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Common variation in the autism risk gene *CNTNAP2*, brain structural connectivity and multisensory speech integration

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ABSTRACT

Three lines of evidence motivated this study. 1) *CNTNAP2* variation is associated with autism risk and speech-language development. 2) *CNTNAP2* variations are associated with differences in white matter (WM) tracts comprising the speech-language circuitry. 3) Children with autism show impairment in multisensory speech perception. Here, we asked whether an autism risk-associated *CNTNAP2* single nucleotide polymorphism in neurotypical adults was associated with multisensory speech perception performance, and whether such a genotype-phenotype association was mediated through white matter tract integrity in speech-language circuitry. Risk genotype at rs7794745 was associated with decreased benefit from visual speech and lower fractional anisotropy (FA) in several WM tracts (right precentral gyrus, left anterior corona radiata, right retrolenticular internal capsule). These structural connectivity differences were found to mediate the effect of genotype on audiovisual speech perception, shedding light on possible pathogenic pathways in autism and biological sources of inter-individual variation in audiovisual speech processing in neurotypicals.

1. Background

Mutations or micro-deletions of the Contactin-associated protein-like 2 gene (*CNTNAP2*) result in a range of severe neurological disorders (Rodenas-Cuadrado, Ho, & Vernes, 2014) and substantial evidence for a connection between *CNTNAP2* and Autism Spectrum Disorder (ASD) has emerged since a recessive mutation in *CNTNAP2* was first identified in an Amish family with a syndromic ASD, some ten years ago now (Strauss et al., 2006). Genome wide association studies (GWAS) have also demonstrated

Abbreviations: A, auditory only; ASD, Autism Spectrum Disorder; AV, audiovisual; BET, Brain Extraction Tool; BIRN, Biomedical Informatics Research Network; CNTNAP2, contactin-associated protein-like 2 gene; dB_A, decibel (a-weighed); DICOM, Digital Imaging and Communications in Medicine; DTI, diffusion tensor imaging; EPI, echo planar imaging; ERP, event- related potential; FA, fractional anisotropy; FLAIR, Fluid Attenuated Inversion Recovery; fMRI, Functional Magnetic Resonance Imaging; FOV, field of view; GWAS, genome wide association study; IFG, inferior frontal gyrus; MHz, megahertz; min, minutes; mm, millimeters; MNI, Montreal Neurological Institute; MPRAGE, Magnetization Prepared Rapid Acquisition Gradient Echo; MRC, Medical Research Counsel; MRI, Magnetic Resonance Imaging; MRRC, Magnetic Resonance Research Center; NifTi, Neuroimaging Informatics Technology Initiative; NN, no noise; Ns., not significant; OLS, Ordinary Least Squares; rlIC, Retrolenticular internal Capsule; RM-ANOVA, Repeated Measures Analysis of variance; RF, radiofrequency; SD, standard deviation; Secs, seconds; SENSE, SENSitivity Encoding; sLF, superior longitudinal fasciculus; SNR, Signal to Noise Ratio; SPL, sound pressure level; SNP, single nucleotide polymorphism; TBSS, tract based spatial statistics; TE, echo time; TFCE, Threshold-Free Cluster Enhancement; TR, repetition time; V, visual only; WM, white matter; 3D, three dimensional.

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increased familial risk for the development of ASD and other intellectual disabilities associated with common single-nucleotide polymorphisms (SNPs) in *CNTNAP2* (Alarcon et al., 2008a, 2008b; Arking et al., 2008a, 2008b). Particularly prevalent in those carrying *CNTNAP2* mutations, apart from generalized intellectual disability, are varieties of language disorder (Rodenas-Cuadrado et al., 2016). Beyond these extreme but rare cases of *CNTNAP2* mutations and deletions, allelic variants commonly expressed in the general population have been associated with language function. Thus, there is a wide range of emergent evidence for association between *CNTNAP2* SNPs and language development, such as age of first word in ASD (Alarcon et al., 2008a, 2008b) and age of first phrase in healthy children (Anney et al., 2012a, 2012b). *CNTNAP2* SNPs and disruptions have also been variously associated with the ability to repeat nonsense words in individuals with dyslexia (Peter et al., 2011a, 2011b), with specific language impairment (Newbury et al., 2011; Vernes et al., 2008), with early language development in the unaffected population (Whitehouse, Bishop, Ang, Pennell, & Fisher, 2011), with speech delay in an ASD boy (Poot et al., 2010) and with stuttering (Petrin et al., 2010). These common allelic variants, when found to confer risk for a given disorder, are typically then referred to as "risk" alleles, but it is important to point out here that the association of particular *CNTNAP2* SNPs with ASD risk should be considered provisional at this stage, since their precise role in the disease remains to be clarified. Hereafter, therefore, when we refer to the association between *CNTNAP2* genotypes and risk, these should be considered presumed.

An event-related potential (ERP) study showed that carriers of the *CNTNAP2* risk SNP at rs7794745 showed aberrant brain responses (P600 effects) to syntactic violations (Kos et al., 2012), further tying this gene to language-related endophenotypes. In a similar vein, a hemodynamic imaging study demonstrated association between an ASD risk SNP on *CNTNAP2* and functional connectivity between frontal and temporal cortices in the human brain, implicating *CNTNAP2* in the organization of language-related neuro-circuitry. There is also evidence from structural neuroimaging, using diffusion tensor imaging (DTI), that *CNTNAP2* SNPs are associated with white matter structural integrity of the uncinate fasciculus (Clemm von Hohenberg et al., 2013), which connects temporal and frontal structures (Olson, Von Der Heide, Alm, & Vyas, 2015), as well as other major white matter pathways such as the dorsal cingulum bundle. Thus, a substantial weight of evidence now points to a role for *CNTNAP2* in the development of structural connectivity within speech-language associated circuits and with the development of speech-language abilities.

Of course, ASD has long been associated with delayed development of speech and language skills (Boucher, 2012; Eigsti, Bennetto, & Dadlani, 2007; Lazenby et al., 2016). Recently, our research group reported that children with ASD show a severe deficit in their abilities to integrate seen and heard speech inputs, such that under noisy environmental conditions, they do not benefit from multisensory audiovisual inputs in the way that their neurotypical peers do (Foxe et al., 2015). Given the association between *CNTNAP2* and ASD, the strong indication that *CNTNAP2* is involved in the development of language-specific neurocircuitry, and our evidence for a multisensory speech impairment in ASD, we set out here to determine if *CNTNAP2* allelic variation in the neurotypical population might be related to multisensory speech integration abilities. We further investigated whether structural variation within white matter tracts connecting major hubs of the perisylvian speech-language circuit (e.g., superior temporal cortex, inferior frontal regions and parts of the motor cortex) played a mediating role in this association. Such a finding would support the crucial role that allelic variation in *CNTNAP2* plays in speech-language development and provide insights into the underlying neural connectivity mediating said development.

2. Methods

2.1. Participants

Thirty male (M_{age} = 26.9; SD_{age} = 6.8) and 28 female (M_{age} = 25.5; SD_{age} = 3.9) healthy, neurotypical, adult native English speakers between 20 and 56 years of age participated in this study. The participants were recruited from the East Bronx, where the Albert Einstein College of Medicine is located, and the greater surrounding areas (mainly Westchester County and Manhattan). All had normal hearing and normal or corrected to normal vision according to self-report. To be included in the TD group, individuals could not have a history of neurological, neuropsychiatric, or neurodevelopmental disorders, or a first degree relative with a neurodevelopmental or neuropsychiatric disorder. All participants provided written informed consent in accordance with the tenets of the 1964 Declaration of Helsinki. All procedures were approved by the institutional review board of the Albert Einstein College of Medicine.

2.2. Genotyping

Fifty-eight subjects were genotyped for SNP rs7794745 and fifty-four were genotyped for the rs2710102 location that were both previously identified as having common variants associated with autism in a genome wide association study (Alarcon et al., 2008a, 2008b). Choice of these two *CNTNAP2* SNPs was specifically based on *a priori* hypotheses about the putative role of *CNTNAP2* in speech-language phenotypes and related neural circuitry/connectivity. This was not an exploratory study whereby all ASD implicated SNPs were assessed. Saliva samples were acquired from all participants (DNA Genotek) & DNA extracted per manufacturer protocols at the Einstein Neurogenomics core (http://bit.ly/ZoS7Hz). DNA quality & concentration were assessed using a Bioanalyzer 2100 (Agilent). The above identified variants were genotyped by real-time PCR using pre-existing Taqman assays on a HT-7900 (Applied Biosystems). Genotype quality was assessed by visual examination of allele clustering & by comparing the observed allele frequencies against data from the 1000 genome project.

At the rs7794745 SNP location, 25 participants were carriers of the presumed non-risk AA genotype, while 25 participants carried the heterozygous AT and 8 the homozygous TT presumed risk-associated genotypes. As mentioned in the introduction, it is important to note that association of *CNTNAP2* alleles with ASD risk, as it has emerged from past work, should be considered "presumed" since their precise role in the disease is not yet fully clarified. Both groups of presumed risk-allele carriers (AT and TT) were combined for all non-risk versus risk group comparisons. Non- risk (M_{age} = 25.8; SD_{age} = 3.9) and risk samples (M_{age} = 26.5; SD_{age} = 6.6) did not differ significantly in regard to their mean age $t(55) = 0.23$; $p = 0.8$ and their age frequency distribution $\chi^2(15, N = 58) = 15.13$, $p = 0.44$. Both groups also did not differ in regard to the number of males (Non-risk: n = 11; Risk: n = 19) and females (Non-risk: n = 14; Risk: n = 14), $\chi^2(1, N = 58) = 1.05$, $p = 0.31$. At the rs2710102 location, only 6 participants were identified as carriers of the homozygous non-risk genotype (TT) whereas 14 were homozygous (CC) and 34 were heterozygous (CT) risk carriers. The subject numbers for the respective subgroups were too small for statistical comparisons and we therefore did not proceed with analysis of the effects of SNP rs2710102.