherence was higher among individuals with the AA genotype ($Mdn = 0.507$) than the GG + GA genotypes ($Mdn = 0.465$), $U = 81.000$, $p = 0.050$. Similarly, for the AU Group, R-Ant coherence was higher among individuals with the AA genotype ($Mdn = 0.582$) than the GG + GA genotypes ($Mdn = 0.520$), $U = 148.000$, $p = 0.065$.

With rs3087889, there was not a consistent pattern for mean coherence values between groups, therefore, we selected an additive model and considered each genotype separately. The Kruskal Wallis test indicated that the R-Ant coherence did not significantly differ among genotypes within either the AU Group ($\chi^2 = 4.486$, $p = 0.106$) or the UVA Group ($\chi^2 = 0.282$, $p = 0.868$).

To assess the association of those rs9074 and rs3087889 polymorphisms on the R-Ant theta coherence we computed a stepwise linear regression analysis combining the two populations predicting R-Ant coherence from both SNPs. A significant regression coefficient was found for only the rs9074 using the recessive model, such that R-Ant theta coherence increased with increasing genetic risk $F(1, 153) = 5.557$, $p = 0.020$, adjusted $R^2 = 0.029$, standardized $\beta = 0.187$. We then computed a stepwise linear regression analysis combining the two populations predicting autistic traits from both SNPs. An association of rs9074 with autistic traits was also present $F(1, 153) = 4.691$, $p = 0.032$, adjusted $R^2 = 0.024$, standardized $\beta = 0.173$. Rs3087889 was again excluded from the model. 2D dot plots depicting R-Ant coherence values and autistic traits grouped by rs9074 SNP allele can be seen in Fig. 2.

Discussion

We evaluated EEG coherence, an estimate of functional neural connectivity, as a quantitative measure for autistic traits in two independent neurotypical populations. We established that autistic traits are positively associated with R-Ant theta coherence and that a KCC2 polymorphism, rs9074, is related both to this coherence and autistic traits. Our results suggest that theta hypersynchrony within the right anterior region during eyes closed rest may be considered as a quantitative measure for autistic traits.

While we find right theta hypersynchronization associated with higher autistic traits, we hypothesized the opposite, as lower theta synchronization (Doesburg et al. 2013; Hornung et al. 2019; Larrain-Valenzuela et al. 2017) and lower synchronization of long range connections in general (Belmonte 2004; Just et al. 2007; Monk et al. 2009; Ouyang et al. 2017; Travers et al. 2012; Vissers et al. 2012) have been previously associated with ASD. This discrepancy might be explained by the characteristics of our study population. Our sample is composed of healthy college or graduate students without any diagnosis of psychiatric or neurologic disorders. Therefore, they may possess compensatory mechanisms to overcome any autistic-like tendencies. In a study by Sperdin et al. (2018), toddlers and preschoolers with ASD with a gaze pattern similar to their typically developing peers showed an increased driving in theta within the left middle cingulate cortex and the right paracentral lobule while watching dynamic social images. These authors proposed this increased theta might be a mechanism to compensate for atypical development of the brain's circuitry over time. Moreover, improvements in autistic children with memory enhancement therapy was related to elevated EEG theta

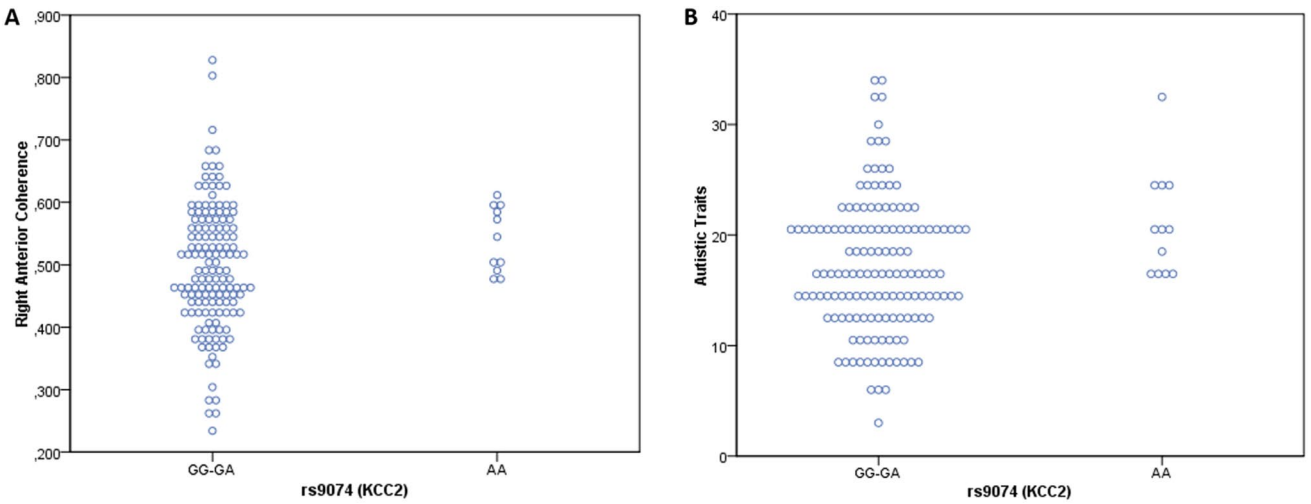

Fig. 2 2D dot plot of **a** right anterior coherence **b** autistic traits grouped for rs9074 (KCC2) alleles

coherence between frontal and posterior brain regions (Chan et al. 2015).

Similar to cognitive alterations in individuals diagnosed with ASD, epileptic patients display symptoms due to alterations within the related circuits. In line with this, approximately 30% of children with autism have epilepsy and 30% of children with epilepsy have autism (Tuchman et al. 2009). Accordingly, chronic seizure activity is related to decreased and altered firing of theta generating GABAergic cells in the septum (Colom et al. 2005; Garrido Sanabria et al. 2006), and temporal lobe epilepsy is thought to arise due to an imbalance in septohippocampal theta (Kitchigina et al. 2013). In a study by Young et al (2018), changes in theta band synchronization during memory tasks were measured with intracranial recordings in epilepsy patients at interictal period. The study showed that there was theta increase at different points in time during task. However, the seizure onset zone was not able to engage the network and increase theta band coherence. They proposed that a failure to hypersynchronize networks in theta frequency might explain the memory problems in epilepsy patients. This study highlights theta hypersynchronization as a necessary component of normal network function. Moreover, theta stimulation is suggested as an antiepileptic treatment (Fisher 2015). Although autism and epilepsy seem to be two distinct phenomenon, both abnormalities are underlined by changes in normal network activity. Seizures are an indicator of abnormal network activity and the most severe of this abnormality would manifest as epilepsy disorder. Therefore, a protective or therapeutic mechanism for epilepsy could have a similar function for autism. Studies show that children with ASD benefit from theta burst stimulation (Abujadi et al. 2018; Jannati et al. 2020). These findings also support that our finding of increase theta coherence in healthy individuals with higher autistic symptoms might be a compensatory mechanism as our sample population is fully functional and able to adapt to the needs of daily life/communication.

There may be two reasons for why only theta increase in the right hemisphere predicts autistic traits. The first explanation is that the AQ measures only core autistic traits, which are social skills, communication and attention related issues. The right hemispheres' dominance for those functions has been well-characterized (Caruana et al. 2015; Dapretto et al. 2006; De Haan et al. 2002; Luyster et al. 2014; Saxe et al. 2009). We hypothesize that coherence within left anterior regions may have emerged as predictive if there were more language-related questions in the AQ. A second possibility is that fasciculus lateralization drives the present results. As coherence must be heavily shaped by structural connectivity of the regions, we assume that the third sub-tract of superior longitudinal fasciculus (SLF III) (Martino and De Lucas 2014) and the arcuate fasciculus linking MTG (at the middle/posterior temporal sections) and dorsolateral prefrontal cortex (DLPFC) (Jung et al. 2017; Martino and De Lucas 2014) are involved in the coherence of R-Ant region. SLF III is right-lateralized, volumetrically larger and exhibits greater extension into the anterior inferior frontal gyrus in the right hemisphere (Hecht et al. 2015). Futhermore, mMTG is connected to hippocampus with higher connectivity in the right hemisphere (Xu et al. 2015). Besides, studies suggest that the mMTG is a hub for synchronization of fronto-parietal regions, and while activation of mMTG activates mainly anterior regions, it deactivates posterior regions (Jung et al. 2017; Xu et al. 2015). Therefore, signal differences would be more pronounced in the right hemisphere, especially within the frontal region. Additionally, in support of our study, during eyes open resting in a control group, theta band coherence was significantly higher in the right hemisphere, while there were no differences between hemispheres for the ASD group (Machado et al. 2015).

We find that the homozygote minor allele of rs9074 polymorphism in KCC2 gene is associated with the highest theta synchronization value, perhaps corroborating a link between ASD and GABA alterations (Delong 2007; Kana et al. 2007; Ma et al. 2005; Orekhova et al. 2008; Piton et al. 2013; van Kooten et al. 2005). Broncel et al. (2019) found very high theta hypersynchronization in rats after epileptic discharges if there is a $GABA_A$ blockage in the medial septum. Rs9074 is a polymorphism located at the 3′ untranslated region—a potentially regulatory region that may change expression of this channel, which would change the degree of inhibitory effect of $GABA_A$ signaling. We also find that rs9074 is not only related to theta coherence but also directly to autistic traits. This possible relation of *KCC2* to ASD has previous been shown by others (Duarte et al. 2013; Merner et al. 2015). This direct relation to autistic traits suggests that *KCC2* may affect other parameters in addition to theta.

There are two main assets of this study. First, ASD is a complex disorder with a continuum among population and we approached the phenotype as a continuum. This approach enables the investigation of the full spectrum of the phenotype of interest and prevents the inclusion of false negatives (including asymptomatic individuals with physiological changes accompanied by a compensatory mechanism) in a control group. In this study, by combining quantitative and continuous measures in an unaffected population, we were able to reveal the role of oscillation alterations and related polymorphisms in autistic traits. Lastly, we directly addressed replicability of our findings by validating our results in two independent populations with different backgrounds and using different collection methods.

We also acknowledge several limitations of the current study. First, although we assessed the continuum of traits in a healthy population, we did not include a patient group and therefore likely did not capture the extreme end of the continuum. In a follow up study, it would be beneficial to

assess autistic traits continuously in a sample of both individuals diagnosed with ASD and unaffected individuals to more fully understand the association between autistic traits and theta coherence within R-Ant region. Secondly, volume conduction effects cause interference from non-neuronal interactions. Yet, although connectivity measures of EEG are blurred with volume conduction effects, coherence measures give information about true neuronal interaction. There are several methods (source reconstruction or phase-lagged interaction measures) to improve the measurement of true connectivity, but unfortunately none of the methods fully mitigate the effects of volume conduction (Bastos and Schoffelen 2016). Among those methods, although coherence is one of the most vulnerable methods to volume conduction effects, it is the most established and used method (Bowyer 2016). One approach to minimize the contribution of volume conduction to the coherence is to consider only long distance electrodes (Srinivasan et al, 2007, see also Methods Sect. 2.3.3). In accordance with this, we only calculated connections between long distance electrodes. In addition, since the same volume conduction effect will be observed in all subjects and all regions, it is possible to examine the observed subject-measurement relationship in this context. In our study, only R-Ant region showed relation to autistic traits among 6 different examined regions and global connections. In this case, we can think that this is a relationship specific to neural connections in this region. Otherwise, if there was a relationship based solely on volume conduction, all regions should be able to predict autistic traits in the same way.

Finally, we acknowledge the small sample size in our assessment of polymorphisms. Further analysis with larger cohorts can be useful. Nevertheless, this does not rule out that we validated the tendency for the *KCC2* minor allele (AA) group to display higher theta coherence and greater autistic traits in the total group.

Conclusion

In conclusion, right anterior theta hypercoherence may serve as a quantitative measure for autistic traits, and *KCC2*—a key modulator of inhibitory GABA—contributes both to theta coherence within this region and autistic traits in healthy adults. Our results highlight the importance of using quantitative measures accompanied with a continuous-variable approach in the general population to unravel the underlying factors contributing to complex disorders like autism.

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Compliance with Ethical Standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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