

Electrophysiological network alterations in adults with copy number variants associated with high neurodevelopmental risk

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Abstract

Rare copy number variants associated with increased risk for neurodevelopmental and psychiatric disorders (referred to as ND-CNVs) are characterized by heterogeneous phenotypes thought to share a considerable degree of overlap. Altered neural integration has often been linked to psychopathology and is a candidate marker for potential convergent mechanisms through which ND-CNVs modify risk; however, the rarity of ND-CNVs means that few studies have assessed their neural correlates. Here, we used magnetoencephalography (MEG) to investigate resting-state oscillatory connectivity in a cohort of 42 adults with ND-CNVs, including deletions or duplications at 22q11.2, 15q11.2, 15q13.3, 16p11.2, 17q12, 1q21.1, 3q29, and 2p16.3, and 42 controls. We observed decreased connectivity between occipital, temporal and parietal areas in participants with ND-CNVs. This pattern was common across genotypes and not exclusively characteristic of 22q11.2 deletions, which were present in a third of our cohort. Furthermore, a data-driven graph theory framework enabled us to successfully distinguish participants with ND-CNVs from unaffected controls using differences in node centrality and network segregation. Together, our results point to alterations in electrophysiological connectivity as a putative common mechanism through which genetic factors confer increased risk for neurodevelopmental and psychiatric disorders.

1 Introduction

2 A number of rare genetic variants occurring through the deletion or duplication of
3 chromosomal segments are associated with significantly increased risk for a range of
4 neurodevelopmental disorders (ND), including schizophrenia, autism spectrum disorder
5 (ASD), and developmental delay¹. Although the underlying mechanisms remain poorly
6 understood, these copy number variants (referred to hereafter as ND-CNVs) are thought to
7 increase the risk for psychopathology through alterations in neural structure and function. Thus,
8 neuroimaging studies in participants with ND-CNVs provide a unique opportunity to study
9 intermediate phenotypes of mental disorders.

10 Furthermore, recent work suggests that CNV-specific phenotypic outcomes are limited,
11 pointing instead to a large degree of similarity across phenotypes associated with different ND-
12 CNVs^{2,3}. Focusing on convergent neural alterations across different genotypes can thus help
13 elucidate the mechanisms linking ND-CNVs at different loci to a shared psychopathology and
14 increase in neurodevelopmental risk.

15 Failures of functional neural integration have long been considered a hallmark of
16 neurodevelopmental disorders such as schizophrenia^{4,5}. In recent years, evidence of disrupted
17 connectivity has also emerged in ASD populations^{6,7} and has been shown to transcend
18 diagnostic boundaries⁸. ND-CNVs are thought to increase disorder risk by acting on large-scale
19 neural integration through molecular and cellular mechanisms⁹. Studying functional network
20 alterations in participants with ND-CNVs could thus help establish their reliability as
21 biomarkers of neurodevelopmental risk. Synchronous oscillatory activity thought to support
22 communication between brain areas is of particular interest as a window into excitatory and
23 inhibitory mechanisms, and can be measured at rest using electro- and magneto-
24 encephalography (EEG/MEG).

25 However, the rarity of ND-CNVs means that evidence of their functional connectivity
26 correlates is scarce. Of the genetic imaging studies conducted so far, most have focused on the
27 22q11.2 deletion syndrome (also known as DiGeorge or velo-cardio-facial syndrome). This
28 deletion is associated with a number of physical phenotype manifestations such as congenital
29 cardiac malformations, as well as high risk for psychopathology, and has long been recognised
30 as a discrete syndrome¹⁰⁻¹³. The presence of a 22q11.2 deletion has been linked to alterations
31 in brain structure and function¹⁴⁻¹⁷, including disrupted structural connectivity^{18,19}. Although
32 fewer studies have investigated functional connectivity, they report similarly disrupted
33 networks using functional MRI²⁰⁻²² and EEG²³.

34 Despite emerging evidence of white matter alterations associated with other ND-CNVs²⁴⁻²⁶,
35 very few studies have investigated their functional correlates. Recent electrophysiological
36 research reported increased beta-band activity in participants with 15q11.2-q13.1
37 duplications^{27,28} and 16p11.2 deletions²⁹, as well as delayed evoked responses in the latter^{24,30}.
38 Based on current evidence it is difficult to assess the extent and specificity of functional
39 connectivity alterations, especially for rarer ND-CNVs.

40 To address this, we investigated oscillatory connectivity measured with MEG in participants
41 with ND-CNVs at nine different loci. Given the common phenotypic outcomes associated with
42 ND-CNVs², this approach can identify convergent endophenotypes of potentially higher
43 clinical relevance. Because a third of participants had 22q11.2 deletions (a sample size twice
44 as large as any other genotype in our cohort), we also assessed alterations in connectivity

45 separately in this subgroup and in the group of participants with other ND-CNVs. This allowed
46 us to directly assess the specificity of the effects, especially considering previous findings of
47 widespread neural alterations associated with 22q11.2 deletions.

48 In both subgroups, we found evidence of disrupted alpha and beta-band oscillatory connectivity
49 in posterior brain regions. Furthermore, using graph theory measures of network topology and
50 information transfer, we were able to identify participants with ND-CNVs based on their
51 individual connectivity maps. The two approaches highlighted common patterns of
52 dysconnectivity in participants with ND-CNVs, as well as specific network features that might
53 be linked to CNV pathogenicity.

54 **Methods**

55 **Participants**

56 MEG data were acquired in 42 adults with ND-CNVs targeted for their high penetrance for
57 neurodevelopmental disorders (22 female; mean age 38.5 ± 12.5 years). ND-CNVs at nine
58 different loci were represented in the cohort, with 14 (33%) participants carrying 22q11.2
59 deletions. Demographic and clinical information for this cohort are provided in the
60 Supplementary Information (Supplementary Table 1).

61 Recruitment was performed through NHS genetics clinics and relevant support groups within
62 the UK. Written consent was obtained in accordance with The Code of Ethics of the World
63 Medical Association (Declaration of Helsinki), and all procedures were approved by the South
64 East Wales Research Ethics Committee.

65 Controls were selected among resting-state datasets acquired at CUBRIC as part of the “100
66 Brains” and “UK MEG Partnership” projects. These cohorts included healthy participants
67 aged 18-65 with no history of neurological or neuropsychiatric disorders, and 42 controls were
68 chosen to match the gender and age of the ND-CNV carriers as closely as possible (22 female;
69 mean age 33.3 ± 9.6 years). These measurements were acquired under protocols approved by
70 the Cardiff University School of Psychology Ethics Committee.

71 Since a third of the ND-CNV cohort consisted of participants with 22q11.2 deletions, we
72 assessed the impact of this subgroup by repeating all analyses on (1) participants with other
73 ND-CNVs (except 22q11.2 deletions) and their matched controls ($N = 56$), and (2) participants
74 with 22q11.2 deletions and their matched controls ($N = 28$).

75 **Genotyping**

76 Participants with ND-CNVs were genotyped using the Illumina HumanCoreExome whole
77 genome SNP array, which contained an additional 27,000 genetic variants at loci previously
78 linked to neurodevelopmental disorders, including CNVs. The raw intensity data was
79 processed using Illumina Genome Studio software (version 2011.1). PennCNV (version 1.0.3)
80 was used to perform CNV calling in order to confirm the presence of the ND-CNV in each
81 case sample, with each CNV being required to span a minimum of 10 informative SNPs and to
82 be at least 10 kb in length. CNV coordinates were specified according to genome version hg19,
83 and the boundaries of each CNV were confirmed by manually inspecting the Log R ratio and
84 B allele frequency plots at each of the genomic regions of interest (Supplementary Table 1).
85 Genetic information was not available for control participants; given the rarity of these
86 genotypes in the general population, they were assumed to carry no ND-CNVs.

87 **Data collection**

88 Five-minute resting-state MEG recordings were made using a 275-channel CTF radial
89 gradiometer system (CTF, Vancouver, Canada) at a sampling rate of 1200 Hz. Three of the
90 sensors were turned off due to excessive noise, and 29 reference channels were recorded to
91 improve noise cancellation³¹. During the recordings, participants were seated upright and
92 fixated their eyes on a red fixation point presented centrally on either a CRT monitor or LCD
93 projector. Three electromagnetic coils were placed at fiducial locations (nasion and pre-
94 auricular) for head localization.

95 To aid in source localization, structural T1-weighted MRI scans were also acquired using a 3T
96 General Electric or Siemens MRI scanner with a 1 mm isotropic FSPGR/MPRAGE pulse
97 sequence.

98 **Data analysis**

99 **Pre-processing**

100 To remove muscle artifacts, a semi-automatic procedure was implemented using the FieldTrip
101 toolbox³² and MATLAB R2015a. Sensor time-series were bandpass-filtered between 110 and
102 140 Hz and z-transformed; segments exceeding a participant-specific z-score threshold were
103 removed. Next, eye movement and cardiac artifacts were projected out of the data using
104 Independent Component Analysis (ICA). Noisy channels exhibiting high variance were also
105 removed from the data where necessary. There was no significant difference in recording
106 duration after artifact rejection between the ND-CNV and control groups ($t(81.8) = 1.61$, $P =$
107 0.11 , mean duration 255.88 ± 29.31 s and 245.33 ± 30.86 s respectively).

108 Head motion was monitored continuously in 18/42 ND-CNV datasets and 40/42 control
109 datasets, and head localization was performed at the start and end of the recording in the
110 remaining datasets. There was no significant difference between the ND-CNV and control
111 groups in maximum head coil displacement between the beginning and end of the recording
112 ($t(79.8) = 0.85$, $P = 0.39$, mean displacement 2.07 ± 3.62 mm and 2.7 ± 3.06 mm respectively).
113 In datasets with continuous head localization, the maximum distance of the head coils from
114 their average position across the entire recording did not significantly differ between groups
115 ($t(39.4) = 1.44$, $P = 0.16$, mean distance 4.74 ± 3.5 mm and 3.21 ± 4.2 mm respectively).

116 Prior to source localization, coregistration was performed by manually marking head coil
117 locations on each participant's MRI using FieldTrip. The data were downsampled to 600 Hz
118 and bandpass-filtered in six different frequency bands: delta (2-4 Hz), theta (4-8 Hz), alpha (8-
119 13 Hz), beta (13-30 Hz), low gamma (40-60 Hz), and high gamma (60-90 Hz).

120 **Estimating functional connectivity**

121 To assess group differences in resting-state connectivity (Figure 1), we focused on amplitude-
122 amplitude coupling of source-localized oscillatory signals³³. Continuous data in each of the six
123 frequency bands were projected into source space using a Linearly Constrained Minimum
124 Variance (LCMV) beamformer. Sources were reconstructed on a 6 mm template grid warped
125 to each participant's MRI, using a multiple local-spheres forward model³⁴. To alleviate the
126 depth bias, beamformer weights were normalized by their vector norm³⁵.

127 Next, 90 nodes corresponding to cortical regions of interest (ROI) in the Automated
128 Anatomical Labelling (AAL) atlas³⁶ were identified by performing a frequency analysis on all
129 sources within each ROI and selecting the source with the largest temporal standard deviation.

130 Continuous virtual sensor timecourses corresponding to the 90 nodes were then reconstructed
131 and bandpass-filtered into the frequency bands of interest.

132 To avoid spurious correlations, the node time-series were orthogonalized using a multivariate
133 symmetric orthogonalization approach³⁷. A Hilbert transform was used to calculate oscillatory
134 amplitude envelopes, which were then despiked using a median filter, downsampled to 1 Hz,
135 and trimmed to avoid filter and edge effects. To obtain connectivity matrices, pairwise
136 correlations were calculated between the 90 Hilbert envelopes. Next, a Fisher transform was
137 applied to obtain z-scores with zero mean and unit variance across connections in each
138 participant's map. This procedure corrected for possible systematic differences across
139 participants, for example due to differences in data quality³⁸.

140 Intracranial volume (ICV), quantified as the number of 1-mm isotropic voxels inside the brain,
141 was smaller in the ND-CNV group than the control group ($t(65.5) = -2.19$, $P = 0.03$), in line
142 with some previous reports³⁹⁻⁴¹. The potential impact of this difference on the MEG results
143 was alleviated through the source localization procedure and the z-scoring of the connectivity
144 matrices.

145 In addition to the six frequency bands listed above, a combined measure of connectivity was
146 obtained by calculating the vector-sum of connectivity matrices across all frequency bands⁴².

147 **Group differences in resting-state connectivity**

148 To reduce the impact of noise, a conservative ranking procedure⁴² was used to threshold the
149 connectivity maps for the purposes of between-group comparisons. This consisted of
150 calculating the rank of each connection in participant-level connectivity matrices and averaging
151 the resulting rank map across participants in each group. Only the top 20% edges in the average
152 rank map were considered "valid" and selected for further analysis. To ensure that large
153 differences in signal across cohorts were not discarded by this procedure, the rank-thresholding
154 procedure was performed separately in each cohort, and connections determined as "valid" in
155 either cohort were included in further analysis.

156 To assess differences between groups, Welch's t-tests were conducted at each valid edge.
157 Significant edges were identified using an uncorrected $\alpha = 0.05$. Correction for multiple
158 comparisons was applied using a randomization procedure with 10000 sign-shuffling iterations
159 and maximal statistic thresholding (omnibus $\alpha = 0.05$)⁴³.

160 Furthermore, the robustness of cohort differences was evaluated through a resampling
161 procedure. Increases and decreases in connectivity between groups were tabulated using
162 random samples of half of each group. This was repeated across 10000 iterations, and edges
163 showing a consistent effect direction across at least 95% of iterations were considered robust.

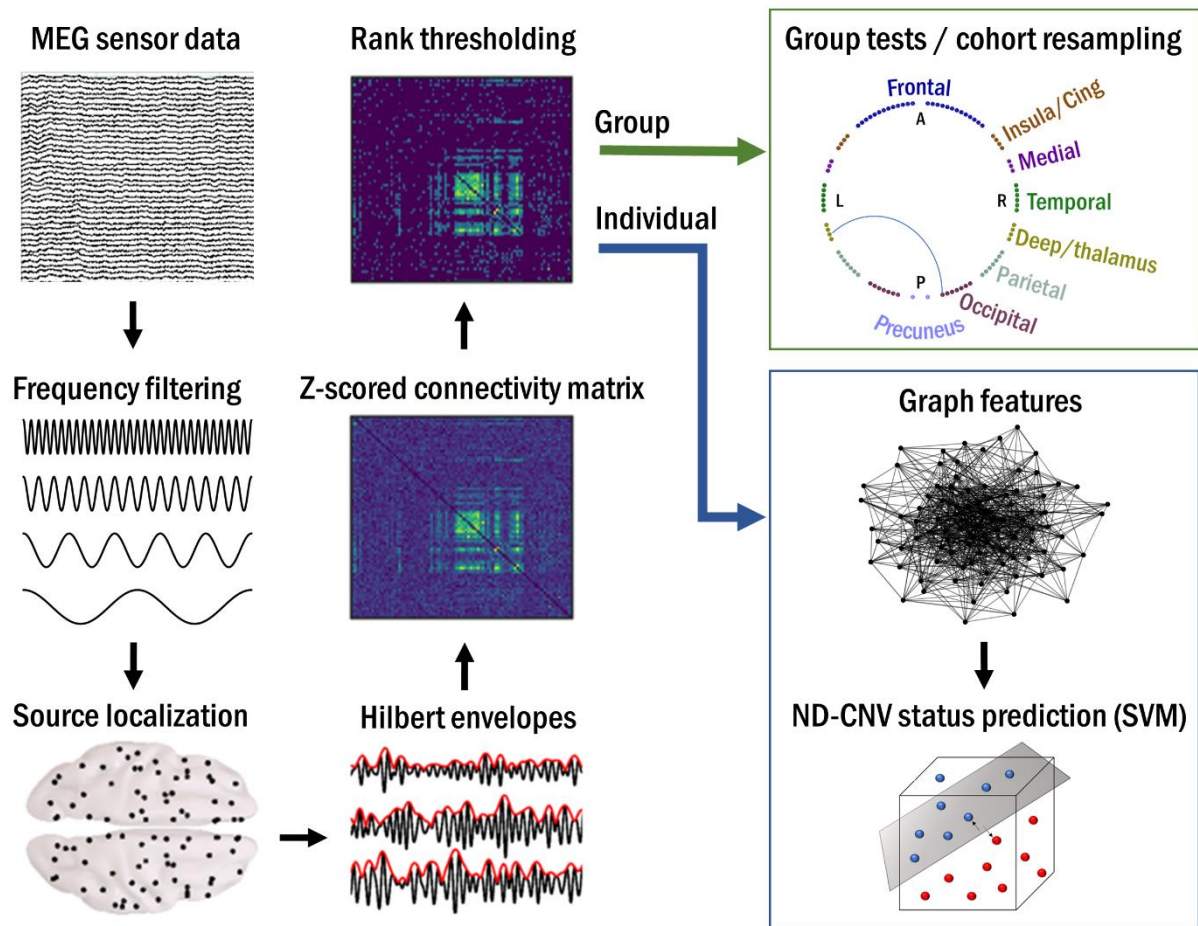


Figure 1. Overview of the analysis pipeline. Resting-state MEG data were preprocessed, filtered into six frequency bands, and projected into source space. Hilbert envelopes were calculated at 90 AAL-atlas-based virtual sensor locations, and correlated to obtain functional connectivity matrices. These were z-scored and rank-thresholded at the group level for between-group analyses, and at the subject level for data-driven prediction of ND-CNV status using graph theory. See Supplementary Figure 1 for the full list of node labels corresponding to the circular plots in this paper.

164 To control for potential confounds (for example, resulting from imperfect age matching
 165 between the ND-CNV and control groups), an additional multiple regression analysis was
 166 performed. Combined-frequency connectivity matrices were entered as response variables with
 167 a categorical predictor (ND-CNV presence) and three covariates (age, gender, and ICV). A
 168 resampling procedure as described above was performed to assess the robustness of between-
 169 group differences. The sign of the regression slope associated with the main predictor was
 170 tabulated across 1000 split-half cohort randomizations. Edges showing consistent effects
 171 across 95% of iterations were considered robust.

172 **Individual networks: identifying participants with ND-CNVs using graph theory**

173 Next, a data-driven graph theory approach was used to assess whether participants with ND-
 174 CNVs could be distinguished from unaffected controls using functional connectivity features.
 175 To this aim, the cohort was divided into training and test sets using an iterated cross-validation
 176 procedure.

177 This analysis focused on individual networks by selecting the top 20% of connections in each
 178 participant's normalized connectivity map as the basis for undirected graphs. Networks were

179 then characterized using six nodal graph theory metrics chosen to capture node connectedness,
180 network integration, and network modularity (Supplementary Table 2). All graph theory
181 analyses were performed using MATLAB R2019a and the Brain Connectivity Toolbox⁴⁴.

182 To discriminate between groups, a linear support vector machine (SVM) classifier⁴⁵ was
183 trained on each of the node metrics. Additionally, a pooled feature vector was created by
184 combining the six metrics to maximize the amount of complementary information input to the
185 classifier. This approach makes use of information across all nodes while avoiding the need for
186 multiple testing.

187 Classification was performed between the ND-CNV and control groups, as well as between the
188 two ND-CNV subgroups (22q11.2 deletions and other ND-CNVs) and their matched controls.
189 To avoid overfitting, model performance was evaluated using 100 iterations of stratified five-
190 fold cross-validation. This entailed iteratively leaving out a fifth of the data for testing and
191 training the model on the remaining data, whilst ensuring balanced group representation in each
192 fold. Performance was quantified using accuracy (proportion correctly classified observations),
193 sensitivity (true positive rate) and specificity (true negative rate) in order to highlight any
194 asymmetries in ND-CNV and control identification. Furthermore, significance was assessed
195 by shuffling the true labels 5000 times and recomputing classifier accuracy to estimate the
196 empirical chance level and calculate a one-tailed p-value⁴³.

197 **Results**

198 **Connectivity alterations associated with ND-CNVs**

199 The analysis of group differences in oscillatory connectivity revealed the largest number of
200 valid connections in the alpha and beta bands (Figure 2). Most of the significantly different
201 connections showed a decrease in oscillatory connectivity between posterior, parietal and
202 temporal nodes in the ND-CNV group, with the exception of a few right-hemisphere edges.
203 More extensive cohort effects were detected using the combined frequency maps (61 edges
204 exceeded the uncorrected threshold, compared to 1, 28 and 42 in the theta, alpha and beta
205 bands). These patterns were robust to random sub-sampling of the cohort, suggesting that they
206 were not driven by individual subjects. A small number of left-hemisphere connections,
207 including the precuneus, early visual cortex, and parietal regions, survived omnibus correction
208 for multiple comparisons.

209 Importantly, a similar pattern of hypoconnectivity was observed even after excluding
210 participants with 22q11.2 deletions and their matched controls (Figure 2B-C). Both ND-CNV
211 subgroups showed decreased posterior connectivity (Figure 3; Supplementary Figure 1),
212 indicating that the overall pattern was not driven by the 22q11.2 deletion group. On the other
213 hand, participants with 22q11.2 deletions exhibited more right-hemisphere hyperconnectivity
214 compared to controls. These effects spanned the precuneus and parietal cortex, as well as
215 frontal regions, suggesting some overlap with the default mode network (DMN).

216 To ensure that these differences were not affected by potential confounds, the cohort
217 resampling tests on combined frequency maps were repeated as a multiple linear regression
218 with age, gender and ICV as covariates. This analysis revealed fewer connections (65 compared
219 to the original 92 in the whole cohort analysis), but largely similar patterns of dysconnectivity
220 (Figure 3).

221 Furthermore, although IQ scores could not be included in this analysis because they were not
 222 available for the control group, IQ scores in the ND-CNV group correlated with connectivity
 223 strength at only four edges (Supplementary Figure 2).

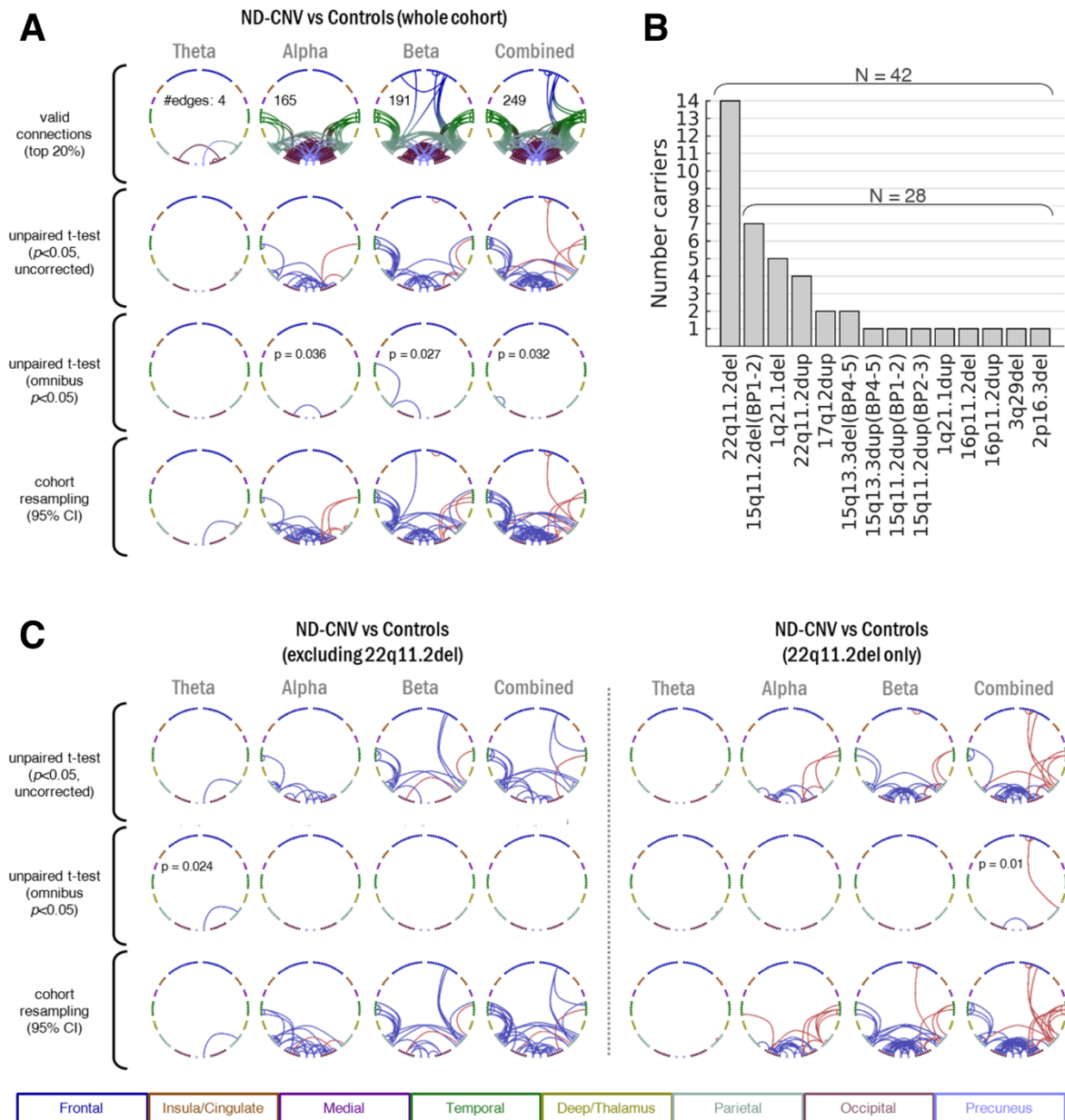


Figure 2. A. Differences in resting-state connectivity (amplitude correlations) between participants with ND-CNVs and controls. Connectivity increases and decreases in the ND-CNV group are shown in red and blue respectively. The rows show (top to bottom): valid connections after mean-rank thresholding in each frequency band; uncorrected ($P < 0.05$) differences between groups; multiple comparison-corrected (omnibus $P < 0.05$) differences between groups; and connections showing consistent increases/decreases in 95% of cohort resampling iterations. **B.** Frequency of deletions (del) and duplications (dup) at each locus in the ND-CNV group. **C.** As in A, for subgroups excluding participants with 22q11.2 deletions and their matched controls (left) or including only participants with 22q11.2 deletions and their matched controls (right). To facilitate comparison, “valid” connections were the same as in A. Only frequency bands with surviving “valid” connections are shown.

224 Network features as predictors of ND-CNV status

225 A graph theory framework was employed to identify participants with ND-CNVs from their
226 functional networks based on combined frequency maps. This approach has the advantage of
227 reducing dimensionality and complements the edge-focused group testing approach described
228 above. Despite methodological differences between the two analyses, a visualization of the
229 nodal graph theory features shows overlap with the nodes highlighted in the group analysis
230 (Supplementary Figures 3-6).

231 Graph theory metrics were successful predictors of ND-CNV participants relative to unaffected
232 controls. The best prediction accuracy was achieved by combining the 6 node features
233 (Supplementary Table 3; Figure 4A; maximum accuracy $71\% \pm 3.44$, $P=0.0002$). However,
234 participants with 22q11.2 deletions were more consistently correctly classified than those with
235 other ND-CNVs (Figure 4B).

236 This was confirmed by subgroup classification analyses, which also pointed to subgroup
237 differences. When excluding participants with 22q11.2 deletions, the best decoding accuracies
238 were achieved using node eccentricities ($65.62\% \pm 3.91$, $P=0.0016$), node degrees, and the joint
239 feature model. On the other hand, all node features were successful in discriminating
240 participants with 22q11.2 deletions from their matched controls, with the best performance
241 obtained using the clustering coefficient ($87.61\% \pm 4.95$, $P=0.0002$).

242 These results point to commonalities in network features (such as decreased centrality) that
243 allow for the successful classification of participants with ND-CNVs across distinct genotypes.
244 On the other hand, the features are specific enough to allow successful discrimination between
245 participants with 22q11.2 deletions and other ND-CNVs (Supplementary Table 3). Given the
246 higher overall burden of 22q11.2 deletions in neurodevelopmental disorders¹, this suggests that
247 increased neurodevelopmental risk may be associated with more salient alterations in network
248 function and may underpin specific genotype effects.

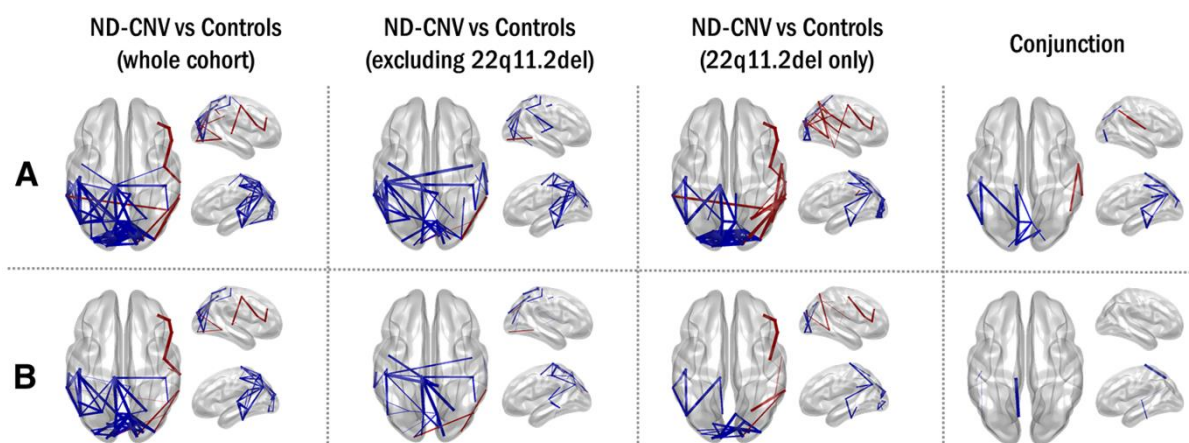


Figure 3. Differences in connectivity are not driven by age, gender, and intracranial volume. Connections meeting the 95% confidence criterion in the cohort resampling test are displayed for all group tests (first three columns). The last column shows supra-threshold connections in both the 22q11.2 deletion group and the other ND-CNV group; they are shown in blue where they are decreased in both groups, and in red where they have opposite signs. Line width increases with effect robustness. **A.** Connections exhibiting robust differences based on the cohort resampling test of combined frequency matrices, plotted on the template brain. **B.** As in A, after including age, gender and intracranial volume as covariates in a multiple linear regression with "ND-CNV presence" as a main categorical predictor.

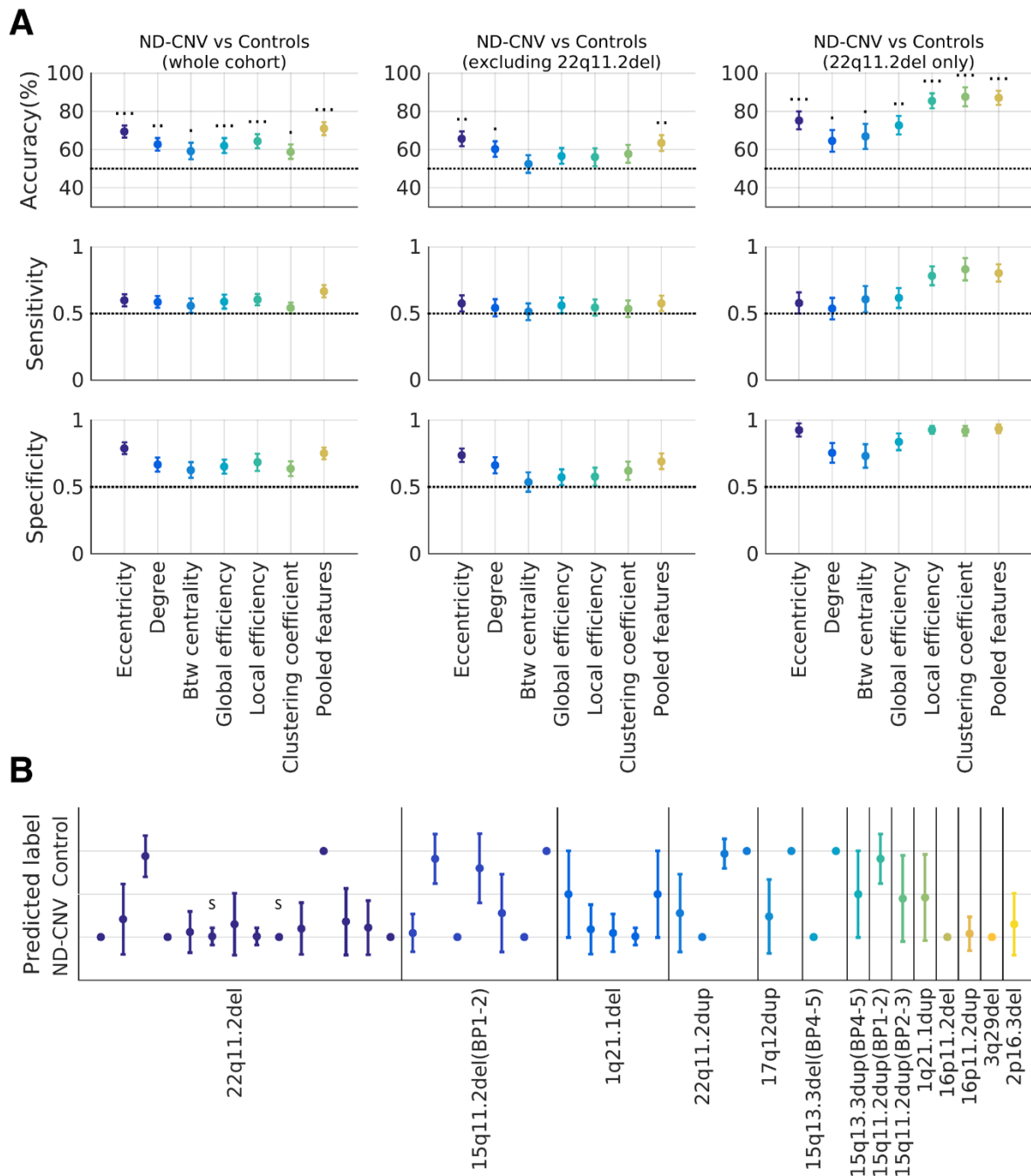


Figure 4. Classifying participants with ND-CNVs and unaffected controls from individual MEG functional networks using graph theory. **A:** Classification performance on the three groups, using different metrics to characterize the networks (eccentricity, degree, betweenness centrality, global and local efficiency, clustering coefficient, and the pooled model). Above-chance accuracies (permutation testing) are marked with 1, 2, and 3 dots respectively for $p < 0.05$, $p < 0.01$, and $p < 0.001$. **B:** How well are different ND-CNVs classified? The plot shows the mean predicted label for each of the 42 participants with ND-CNVs across 100 cross-validation iterations using pooled node features. Participants with 22q11.2 deletions are most consistently correctly identified. Two participants with schizoaffective disorder diagnoses are marked with "S".

249 Discussion

250 To our knowledge, the present study provides the first insight into oscillatory connectivity
251 alterations in people with rare ND-CNVs. Using both an established group analysis pipeline
252 and a data-driven graph theory framework, we uncovered a pattern of functional
253 dysconnectivity affecting posterior regions in participants with ND-CNVs. These patterns were
254 robust to effects of age, gender, and intracranial volume, and emerged despite a conservative
255 thresholding approach restricted to the most reproducible connections.

256 Effects originated in low frequency bands, particularly alpha and beta, which rely on
257 excitatory-inhibitory balance and are thought to underpin long-range communication between
258 brain areas⁴⁶. Connections linking parietal, temporal, and occipital areas were most consistently
259 affected in both the 22q11.2 deletion group and the other ND-CNV group. Similar patterns
260 have been previously reported in schizophrenia patients⁴⁷, including alpha-band parietal
261 hypoconnectivity in first-episode schizophrenia⁴⁸. Furthermore, posterior structural network
262 alterations have been identified as an early marker of ASD⁴⁹, suggesting a link between such
263 alterations and increased neurodevelopmental risk.

264 Similar connectivity changes in the visual processing system and the default mode network
265 have been shown in people with 22q11.2 deletions using structural and functional MRI⁵⁰⁻⁵².
266 Here, we found that these effects are not restricted to the 22q11.2 deletion group, suggesting
267 that long-range connectivity could act as a common marker across genetic variants. Although
268 non-invasive measurements cannot provide direct mechanistic insight, this is consistent with
269 alterations in excitatory-inhibitory balance as a mechanism for pleiotropic genetic effects
270 underlying neurodevelopmental disorders⁵³⁻⁵⁵. This is thought to occur through increased
271 excitation or disinhibition caused by gene haploinsufficiency and mediated by impaired GABA
272 and NMDA receptor function^{56,57}.

273 Despite sample size limitations, differences between the two subgroups also point to effects
274 specific to the highly penetrant 22q11.2 deletions. Hypoconnectivity was more extensive in
275 people with other ND-CNVs, while the 22q11.2 deletion group exhibited more focused
276 patterns; these were robust to cohort resampling, suggesting that they are unlikely to be driven
277 by individual cases. These differences were reflected in the graph theory analysis. Although all
278 network features were altered in the 22q11.2 deletion group, their increased modularity was
279 particularly discriminative, in line with previous reports of increased structural network
280 segregation in people with 22q11.2 deletions^{18,22,58}. For other ND-CNVs, the only predictive
281 features were centrality measures (specifically the node eccentricity and degree), reflecting
282 hypoconnectivity in the ND-CNV cohort. Between and within-group classification results
283 (Supplementary Table 3) highlight the ability of graph theory metrics to capture both
284 convergent and specific network alterations, which could help elucidate the link between CNV
285 pathogenicity and neural system function.

286 Although the present study was able to evaluate ND-CNV effects independently of the
287 contribution of highly penetrant 22q11.2 deletions, the limited sample size remains a concern
288 common in CNV imaging research. The high genotype variability within the cohort makes the
289 specificity of these effects difficult to assess, particularly with regard to differences between
290 the 22q11.2 deletion group and other ND-CNVs. To overcome this, the generalizability of the
291 “fingerprint” obtained by combining graph theory metrics in a machine learning framework
292 could be verified in studies recruiting larger samples of participants with ND-CNVs, for
293 example through large multi-site collaborations.

294 In summary, the present study assessed oscillatory long-range connectivity as a potential
295 marker of pathogenic genetic effects across a range of rare ND-CNVs. Occipital, parietal and
296 temporal brain areas were characterized by consistent hypoconnectivity across genotypes,
297 which was not exclusively driven by the presence of a large number of participants with highly-
298 penetrant 22q11.2 deletions. Functional networks in the ND-CNV group exhibited decreased
299 node centrality and alterations in network efficiency and structure. Furthermore, features
300 specific to highly penetrant variants were present alongside convergent network alterations and
301 enabled successful ND-CNV classification. These results are consistent with a common
302 mechanism for genetic risk, based on an altered balance between excitatory and inhibitory
303 synaptic processes and leading to network dysfunction. We propose that these functional
304 connectivity alterations are an intermediate phenotype on the pathway from synaptic molecular
305 changes to disruption of cognitive function and psychotic illness.

Conflict of interest

The authors declare no competing interests.

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