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Published in:
Schizophrenia Bulletin

Link to article, DOI:
[10.1093/schbul/sbx058](https://doi.org/10.1093/schbul/sbx058)

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Larsen, K. M., Pellegrino, G., Birknow, M. R., Kjær, T. N., Baaré, W. F. C., Didriksen, M., Olsen, L., Werge, T., Mørup, M., & Siebner, H. R. (2017). 22q11.2 Deletion Syndrome Is Associated With Impaired Auditory Steady-State Gamma Response. *Schizophrenia Bulletin*, 44(2), 388-397. Article sbx058.
<https://doi.org/10.1093/schbul/sbx058>

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22q11.2 Deletion Syndrome Is Associated With Impaired Auditory Steady-State Gamma Response

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Background: The 22q11.2 deletion syndrome confers a markedly increased risk for schizophrenia. 22q11.2 deletion carriers without manifest psychotic disorder offer the possibility to identify functional abnormalities that precede clinical onset. Since schizophrenia is associated with a reduced cortical gamma response to auditory stimulation at 40 Hz, we hypothesized that the 40 Hz auditory steady-state response (ASSR) may be attenuated in nonpsychotic individuals with a 22q11.2 deletion. **Methods:** Eighteen young nonpsychotic 22q11.2 deletion carriers and a control group of 27 noncarriers with comparable age range (12–25 years) and sex ratio underwent 128-channel EEG. We recorded the cortical ASSR to a 40 Hz train of clicks, given either at a regular inter-stimulus interval of 25 ms or at irregular intervals jittered between 11 and 37 ms. **Results:** Healthy noncarriers expressed a stable ASSR to regular but not in the irregular 40 Hz click stimulation. Both gamma power and inter-trial phase coherence of the ASSR were markedly reduced in the 22q11.2 deletion group. The ability to phase lock cortical gamma activity to regular auditory 40 Hz stimulation correlated with the individual expression of negative symptoms in deletion carriers ($\rho = -0.487$, $P = .041$). **Conclusions:** Nonpsychotic 22q11.2 deletion carriers lack efficient phase locking of evoked gamma activity to regular 40 Hz auditory stimulation. This abnormality indicates a dysfunction of fast intracortical oscillatory processing in the gamma-band. Since ASSR was attenuated in nonpsychotic deletion carriers, ASSR deficiency may constitute a premorbid risk marker of schizophrenia.

Key words: 22q11.2 deletion syndrome/gamma band/EEG/oscillations/schizophrenia

Introduction

The 22q11.2 deletion is the most common copy number variant in humans with an estimated prevalence of 1:2000 to 1:4000.^{1–3} 22q11.2 deletions are associated with a highly variable clinical phenotype, including a range of somatic disorders, learning problems, cognitive deficits,^{4,5} and hearing loss.⁶ Individuals carrying a 22q11.2 deletion have an increased risk of developing schizophrenia and other psychiatric disorders such as autism spectrum disorder and attention deficit hyperactivity disorder.^{7–9} According to the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome (22q11.2DS), the prevalence of schizophrenia-spectrum disorder is 24% in adolescent and 41% in adult 22q11.2 carriers.⁸ A recent nationwide Danish Registry study showed that the risk of developing a schizophrenia-spectrum disorder in individuals previously diagnosed with 22q11.2DS was approximately 8 times higher than in the general population.¹⁰ Since the 22q11.2DS confers a substantial risk for schizophrenia, the identification of neurophysiologic abnormalities in nonpsychotic 22q11.2 deletion carriers may reveal important insights into the pathogenesis of schizophrenia and assist the search of early markers. Investigations of high-risk groups are very appealing from a clinical perspective as they not only can provide a

better knowledge of the disease evolution, but potentially can lead to strategies for prevention or early treatment.

Auditory steady-state responses (ASSRs) evoked by brief tones or clicks at a repetition rate of 40 Hz provide a readily available, noninvasive means of probing impaired neural gamma synchrony in the auditory system.¹¹ A seminal ASSR study showed that the ASSR was selectively reduced in patients with schizophrenia when auditory stimulation was applied at 40 Hz, but not at 20 Hz or 30 Hz.¹² Later studies reported reduced expression and phase locking of steady-state gamma oscillations in patients with schizophrenia.^{13–16} A recent meta-analysis concludes that decreased 40 Hz ASSR is a robust finding in schizophrenia.¹⁷ The ASSR is further observed reduced in first-episode psychosis,^{18,19} as well as in nonaffected first degree relatives.^{20,21} So far, there have been no studies looking at ASSR in 22q11.2DS. However, one study has shown reduced ASSR in an ultra-high-risk group (UHR),²² where subjects who met criteria for attenuated psychotic symptoms, brief intermittent psychotic symptoms and genetic risk deterioration were included in the study based on the structured interview for prodromal symptoms (SIPS).

Linking ASSR and clinical symptoms in schizophrenia, inter-trial phase coherence (ITPC) has shown to be positive correlated with the presence of positive symptoms.¹⁹ The same group also reported a positive correlation between left-hemispheric ITPC and the expression of auditory hallucination.¹⁵ Moreover, a negative correlation between the ASSR amplitude at 80 Hz and negative symptoms has been shown.²³ In the UHR-study,²² the power of the ASSR at 40 Hz was negatively correlated with the individual PANSS scores for positive symptoms, but not for negative symptoms.

In schizophrenia, impaired neural processing in cortical circuits has been attributed to dysfunctional oscillatory cortical activity in the gamma-band (30–100 Hz) and underlying abnormalities in GABAergic and glutamatergic neurotransmission.^{12,24–26} Cortical gamma oscillations rely on the integrity of fast-spiking GABAergic interneurons which exert a finely timed inhibition onto the pyramidal cells and other inhibitory interneurons.^{27–29} Disturbed interactions of these circuits are thought to critically contribute to pathogenesis and cognitive impairment in schizophrenia.^{30,31} In agreement with the notion of a GABAergic dysfunction, magnetic resonance spectroscopy revealed that gamma-aminobutyric acid (GABA) levels in visual cortex are reduced by 13% in patients with schizophrenia as compared to healthy controls.³² Likewise, prefrontal GABA levels were reduced in patients with schizophrenia as compared to controls using magnetic resonance spectroscopy at 7 T.³³ Accordingly, postmortem studies of 22q11.2 carriers have shown reduced levels of GABA³⁴ at sites of cortical malfunction. Recent studies have provided converging evidence that cortical gamma oscillatory activity critically depend on the function of N-methyl-D-aspartate (NMDA) receptors located on parvalbumin-positive, fast-spiking,

GABAergic interneurons.^{35–40} The emerging links between abnormal cortical gamma oscillatory activity and specific cellular and subcellular mechanisms constitute a potential target for future treatments of schizophrenia.^{24,28,41}

In murine models of 22q11.2DS, a disruption of glutamatergic synaptic transmission within the auditory cortex has been found.⁴² Further, in a mouse model of 22q11.2DS increased acoustic startle response as well as prepulse inhibition (PPI) deficits was found.⁴³ Since no difference in auditory brain stem responses were found, the deficits in PPI could not account for this. One study investigated auditory gating in 22q11.2DS.⁴⁴ While P50 was found to be normal, 22q11.2DS carriers showed increased amplitudes of the first N1 component at central electrodes, suggesting abnormal higher order processing.

Using a neurogenetically informed approach, we recorded the ASSR in nonpsychotic carriers of a 22q11.2 deletion and healthy controls without such deletion with comparable age range and sex ratio. The auditory stimulation was applied regularly and irregularly, testing whether temporal regularity of the 40 Hz train was critical to evoke ASSR. In this way, we could test for differences in steady state as well as transient (nonsteady state) responses. We expected that the 22q11.2 deletion carriers express a reduced cortical gamma response to regular auditory stimulation at 40 Hz as a potential risk marker of schizophrenia, showing a reduction in power and reduced phase synchronization of the ASSR relative to healthy noncarriers. Further, we expected the response to irregular stimulation to be matched between groups. We further explored whether the ASSR alterations in 22q11.2 deletion carriers were correlated with symptom severity.

Materials and Methods

Subjects

Eighteen young individuals 12–25 years with a verified 3 MB deletion at chromosome 22q11.2 and 27 healthy individuals without a 22q11.2 deletion participated in the study. Groups were comparable with respect to sex ratio (controls: 18 males and 9 females, carriers: 13 males and 5 females, $\chi^2 = 0.54$, $P = .46$) and age distribution (controls: mean age = 15.96 years, standard deviation (SD) = 2.71 years; carriers: mean age = 15.39 years, SD = 2.45 years, $t_{43} = 0.72$, $P = .47$). All participants were evaluated for the presence of current psychiatric disorders and diagnoses were given according to the ICD-10 diagnostic system if clinical criteria were met. For more information on diagnose criteria, see supplementary material. Carriers of the 22q11.2 deletion with a known history of schizophrenia were excluded. The Structured Interview for Prodromal Syndromes (SIPS)^{45,46} was used to rate the severity of: positive, negative, disorganized, and general symptoms, see supplementary materials for detailed information.

The following exclusion criteria were applied to controls: (a) schizophrenia, schizotypal and delusional disorders (ICD10 DF20-29); (b) bipolar disorder (ICD10 DF30-31); (c) depression (ICD10 DF32-33) except for a past episode of mild or moderate depression (ICD10 DF 32.0 or 32.1); (d) substance abuse; or (e) a first degree relative with a psychotic illness. All participants underwent a verbal and written informed consent process. Participants under the age of 18 provided a verbal assent while their parent's completed written consent. The study was approved by the Regional Ethical Committee of Copenhagen (project id: H-3-2012-136) and the Danish Data protection Agency (project id: 2007-58-0015). All participants are part of a larger nationwide study and underwent extended cognitive, genetic, and clinical testing, described in detail by Schmock et al.⁴⁷ Since 22q11.2DS is associated with hearing loss, audiometric testing was performed prior to the experiment to confirm that participants were able to hear the click stimuli (20 dB random test Oscilla USB-310 Tablet screening audiometer), see supplementary materials for more details.

ASSR Paradigm

To evoke steady-state gamma activity, subjects were presented with a train of short clicks delivered at a mean click-repetition frequency of 40 Hz.¹² Each click lasted

1 ms and each click train lasted 1 s, followed by a pause of 2 s, resulting in a stimulus onset asynchrony of 3 s (figure 1). The stimuli were delivered binaurally via insert-earphones at a sound pressure level of 85 dB (E-A-RTONE 3A), using the MatLab-based Cogent 2000 toolbox as presentation software (http://www.vislab.ucl.ac.uk/cogent_2000.php). We used an external soundcard (RME Babyface 22-Channel, 192 kHz Bus-powered, Haimhausen, Germany). During ASSR recording, subjects sat in a comfortable chair, were instructed to relax and to constantly look at a fixation cross on the screen in front of them without paying particular attention to the sounds.

We applied regular and irregular 40 Hz trains. In the regular condition, clicks had a constant inter-click interval of 25 ms (ie, regular 40 Hz train). In the irregular condition, the inter-click interval was randomly jittered between 11 and 37 ms while maintaining a mean click frequency of 40 Hz. We introduced this condition to record the cortical response evoked by click stimulation that was matched for all acoustic features except regularity. Thus, the irregular condition served as a control condition testing whether the temporal regularity of the 40 Hz train was critical to evoke abnormal auditory cortical processing restricted to ASSR. The temporal structure of the irregular click train was kept constant within subjects, but changed randomly across subjects. For regular and

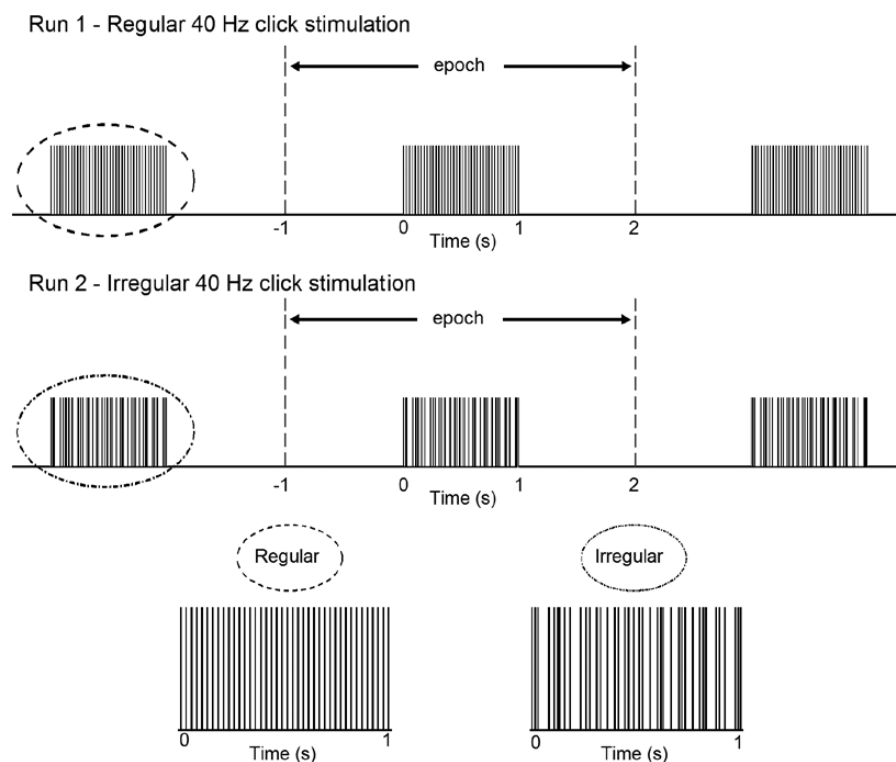


Fig. 1. Temporal structure of the auditory stimulation paradigm to evoke 40 Hz auditory steady-state responses. Regular (run 1) and irregular (run 2) 40 Hz click trains were given in separate experimental blocks. Each click train had duration of 1 s, followed by a pause of 2 s. Clicks were applied every 25 ms at a constant frequency of 40 Hz in the regular condition and randomly jittered from 11 to 37 ms in the irregular condition. An epoch covered the period of 40 Hz stimulation and a prestimulus window of -1000 to 2000 ms.

irregular 40 Hz stimulation, ASSR were recorded in 2 separate runs, consisting of 120 trials (figure 1). A single run lasted 6 min.

EEG Recordings and Preprocessing

EEG data was recorded using a 128 channel ActiveTwo Biosemi System (BioSemi, Amsterdam, Netherlands) at a sampling frequency of 4096 Hz. Preprocessing and analysis were carried out using the fieldtrip toolbox.⁴⁸ The preprocessing steps consisted of referencing to the average of the 2 mastoids, band-pass filtering using the interval [1–130] Hz, notch filtering using the interval [48–52] Hz to attenuate 50 Hz line noise, down-sampling to 1024 Hz, and finally epoching with a peri-stimulus window of –1000 to 2000 ms. Epochs were baseline corrected using the average over the time window –1000 to –300 ms. Artifact removal was performed using a simple threshold approach where epochs were rejected if their values exceeded $\pm 100 \mu\text{V}$. No channels were discarded.

Time-Frequency Analysis

The epoched data were wavelet-transformed using a Complex Morlet wavelet with 12 cycles, as implemented in the Fieldtrip toolbox.⁴⁸ The frequency band of interest covered frequencies from 10 to 60 Hz at 1 Hz resolution. The inter-trial phase coherence (ITPC) and power were extracted from the wavelet coefficients. The amplitude of the ITPC reflects the phase consistency across trials for a given channel, time and frequency point and can take values between 0 (no phase consistency) and 1 (perfect phase consistency). The ITPC and power amplitude as recorded from Cz were averaged in the 300–700 ms time window of auditory stimulation over the frequencies 36–44 Hz.

Statistical Analysis

Two sample *t*-test and Wilcoxon rank sum test was used to test for case–control differences in IQ and the 4 domain scores obtained from the SIPS interview, respectively. Group differences in ASSR were assessed using a hypothesis-driven approach testing for differences in raw ITPC amplitude as well as power values between groups from one single EEG channel (Cz according to the international 10–20 system, see figure 2 where channel Cz is marked). We chose Cz because we had a priori knowledge that the sensors of the mid-central regions express the highest gamma response to regular click stimulation at 40 Hz.⁴⁹ Two separate repeated-measures analysis of covariance (rm-ANCOVA) were therefore computed with ITPC or power as dependent variables. The rm-ANCOVA included the factors group (2 levels: deletion carriers and noncarriers) and the within-subject factor condition (2 levels: regular and irregular), as well as age and sex as covariates. We post hoc added hearing thresholds observed as a mean over frequencies from the 20 dB

random test as covariate. Information regarding post hoc test for diagnosis and IQ can be found in the supplementary material.

Greenhouse-Geisser correction for nonsphericity was applied if necessary. Follow-up *t*-tests were performed for significant interactions. If equality of variance were not met (assessed with Levene's test), separate variance *t*-tests were performed when necessary. Bonferroni correction for multiple comparisons was applied if necessary and significance levels were corrected accordingly.

Two subjects belonging to the 22q11.2 group completed only the regular condition because of exhaustion after this session. One healthy noncarrier had to be discarded from the regular condition due to problems with the trigger. The results are thus based on the ASSR of 18 deletion carriers for the regular condition, 16 deletion carriers for the irregular condition, 26 healthy noncarriers for the regular condition and 27 healthy noncarriers for the irregular condition. For the rm-ANCOVA the results are based on 16 deletion carriers and 26 nondeletion carriers.

Nonparametric Spearman's rank correlations analysis was performed to test for correlations between individual ASSR measures and negative symptoms score. Only correlations with negative symptoms were carried out since the presentation of positive, disorganized and generalized symptoms were skewed in the present group (5 out of 18 (28%) of the 22q11.2 deletion carriers had a score of 0 in the positive symptoms, 5 out of 18 (28%) in the disorganized and 11 out of 18 (61%) in generalized symptoms). For all analyses, significance threshold was set at $P < .05$.

Results

The 22q11.2DS group had an IQ (median = 82.0, 90th percentile = 94.8, 10th percentile = 63.6) that was significantly lower than the IQ in the control group (median = 108.0, 90th percentile = 127, 10th percentile = 95.2, $t_{43} = -7.05$, $P < .001$). The included raw sum of negative symptoms for the 22q11.2 carriers ranged from 1 to 16 (mean = 7 and SD = 3.5), from 0 to 12 for positive symptoms (mean = 2.7, SD = 3.1), from 0 to 6 for disorganized (mean = 1.8, SD = 1.8) and from 0 to 7 for generalized symptoms (mean = 1.0, SD = 1.9).

Within the 22q11.2 deletion carriers, one was diagnosed with affective disorder, 2 with disturbance of activity/attention deficit disorder without hyperactivity, 6 with anxiety or phobia and one with both autism spectrum disorder and anxiety or phobia. None of the participants had psychosis, but the 22q11.2DS cohort had significantly elevated raw SIPS scores for negative ($W = 477$, $P < .001$), positive ($W = 350.5$, $P = .008$), disorganized ($W = 404$, $P < .001$) and generalized ($W = 312.5$, $P = .027$) symptoms, relative to the control group. Fifteen of the 22q11.2 deletion carriers had negative symptoms within the attenuated level (3–5) in one or more of the negative

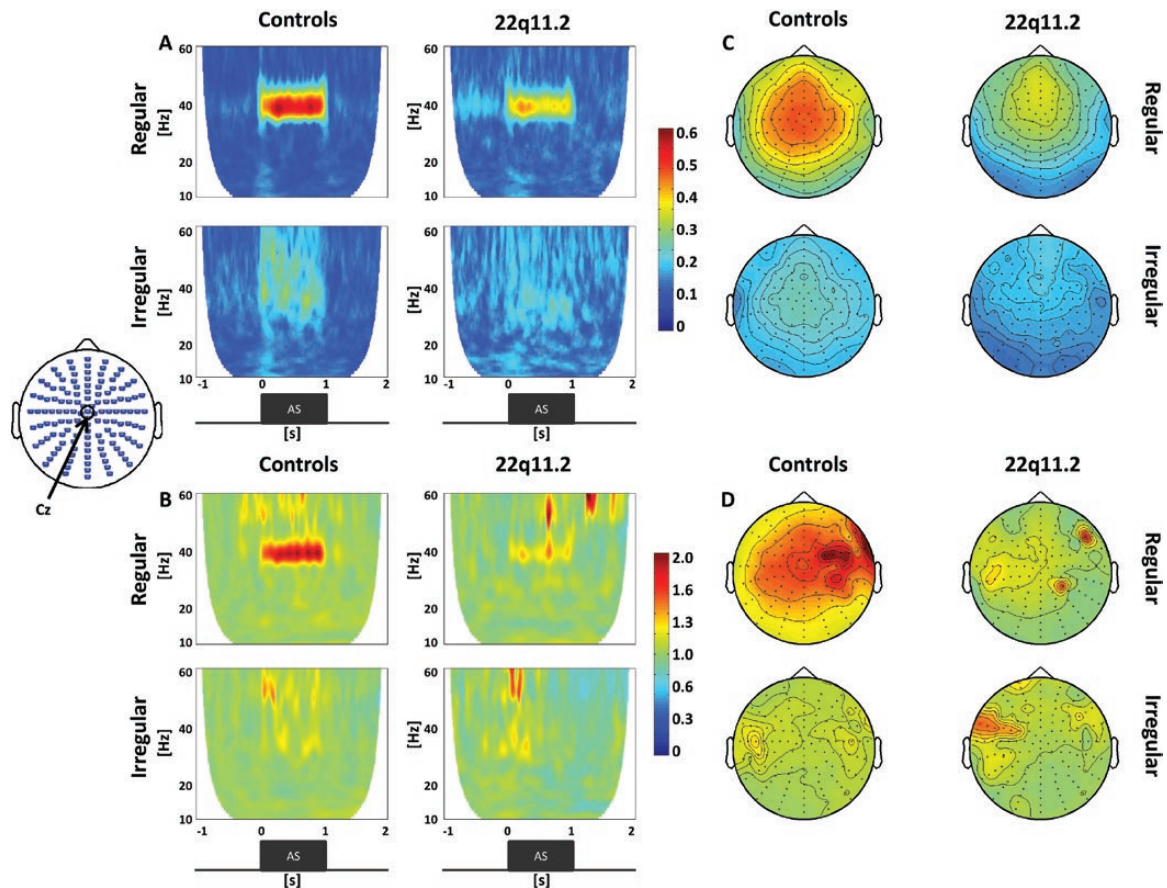


Fig. 2. Group time frequency plots of the ASSR within the 10–60 Hz range from electrode Cz. (A) Group ITPC of the ASSR for the regular (top) and irregular (bottom), for both noncarriers (left) and 22q11.2 carriers (right). (B) Group power of the ASSR, again shown for both conditions and groups. In both (A) and (B), the time stamp 0 indicates the onset of the click train. Duration of the click train is illustrated by the grey box. In the time-frequency response a cut-off can be seen (ie, white area without data in the color plots) due to the length of the wavelet being longer for the lower frequencies as compared to higher frequencies. Consequently, not all time point values can be estimated. (C) Topographical maps for ITPC presented for controls, 22q11.2 and both conditions as in A in the time window 300–700 ms and frequency window 36–44 Hz. (D) Topographical maps for power presented for controls, 22q11.2 and both conditions as in B in the time window 300–700 ms and frequency window 36–44 Hz.

symptoms categories, but only 3 individuals had one or more psychotic and/or disorganized symptoms within the attenuated level. Generalized symptoms at the attenuated level were only seen in 2 of the 22q11.2 deletion carriers. Apart from one subject taking 20 mg of retalin, none of the carriers took medication acting on the central nervous system.

The 20 dB random test, revealed that subjects were able to detect the tones used for eliciting ASSR. Across frequencies the observed threshold levels were (mean = 20.5, SD = 0.7) for controls and (mean = 24.6, SD = 3.6) for 22q11.2. In 2 22q11.2 deletion carriers, the dB level of the click trains was decreased from 85 dB to 80 dB because they reported that the stimulus was uncomfortable at 85 dB.

Mean ITPC and power frequency plots of the ASSR for healthy 22q11.2 deletion carriers and noncarriers are shown in figures 2A and 2B, respectively. Healthy controls without a 22q11.2 deletion showed a clearly discernible

ASSR at around 40 Hz which was temporally confined to the time of regular click stimulation (figure 2). This response critically relied on the regularity of the 40 Hz acoustic train, because jittering the frequency around 40 Hz, as expected reduced the ASSR (figures 2 and 3). Nonpsychotic carriers showed a clear attenuation of the ASSR to regular 40 Hz click stimulation (figures 2 and 3) when comparing to the healthy noncarriers. The response to the irregular 40 Hz click train stimulation was similar in both groups.

Inter-trial Phase Coherence

Repeated-measures-ANCOVA revealed that mean ITPC across the 2 conditions was significantly lower for the 22q11.2 group compared to the noncarrier group ($F_{1,38} = 7.1$, $P = .011$, figure 3A). Moreover, we observed a significant effect of condition ($F_{1,38} = 6.3$, $P = .016$), showing that the regular condition was overall much more effective

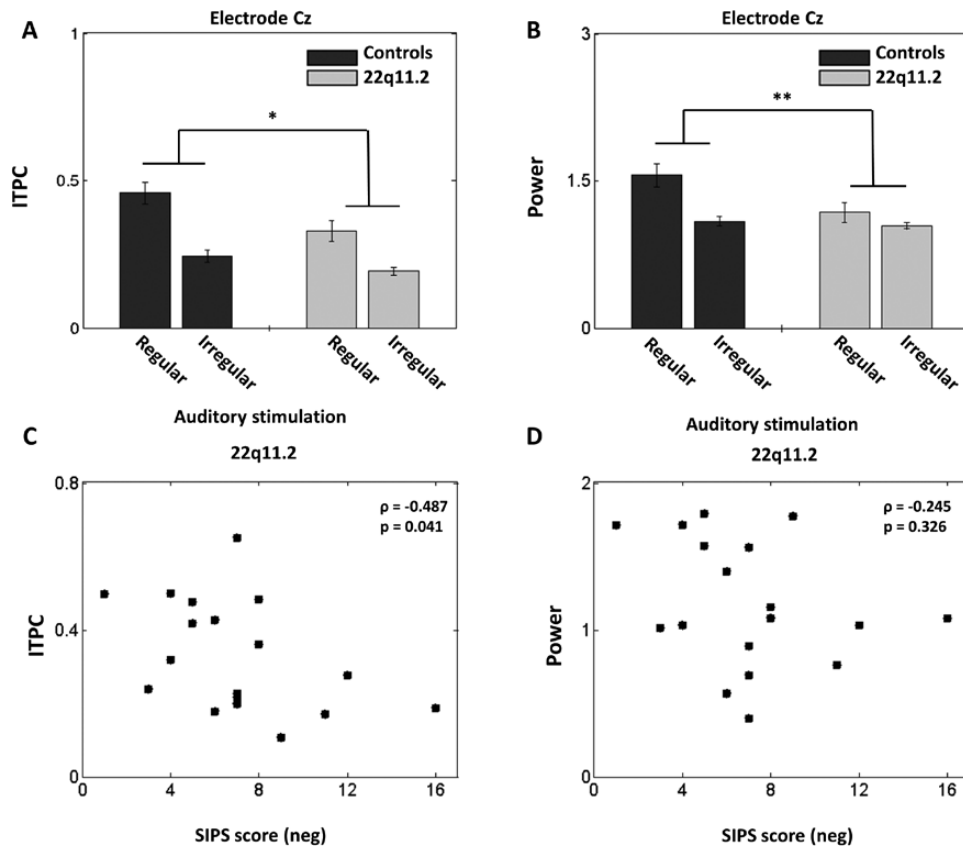


Fig. 3. Group data of the ASSR. Mean ITPC (A) and mean gamma power (B) of the ASSR evoked by regular or irregular 40 Hz click trains in 22q11.2 carriers (light gray) and healthy noncarriers (dark gray). Asterisks indicate significant between-group difference (* $P < .05$, ** $P < .01$). Error bars equal SEM (standard error of the mean). (C) and (D) shows ITPC and power as a function of SIPS negative subscale scores, respectively. Since the nonparametric spearman rank correlation was used, there is no regression line.

than the irregular. There was no significant group-by-condition interaction ($F_{1,38} = 2.4$, $P = .127$). Overall the mean value of ITPC in the regular condition was reduced by 28% in the deletion carriers compared to the noncarriers and by 21% in the irregular condition (figure 3A). ITPC decreased with age ($F_{1,38} = 6.4$, $P = .016$) and age showed a significant interaction with condition ($F_{1,38} = 4.7$, $P = .037$) attributable by younger participants having a higher ITPC score in the regular condition compared to older participants while younger and older participants showed similar values for the irregular condition. No effect of sex ($F_{1,38} = 2.0$, $P = .169$) or interaction (condition-by-sex, $F_{1,38} = 1.6$, $P = .209$) was observed. Adding hearing threshold as covariates the group effect did not change significance.

Power

Repeated-measures-ANCOVA also showed a significant group difference in power ($F_{1,38} = 7.6$, $P = .009$) which reflected a relative reduction of the ASSR power in deletion carriers (figure 3B). There was also a main effect for condition ($F_{1,38} = 4.6$, $P = .038$) and a significant interaction between group and condition ($F_{1,38} = 6.1$, $P = .018$) which was due to a stronger decrease in power for the regular condition in deletion carriers relative to noncarriers (figure 3B).

Post hoc pair-wise comparisons confirmed that 22q11.2 deletion carriers had a lower power for the regular condition as compared to healthy noncarriers ($t_{40} = 2.4$, $P = .022$, $\alpha = 0.025$), while power did not differ from healthy noncarriers in the irregular condition ($t_{40} = 2.9$, $P = .770$). Overall the mean value of power in the regular condition was reduced by 24% in the deletion carriers compared to the noncarriers, but only by 4% in the irregular condition.

We also found a significant effect for age with lower ASSR power at higher age ($F_{1,38} = 5.7$, $P = .022$). The age effect interacted with condition ($F_{1,38} = 5.3$, $P = .027$). Inspection of the individual data revealed that younger participants expressed a higher power in the regular condition compared to older participants, while power was not influenced by age in the irregular condition. There was no effect of sex ($F_{1,38} = 0.8$, $P = .368$) or interaction between sex and condition observed ($F_{1,38} = 1.2$, $P = .283$).

For post hoc tests on diagnosis and IQ, see supplementary material.

Correlation With Negative Symptoms

In carriers with a 22q11.2 deletion, individual ITPC for the regular condition showed a negative correlation with the individual SIPS scores of negative symptoms

(Spearman: $\rho = -0.487$, $P = .041$). The correlation remained significant after including age as covariate ($\rho = -0.493$, $P = .045$). For comparison, we also calculated the correlation between ITPC for the irregular condition and negative symptom scores and found no correlation ($\rho = -0.146$, $P = .591$). No correlation was found between ASSR power and negative symptom scores (Spearman: $\rho = -0.245$, $P = .326$).

Discussion

This study, to our knowledge, provides first-time evidence that the cortical steady-state response to auditory 40 Hz stimulation is impaired in a genetically defined group with a markedly increased risk for schizophrenia. In nonpsychotic 22q11.2 deletion carriers, the gamma power of the ASSR was significantly reduced by 24% during regular 40 Hz click stimulation relative to healthy controls without 22q11.2 deletion. We further detected a reduced phase precision of the ASSR in nonpsychotic 22q11.2 deletion carriers who showed on average a relative reduction of 28% in trial-to-trial phase synchronization as compared to the control group. The ability to phase lock the auditory evoked gamma activity to the 40 Hz click train stimulation was negatively correlated with the negative symptom scores of the 22q11.2 carriers. The results confirm and extend previous ASSR studies in nonpsychotic first-degree relatives of patients with schizophrenia.^{20,21} Together, these findings show that a deficient ASSR may be a useful premorbid risk marker for schizophrenia. In carriers with a 22q11.2 deletion, reduced trial-to-trial phase synchronization correlated with the presence of elevated negative symptoms. Since negative symptoms represent an important facet of the symptomatology in schizophrenia these findings corroborate the notion that abnormal synchronization of gamma-band activity may play an important role in the generation of the negative symptoms in schizophrenia.

GABA_A receptor-mediated inhibition plays a substantial role in the underlying mechanisms of gamma oscillations.⁵⁰ GABA-mediated inhibition results in decreased gamma oscillations and cognitive impairment, for a review see.^{30,51} Recent studies in rodents point to a critical functional role of NMDA receptors located on fast-spiking parvalbumin positive, GABAergic interneurons^{39,40,52,53} in the generation of cortical gamma oscillations, suggesting that the ASSR may be used as biomarker for cortical NMDA function.³⁷

The ASSR is reduced in schizophrenia^{14–16} as well as in first-episode psychosis.^{18,19} This might be caused by a reduced concentration of GABA or dysfunctional GABA release^{24,54} or a dysfunction of the NMDA receptors controlling the fast-spiking GABAergic interneurons.⁵⁵ In addition, electrophysiological findings have indicated that evoked gamma power increases during childhood and adolescence.^{56–58} It is thus believed that neural synchrony

continues to develop until early adulthood,⁵⁸ which might be linked to a gradual developmental switch in expression of the gamma-1 relative to the gamma-2 subunit of the GABA receptors, causing a more rapid and precise inhibition of pyramidal target cells.⁵⁹ It therefore remains to be clarified, whether the abnormal ASSR in 22q11.2 deletion carriers is caused by a GABA-related or NMDA receptor related dysfunction or both.

Although we speculate that the present finding of a reduced ASSR in 22q11.2 deletion carriers could be due to a reduced level of GABA as observed in schizophrenia, 22q11.2DS results in a wide range of neurobiological abnormalities. Other neurobiological sequelae, such as hearing loss,⁶ PRODH deletion effects on glutamatergic transmission,⁶⁰ and ZDHHC8 deletion effects on development of neural function,^{60,61} might also affect the ASSR in 22q11.2 deletion carriers. At variance with 2 previous reports,^{56,57} we found that the power and ITPC of ASSR decreased with age. However, the age range in the present study (12–25 years, with the majority being between 12 and 18 years) differs markedly from the age ranges in previous reports. In one study, 3 groups were tested at an age of 10, 11.5, and 19–45 years.⁵⁶ In a MEG study on the age effect on ASSR, age distribution was large, ranging from 5 to 52 years.⁵⁷ Since our statistical analysis revealed group differences in ITPC and power in a model that controlled for the effect of age, the observed abnormality in ASSR in deletion carriers cannot be attributed to the age of our participants. Since it can be expected that a proportion of 22q11.2 carriers will develop schizophrenia at a later time in life,⁸ repeated measurements of ASSR in the same subjects during adolescence and early adulthood might provide more fine-grained insights into age-related developmental trajectories of ASSR abnormalities and their relation to the clinical manifestation of schizophrenia.

The reduction of ITPC in 22q11.2 deletion carriers was observed across the 2 conditions; regular and irregular. However, the reduction of power was observed only in the regular condition. This is an interesting finding, showing that even transient gamma responses during irregular broad-band gamma stimulation are less efficiently aligned in deletion carriers. ITPC correlated negatively with the negative symptoms of the 22q11.2 deletion carriers. However, given the exploratory nature of the correlational analyses and the relatively small sample size this finding should be considered preliminary and needs to be replicated in larger studies before any strong conclusions can be drawn. The small sample size of nonpsychotic 22q11.2 deletion carriers is a limitation of the study. The abnormalities in ASSR found in 18 deletion carriers warrant replication in larger cohorts.

In conclusion, this study presents first time evidence that young nonpsychotic 22q11.2 carriers lack the auditory steady state gamma response. This highlights the emerging importance of gamma-band oscillations in

understanding the neurophysiological characteristics of schizophrenia. Further, the observed results in a non-psychotic high risk group indicate that dysfunctional gamma responses from an auditory steady state gamma paradigm could potentially serve as an early risk marker for schizophrenia if confirmed by longitudinal studies. Reliable functional risk markers that can support early diagnostics may assist clinical decision-making for targeted therapy. This is relevant because recent results found a positive effect of early treatment on clinical and function status.^{62,63}

Funding

This study was funded by the Lundbeck Foundation, Denmark (R155-2014-1724); Lundbeck Foundation [Grant of Excellence “ContAct” R59 A5399]; Lundbeck Foundation fellowship (R105-9813); The Capital Region’s Research Foundation for Mental Health Research.

Acknowledgments

The authors would like to thank all the participant 22q11DS and families for taking the time to participate in our study and the Danish National Association for their strong support of our work. The authors highly appreciate the efforts of Anders Vangkilde and Henriette Schmock for their dedicated assistance in the recruitment and clinical assessments. The authors would also like to thank Lars Ewald for technical support. We would also like to express our gratitude to the staff involved in the Danish Blood Donor Study, Capital Region Blood bank, Glostrup as well as <http://www.forsogsperson.dk/>. H.R.S. received honoraria as speaker from Lundbeck A/S, Valby, Denmark, Biogen Idec, Denmark A/S, Genzyme, Denmark and MerckSerono, Denmark, honoraria as editor from Elsevier Publishers, Amsterdam, The Netherlands and Springer Publishing, Stuttgart, Germany, travel support from MagVenture, Denmark, and grant support from Biogen Idec, Denmark A/S. M.R.B. is a prior employee at H. Lundbeck A/S, Denmark and received financial support for her PhD from the Innovation Fund Denmark. M.D. is employed with Lundbeck A/S. The authors declare no further biomedical financial interests or potential conflicts of interest. This article is distributed under the Danish legislation governed by the Privacy Act (act# 429, 31/05/2000), which does not permit data sharing at publicly available repositories or in raw formats. Summary statistics can be obtained through contact with the corresponding author (melissal@drcmr.dk).

References

- Oskarsdóttir S, Vujic M, Fasth A. Incidence and prevalence of the 22q11 deletion syndrome: a population-based study in Western Sweden. *Arch Dis Child*. 2004;89:148–151.
- Goodship J, Cross I, LiLing J, Wren C. A population study of chromosome 22q11 deletions in infancy. *Arch Dis Child*. 1998;79:348–351.
- Shprintzen RJ. Deletion 22q11.2 (velo-cardio-facial syndrome DiGeorge syndrome). In: Cassidy S, Allanson JE, eds. *Management of Genetic Syndromes*. Vol 3. New York/Hoboken, NJ: John Wiley; 2010:263–284.
- Robin NH, Shprintzen RJ. Defining the clinical spectrum of deletion 22q11.2. *J Pediatr*. 2005;147:90–96.
- Karayorgou M, Simon TJ, Gogos JA. 22q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. *Nat Rev Neurosci*. 2010;11:402–416.
- Jiramongkolchai P, Kumar MS, Chinnadurai S, Wootten CT, Goudy SL. Prevalence of hearing loss in children with 22q11.2 deletion syndrome. *Int J Pediatr Otorhinolaryngol*. 2016;87:130–133.
- Bassett AS, Marshall CR, Lionel AC, Chow EW, Scherer SW. Copy number variations and risk for schizophrenia in 22q11.2 deletion syndrome. *Hum Mol Genet*. 2008;17:4045–4053.
- Schneider M, Debbané M, Bassett AS, et al.; International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. Psychiatric disorders from childhood to adulthood in 22q11.2 deletion syndrome: results from the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry*. 2014;171:627–639.
- Purcell SM, Wray NR, Stone JL, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009;460:748–752.
- Vangkilde A, Olsen L, Hoeffding LK, et al. Schizophrenia spectrum disorders in a Danish 22q11.2 deletion syndrome cohort compared to the total danish population—a nationwide register study. *Schizophr Bull*. 2016;42:824–831.
- Plourde G, Stapells DR, Picton TW. The human auditory steady-state evoked potentials. *Acta Otolaryngol Suppl*. 1991;491:153–160.
- Kwon JS, O’Donnell BF, Wallenstein GV, et al. Gamma frequency-range abnormalities to auditory stimulation in schizophrenia. *Arch Gen Psychiatry*. 1999;56:1001–1005.
- Light GA, Hsu JL, Hsieh MH, et al. Gamma band oscillations reveal neural network cortical coherence dysfunction in schizophrenia patients. *Biol Psychiatry*. 2006;60:1231–1240.
- Krishnan GP, Hetrick WP, Brenner CA, Shekhar A, Steffen AN, O’Donnell BF. Steady state and induced auditory gamma deficits in schizophrenia. *Neuroimage*. 2009;47:1711–1719.
- Spencer KM, Niznikiewicz MA, Nestor PG, Shenton ME, McCarley RW. Left auditory cortex gamma synchronization and auditory hallucination symptoms in schizophrenia. *BMC Neurosci*. 2009;10:85.
- Brenner CA, Sporns O, Lysaker PH, O’Donnell BF. EEG synchronization to modulated auditory tones in schizophrenia, schizoaffective disorder, and schizotypal personality disorder. *Am J Psychiatry*. 2003;160:2238–2240.
- Thuné H, Recasens M, Uhlhaas PJ. The 40-Hz auditory steady-state response in patients with schizophrenia: a meta-analysis. *JAMA Psychiatry*. 2016;73:1145–1153.
- Symond MP, Symond MB, Harris AW, Gordon E, Williams LM. “Gamma synchrony” in first-episode schizophrenia: a disorder of temporal connectivity? *Am J Psychiatry*. 2005;162:459–465.
- Spencer KM, Salisbury DF, Shenton ME, McCarley RW. Gamma-band auditory steady-state responses are impaired in first episode psychosis. *Biol Psychiatry*. 2008;64:369–375.
- Hong LE, Summerfelt A, McMahon R, et al. Evoked gamma band synchronization and the liability for schizophrenia. *Schizophr Res*. 2004;70:293–302.

21. Rass O, Forsyth JK, Krishnan GP, et al. Auditory steady state response in the schizophrenia, first-degree relatives, and schizotypal personality disorder. *Schizophr Res*. 2012;136:143–149.
22. Tada M, Nagai T, Kirihaara K, et al. Differential alterations of auditory gamma oscillatory responses between pre-onset high-risk individuals and first-episode schizophrenia. *Cereb Cortex*. 2014;26:1027–1035.
23. Hamm JP, Gilmore CS, Picchetti NA, Sponheim SR, Clementz BA. Abnormalities of neuronal oscillations and temporal integration to low- and high-frequency auditory stimulation in schizophrenia. *Biol Psychiatry*. 2011;69:989–996.
24. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci*. 2005;6:312–324.
25. Spencer KM, Nestor PG, Niznikiewicz MA, Salisbury DF, Shenton ME, McCarley RW. Abnormal neural synchrony in schizophrenia. *J Neurosci*. 2003;23:7407–7411.
26. Uhlhaas PJ, Singer W. Oscillations and neuronal dynamics in schizophrenia: the search for basic symptoms and translational opportunities. *Biol Psychiatry*. 2015;77:1001–1009.
27. Traub RD, Cunningham MO, Gloveli T, et al. GABA-enhanced collective behavior in neuronal axons underlies persistent gamma-frequency oscillations. *Proc Natl Acad Sci U S A*. 2003;100:11047–11052.
28. Bartos M, Vida I, Jonas P. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci*. 2007;8:45–56.
29. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature*. 2009;459:698–702.
30. Gonzalez-Burgos G, Fish KN, Lewis DA. GABA neuron alterations, cortical circuit dysfunction and cognitive deficits in schizophrenia. *Neural Plast*. 2011;2011:723184.
31. Woo TU, Spencer K, McCarley RW. Gamma oscillation deficits and the onset and early progression of schizophrenia. *Harv Rev Psychiatry*. 2010;18:173–189.
32. Yoon JH, Maddock RJ, Rokem A, et al. GABA concentration is reduced in visual cortex in schizophrenia and correlates with orientation-specific surround suppression. *J Neurosci*. 2010;30:3777–3781.
33. Marsman A, Mandl RC, Klomp DW, et al. GABA and glutamate in schizophrenia: a 7 T ¹H-MRS study. *Neuroimage Clin*. 2014;6:398–407.
34. Mori T, Mori K, Fujii E, et al. Neuroradiological and neurofunctional examinations for patients with 22q11.2 deletion. *Neuropediatrics*. 2011;42:215–221.
35. Sullivan EM, Timi P, Hong LE, O'Donnell P. Effects of NMDA and GABA-A receptor antagonism on auditory steady-state synchronization in awake behaving rats. *Int J Neuropsychopharmacol*. 2015;18:pyu118.
36. Zhang J, Ma L, Li W, Yang P, Qin L. Cholinergic modulation of auditory steady-state response in the auditory cortex of the freely moving rat. *Neuroscience*. 2016;324:29–39.
37. Sivarao DV. The 40-Hz auditory steady-state response: a selective biomarker for cortical NMDA function. *Ann N Y Acad Sci*. 2015;1344:27–36.
38. Sivarao DV, Chen P, Senapati A, et al. 40 Hz auditory steady-state response is a pharmacodynamic biomarker for cortical NMDA receptors. *Neuropsychopharmacology*. 2016;41:2232–2240.
39. Nakao K, Nakazawa K. Brain state-dependent abnormal LFP activity in the auditory cortex of a schizophrenia mouse model. *Front Neurosci*. 2014;8:168.
40. Carlén M, Meletis K, Siegle JH, et al. A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. *Mol Psychiatry*. 2012;17:537–548.
41. Vierling-Claassen D, Siekmeier P, Stufflebeam S, Kopell N. Modeling GABA alterations in schizophrenia: a link between impaired inhibition and altered gamma and beta range auditory entrainment. *J Neurophysiol*. 2008;99:2656–2671.
42. Chun S, Westmoreland JJ, Bayazitov IT, et al. Specific disruption of thalamic inputs to the auditory cortex in schizophrenia models. *Science*. 2014;344:1178–1182.
43. Didriksen M, Fejgin K, Nilsson SRO, et al. Persistent gating deficit and increased sensitivity to NMDA receptor antagonism after puberty in a new mouse model of the human 22q11.2 microdeletion syndrome: a study in male mice. *J Psychiatry Neurosci*. 2016;41:150381.
44. Rihs TA, Tomescu MI, Britz J, et al. Altered auditory processing in frontal and left temporal cortex in 22q11.2 deletion syndrome: a group at high genetic risk for schizophrenia. *Psychiatry Res*. 2013;212:141–149.
45. McGlashan TH, Miller TJ, Woods SW, Rosen JL, Hoffmann RE, Davidson L. *Structured Interview for Prodromal Syndromes (SIPS). Norsk Version 5.0*. Norge: Stavanger Universitetssjukehus; 2012.
46. Miller TJ, McGlashan TH, Rosen JL, et al. Prodromal assessment with the structured interview for prodromal syndromes and the scale of prodromal symptoms: predictive validity, interrater reliability, and training to reliability. *Schizophr Bull*. 2003;29:703–715.
47. Schmock H, Vangkilde A, Larsen KM, et al. The Danish 22q11 research initiative. *BMC Psychiatry*. 2015;15:220.
48. Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci*. 2011;2011:156869.
49. Başar E. A review of gamma oscillations in healthy subjects and in cognitive impairment. *Int J Psychophysiol*. 2013;90:99–117.
50. Buzsáki G, Wang XJ. Mechanisms of gamma oscillations. *Annu Rev Neurosci*. 2012;35:203–225.
51. Jädi MP, Behrens MM, Sejnowski TJ. Abnormal gamma oscillations in N-methyl-D-aspartate receptor hypofunction models of schizophrenia. *Biol Psychiatry*. 2016;79:716–726.
52. Cardin JA. Dissecting local circuits in vivo: integrated optogenetic and electrophysiology approaches for exploring inhibitory regulation of cortical activity. *J Physiol Paris*. 2012;106:104–111.
53. Sohal VS. Insights into cortical oscillations arising from optogenetic studies. *Biol Psychiatry*. 2012;71:1039–1045.
54. Lisman JE, Coyle JT, Green RW, et al. Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci*. 2008;31:234–242.
55. Hamm JP, Gilmore CS, Clementz BA. Augmented gamma band auditory steady-state responses: support for NMDA hypofunction in schizophrenia. *Schizophr Res*. 2012;138:1–7.
56. Poulsen C, Picton TW, Paus T. Age-related changes in transient and oscillatory brain responses to auditory stimulation during early adolescence. *Dev Sci*. 2009;12:220–235.
57. Rojas DC, Maharajh K, Teale PD, et al. Development of the 40 Hz steady state auditory evoked magnetic field from ages 5 to 52. *Clin Neurophysiol*. 2006;117:110–117.
58. Uhlhaas PJ, Roux F, Rodriguez E, Rotarska-Jagiela A, Singer W. Neural synchrony and the development of cortical networks. *Trends Cogn Sci*. 2010;14:72–80.
59. Hashimoto T, Nguyen QL, Rotaru D, et al. Protracted developmental trajectories of GABA_A receptor α 1 and

- alpha2 subunit expression in primate prefrontal cortex. *Biol Psychiatry*. 2009;65:1015–1023.
60. Jonas RK, Montojo CA, Bearden CE. The 22q11.2 deletion syndrome as a window into complex neuropsychiatric disorders over the lifespan. *Biol Psychiatry*. 2014;75:351–360.
61. Squarcione C, Torti MC, Di Fabio F, Biondi M. 22q11 deletion syndrome: a review of the neuropsychiatric features and their neurobiological basis. *Neuropsychiatr Dis Treat*. 2013;9:1873–1884.
62. Larsen TK, Melle I, Auestad B, et al. Early detection of psychosis: positive effects on 5-year outcome. *Psychol Med*. 2010;41:1461–1469.
63. Hegelstad WT, Larsen TK, Auestad B, et al. Long-term follow-up of the TIPS early detection in psychosis study: effects on 10-year outcome. *Am J Psychiatry*. 2012;169:374–380.