Agenesis of the Corpus Callosum and Autism: a comprehensive comparison

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Running Title: Autism Symptoms in Callosal Agenesis

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Abstract

The corpus callosum, whose roughly 200 million axons constitute the largest white matter tract in the human brain, remains enigmatic in its contribution to cognition and behavior. Agenesis of the corpus callosum is a rare congenital condition in which the corpus callosum fails to develop; such individuals exhibit localized deficits in non-literal language comprehension, humor, theory of mind, and social reasoning. These findings together with parent reports suggest that behavioral and cognitive impairments in people with callosal agenesis may overlap with the profile of autism spectrum disorders, particularly with respect to impairments in social interaction and communication. To provide a comprehensive test of this hypothesis, we directly compared a group of 26 adults with callosal agenesis to a group of 28 adults with a diagnosis of autism spectrum disorder but no neurological abnormality. All participants had full-scale intelligence quotient scores above 78 and groups were matched on age, handedness, and gender ratio. Using the Autism Diagnostic Observation Schedule together with current clinical presentation to assess autistic symptomatology, we found that 8/26 (about a third) of agenesis subjects presented with autism. However, more formal diagnosis additionally involving recollective parent-report measures regarding childhood behavior showed that only 3/22 met complete formal criteria for an autism spectrum disorder (parent reports were unavailable for 4 subjects). We found no relationship between intelligence quotient and autism symptomatology in callosal agenesis, nor evidence that the presence of any residual corpus callosum differentiated those who exhibited current autism spectrum symptoms from those who did not. Relative to the autism spectrum comparison group, parent ratings of childhood behavior indicated children with agenesis were less likely to meet diagnostic criteria for autism, even for those who met autism spectrum criteria as adults, and even though there was no group

difference in parent report of current behaviors. The findings suggest two broad conclusions. First, they support the hypothesis that congenital disruption of the corpus callosum constitutes a major risk factor for developing autism. Second, they quantify specific features that distinguish autistic behavior associated with callosal agenesis from autism more generally. Taken together, these two findings also leverage specific questions for future investigation: what are the distal causes (genetic and environmental) determining both callosal agenesis and its autistic features, and what are the proximal mechanisms by which absence of the callosum might generate autistic symptomatology?

Abbreviations

ADI-R: Autism Diagnostic Interview-Revised

ADOS: Autism Diagnostic Observation Schedule

AgCC: Agenesis of the Corpus Callosum (participant population recruited for study)

AgCC$^+$: AgCC 'plus' met ASD criteria on ADOS (subgroup of AgCC as defined in this study)

AgCC$^-$: AgCC without evidence of ASD on ADOS (subgroup of AgCC as defined in this study)

ASD: Autism Spectrum Disorder (participant population recruited for study)

CI: Confidence Interval

IQ: Intelligence Quotient

LSAS: Liebowitz Social Anxiety Scale

SCQ: Social Communication Questionnaire

SRS-2: Social Responsiveness Scale -2

Introduction

Agenesis of the corpus callosum (AgCC), a failure to develop the large bundle of fibers that connects the cerebral hemispheres, occurs in at least 1:4000 individuals (Glass et al., 2008). Current evidence suggests that a combination of genetic mechanisms, including single-gene Mendelian and single-gene sporadic mutations, as well as more complex genetics (which may have a mixture of inherited and sporadic mutations) may have a role in the etiology of AgCC (Paul et al., 2007, Sajan et al., 2013). One highly informative mouse model of the developmental and molecular bases of AgCC, the BTBR inbred strain, is also widely used as a mouse model of autism (Wahlsten et al., 2003). Yet the cause of AgCC is identifiable for only 30-45% of human individuals (~10% have chromosomal anomalies and the remaining 20-35% have recognizable genetic syndromes (Bedeschi et al., 2006)). In these individuals, it is common also to see concomitant structural abnormalities such as polymicrogyria, pachygyria (abnormally broad gyri), heterotopias, and variability of anterior commissure structure (Shevell, 2002, Goodyear et al., 2001, Hetts et al., 2006). In the remaining 55 – 70% of individuals with AgCC, the cause is unknown and callosal agenesis is often an isolated neurological finding.

In a human fetus, rudimentary callosal fibers cross the midline by the twelfth post-conceptual week (Rakic and Yakovlev, 1968, Kier and Truwit, 1996). AgCC results when some or all of the ~200 million callosal axons fail to cross the midline, and are instead redirected into atypical fiber bundles (so-called Probst Bundles) running anterior to posterior just lateral to the interhemispheric fissure and medial to the cingulum bundle (i.e. along the medial wall of each hemisphere) (Probst, 1972). AgCC is also typically accompanied by a characteristic dilatation of posterior lateral ventricles (colpocephaly) (Barkovich and Norman, 1988). Neural

functioning in isolated AgCC demonstrates the remarkable plasticity of the developing human brain, as well as the vulnerability of higher-order cognition. Despite complete absence of callosal connections, functional brain connectivity during rest is remarkably intact in isolated AgCC – indeed, adult brains with AgCC generate an entirely typical, bilateral, set of resting-state functional brain networks (Tyszka et al., 2011). However, interhemispheric functional connectivity in AgCC appears to be more varied during cognitive tasks. For example, EEG recordings of visual evoked potentials demonstrate that visual signals do not propagate across the midline in AgCC (Brown et al., 1999), a pattern also evident in patients with surgical callosotomy. Despite this sensory disconnection, individuals with AgCC are capable of comparing simple visual stimuli such as letters and colors across visual fields and can conduct simple bimanual tasks normally (e.g., (Brown et al., 1999, Mueller et al., 2009)), indicating that simple conceptual and motor information can be transferred between the hemispheres in AgCC, perhaps via other connecting pathways such as the anterior commissure (Hsu et al., 2013, Barr et al., 2005). In general, individuals with AgCC do not exhibit the classic disconnection features found in patients with surgical resection of the corpus callosum, but do appear to have subtle limitations in interhemispheric transfer.

The limits of compensation for callosal disconnection in AgCC become more evident on tasks that require complex cognitive operations, demand rapid processing, and rely less on prior experience (Brown et al., 2001, Jeeves, 1979, Meerwaldt, 1983, Sauerwein and Lassonde, 1983) (for review see (Paul et al., 2007)). While AgCC does not appear to have a direct or dramatic impact on general cognitive ability (Chiarello, 1980, Sauerwein et al., 1994) or basic language skills (Liederman et al., 1985, Temple et al., 1989, Temple et al., 1990, Sauerwein et al., 1994),

subtle deficits are evident in comprehension of syntax and linguistic pragmatics (including idioms, proverbs and vocal prosody) as well as humor and other nonliteral language forms. These communicative deficits seem to arise from difficulties in over-riding literal interpretation together with difficulties in using context to infer meaning (Brown et al., 2005, Paul et al., 2003, Banich and Brown, 2000, Sanders, 1989, Huber-Okrainec et al., 2005). Additional deficits in AgCC include impairments in social problem solving (Symington et al., 2010), abstract reasoning (Brown and Paul, 2000), generalization (Solursh et al., 1965) and processing speed (Marco et al., 2012). Overall, AgCC often involves pronounced real-world social impairments similar to those seen in high-functioning people with autism, encompassing diminished social self-awareness (Brown and Paul, 2000), difficulty imagining the social perspective of others (Turk et al., 2010, Symington et al., 2010), poor conversation skills (O'Brien, 1994) and restricted verbal expression of emotional experience (similar to alexithymia) (Buchanan et al., 1980, O'Brien, 1994).

Autism spectrum disorder (ASD) is a complex neuropsychiatric disorder whose diagnosis is based on deficits in communication and social interaction, as well as patterns of stereotyped, restricted and repetitive behaviours. Like AgCC, autism is a developmental disorder, and like AgCC it has a considerable, but complex and polygenic, heritable component (Parikshak et al., 2013). A current leading hypothesis is that autism arises from an atypical developmental course of connectivity and synapse formation in the brain, leading to its conceptualization as a category of disconnection disease (Belmonte et al., 2004, Geschwind and Levitt, 2007, Just et al., 2004). There is considerable heterogeneity among individuals with autism, and considerable heterogeneity in research findings of connectivity (e.g., (Tyszka et al., 2013, Redcay et al.,

2013, Keown et al., 2013, Bellani et al., 2013)), leaving open the possibility that globally abnormal connectivity, or particular patterns of abnormal connectivity, may apply only to certain subtypes of autism.

There is more specific evidence implicating the corpus callosum in the abnormal connectivity of autism. While some studies report reduced corpus callosum cross-sectional volume throughout the callosum in autism (Alexander et al., 2007, Boger-Megiddo et al., 2006, Egaas et al., 1995, Manes et al., 1999), others indicate involvement of select callosal regions [anterior (Manes et al., 1999, Kilian et al., 2008, Vidal et al., 2006, Just et al., 2007), midbody (Manes et al., 1999, Kilian et al., 2008, Piven et al., 1997), and posterior regions (Egaas et al., 1995, Piven et al., 1997, Vidal et al., 2006, Just et al., 2007) have all been implicated]. Similarly, one study of ASD found evidence of reduced fractional anisotropy of fibers (a measure of their organization in bundles) throughout the corpus callosum (Keller et al., 2007), while others found reductions only in the genu (Alexander et al., 2007, Keller et al., 2007, Barnea-Goraly et al., 2004) and splenium (Alexander et al., 2007, Keller et al., 2007). Complementing these abnormalities in white-matter connectivity are their correlations with deficits in processing speed (Alexander et al., 2007), as well as impairments on cognitive tasks thought to require interhemispheric transfer of information (Minshew et al., 1997, Nyden et al., 2004), and decreased functional coupling between specific brain regions during various cognitive activation tasks (Just et al., 2004, Koshino et al., 2005, Kleinhans et al., 2008).

The above observations motivate the prediction that there might be overlap in the cognitive and behavioral difficulties seen in people with autism, and in people with AgCC. Indeed, there have been a few published attempts to identify the prevalence of ASD diagnoses in AgCC, although

these have relied largely upon parent reports of clinical diagnoses and parent questionnaires about potential autism symptoms, and have typically included a very wide range of participants, limiting quantitative characterization and specificity. For instance, surveys completed by caregivers of 720 children and adults with AgCC, indicated that 77% of the individuals with AgCC were considered to have some form of developmental delay (Moes et al., 2009). Seventy-six out of the 720 were reportedly diagnosed with autism or Asperger's disorder (10.6%). Follow-up studies in a subset from that survey, consisting of 61 children with AgCC aged 2 - 11 years, none of whom exhibited significant delays in motor and developmental milestones, showed that 23% had received a diagnosis of autism or Asperger's syndrome (Badaruddin et al., 2007). In these children with limited developmental delays, the percentages of subjects who met specific autism criteria were highest in the social (34 - 46%) and communication domains (25 - 51%). A more recent study used the Autism Quotient questionnaire to screen for autism symptoms in AgCC. Out of 106 participants, 45% of children, 35% of adolescents and 18% of adults exceeded the cut-off score on the Autism Quotient (a questionnaire-based screening instrument for autism) suggesting the presence of autism symptoms, but an autism spectrum diagnosis was not verified by clinical assessment or observation (Lau et al., 2012). Similarly, in an ongoing study of 16 individuals with AgCC, five out of ten children in the six- to seventeen-year-old range scored close to or above the cut-off for autism classification on parent report measures (Booth et al., 2011). In the latter sample, the children with autism symptoms were older (13 – 17 years old) than those who did not show autism symptoms (seven – twelve years old). Most recently, a detailed case study of an adult male with partial AgCC who was also diagnosed with ASD reported lower performance across a variety of self-referential and social-cognitive measures than either neurotypical controls or adults with ASD who do not have

AgCC, although the degree to which he benefitted from self-referencing in a memory task was similar to the ASD group (Lombardo et al., 2012). In sum, while parent reports from prior to 2000 (when the survey was conducted (Moes et al., 2009)) indicate that only approximately 10% of individuals with AgCC received a formal ASD diagnosis, more recent results from screening instruments suggest that 35 - 50% of children and adolescents with AgCC, and approximately 20% of adults, exhibit significant autistic symptomatology.

These prior studies provide initial information about possible autism symptomatology in AgCC, but leave open two important topics: 1) a formal diagnosis of autism, as well as 2) finer differentiation and detailed characterization of the behavioral phenotype in AgCC. The present study addresses both of these issues in a very well characterized sample of 26 individuals with isolated AgCC.

Our first aim was to clarify the rate of formal autism diagnosis in adolescents and adults with AgCC. Autism can be diagnosed if an individual exhibits symptoms in two domains: a) social communication and b) restricted and repetitive behaviors, and if these symptoms are present early in life (typically prior to age three) and result in significant functional impairment. The present study characterized current behavior using (a) the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2012), one of the best observational diagnostic measures available and considered to be the gold standard in research studies, and (b) one or more parent report measures (Social Communication Questionnaire, SCQ (Rutter et al., 2003a) or Autism Diagnostic Interview – Revised, ADI-R (Rutter et al., 2003b)). Information from clinical interview with the participant and, if available a parent was also used to augment and validate

outcomes of these measures. Diagnostic accuracy of autism is typically improved through use of both an observational and parent-report measure (Corsello et al., 2007, Risi et al., 2006). When assessing adults, parent report provides important diagnostic information regarding symptomatology during early childhood; however, recollective parent reports may be compromised by memory decay or distortion over time. All participants with AgCC received the ADOS, but parent report (SCQ or ADI-R) was unavailable for four of these individuals, which reduced our sample size for formal autism assessment to 22 participants with AgCC.

Our second aim was to provide detailed characterization of autism symptomatology in AgCC, as well as to quantify the overlap of social skills deficits between AgCC and autism. For this analysis, we included all 26 individuals with AgCC and divided the group according to their observed autism symptomatology, distinguishing those who met ADOS criteria for autism ($AgCC^+$) from those who did not ($AgCC^-$). We first examined the possibility that callosal malformation (partial or complete AgCC) or demographic factors might account for the subgroup distinction. Behavioral variations among these two subgroups and a sample of individuals with a pure diagnosis of an ASD (with no AgCC) were assessed using autism diagnostic measures (ADOS, SCQ, ADI-R) as well as several additional research measures of social and emotional processing. From the pattern of differences across these groups, we sought to discern which, if any, social skills deficits are most strongly associated with callosal disconnection, which appear to be more clearly linked to autism (apart from callosal disconnection), and which might distinguish those people with AgCC who meet behavioral criteria for autism versus those who do not. Our study constitutes the first comprehensive direct

comparison of autism and AgCC, and the first detailed characterization of autism symptomatology in AgCC.

Materials and Methods

Participants

We studied two distinct groups of participants, one neurologically defined and the second psychiatrically defined: 26 with AgCC, and 28 with ASD. The AgCC group included twenty-one with complete agenesis of the corpus callosum and five with partial agenesis (Figure 1). Participants with AgCC were still included if they had structural findings that commonly co-occur with AgCC: colpocephaly, Probst bundles, interhemispheric cysts and occasional small heterotopias. Potential participants with other structural brain abnormalities such as frontal lobe dysgenesis and large regions of heterotopia, as well as those with known genetic syndromes were not included. Presence of anterior commissure was confirmed in all participants with AgCC. For the five participants with partial AgCC, the percent of residual callosum was estimated by visual inspection of callosal cross-sectional area in midline sagittal T-1 MRI images. Residual callosum was less than 10% of normal size in two participants, 10-25% in one participant, and 25-75% in two others. Diffusion MRI studies indicate that structural connectivity of the remnant corpus callosum in partial AgCC can be highly variable (Wahl et al., 2009). Therefore, the location of residual callosum cannot be assumed to represent the same connectivity as that region within an intact corpus callosum. Analysis of individual connectivity patterns in our participants with partial AgCC was beyond the scope of this study.

AgCC participants were recruited through the National Organization for Disorders of the Corpus Callosum and the Caltech Corpus Callosum Research Program and were enrolled into an ongoing research program on agenesis of the corpus callosum at Caltech. AgCC diagnosis was confirmed by structural MRI, and background information was gathered as part of the larger research program. Twenty-eight high-functioning individuals with an ASD were recruited from an existing research registry at Caltech. These individuals all had a DSM-IV-TR diagnosis of autism or Asperger syndrome, which was confirmed on the ADOS. None of these individuals showed evidence of callosal agenesis, dysgenesis, or other visually apparent malformation on structural MRI.

Exclusionary criteria for both groups included English as a second language, history of moderate-to-severe head injury, major neurological disorder not associated with AgCC, intractable epilepsy, major psychiatric illness other than ASD and comorbid depression/anxiety, and drug abuse as assessed by clinical interview. To avoid confounding effects due to borderline general intellectual function, full scale IQ greater than, or equal to, 70 was required. Full scale IQ was measured using Wechsler intelligence tests (Wechsler Adult Intelligence Scale-III (Fowler et al., 1988) n=23 AgCC; Wechsler Intelligence Scale for Children-III, (Wechsler, 1991), n=1 AgCC; Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999), n=2 AgCC and n=28 ASD). The Wechsler Abbreviated Scale of Intelligence and Wechsler Adult Intelligence Scale-III are reliably correlated (full scale IQ r = .87; verbal IQ r = .88; performance IQ r = .84). Depression and anxiety symptoms are commonly co-morbid with ASD (Matson and Williams, 2013, Mazzone et al., 2012) so rather than excluding participants with these symptoms, we confirmed that groups did not differ on indicators of depression (Beck

Depression Inventory-II (Beck et al., 1996), a self-report questionnaire that examines current depressive symptomatology from the prior two weeks), anxiety (State-Trait Anxiety Inventory (Spielberger et al., 1983), a self-report questionnaire that differentiates between state and trait anxiety) and current mood (Positive and Negative Affect Schedule (Crawford and Henry, 2004), a self-report measure of state affect).

Demographic data for both groups is available in Table 1. Groups were compared using student's t-test (for pairwise parametric contrasts) and Fischer's exact test (for testing differences in proportions; 3x2 for handedness, 2x2 for gender, 4x2 for education). The AgCC and ASD groups did not differ significantly on age, $d = .08$, gender, $p = .13$, or education, $p = .07$, nor did they differ on basic measures of mood and anxiety: Positive and Negative Affect Schedule (Negative, $d = .02$; Positive, $d = .09$), State-Trait Anxiety Inventory (Trait, $d = .34$; State, $d = .56$) and Beck Depression Inventory-II, $d = .23$. However the ASD group had significantly higher IQ scores: full scale IQ, $t(52) = 3.97$, $p < .001$, $d = 1.10$, performance IQ, $t(52) = 3.53$, $p = .001$, $d = .98$, and verbal IQ, $t(52) = 2.97$, $p = .005$, $d = .82$. The groups also differed on handedness, $p < .001$.

All participants gave informed consent (assent with parent consent for four minors) under a protocol approved by the Human Subjects Protection committee of the California Institute of Technology, and the study adhered to American Psychological Association ethical principles.

Measures

Autism Diagnostic Instruments: The Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2001) (Lord et al., 1989) is a standardized observational diagnostic measure. It is organized into four separate modules, based on the age and expressive language level of the individual being assessed. Module 4, designed for verbally fluent adolescents and adults, was administered to all participants in this study. The ADOS consists of communication and social behavior items that are scored from zero (no abnormality) to three (severe abnormality). A select subset of items is included in a diagnostic algorithm that results in a classification of autism, autism spectrum, or nonspectrum. A participant meets criteria for a classification of autism or an autism spectrum disorder if the scores in the social and communication domains and the total on the algorithm meet or exceed cut-off scores. Validity of Module 4 of the ADOS is strong, with sensitivity of 90% and specificity of 93% when differentiating autism and autism spectrum disorders from nonspectrum disorders (Lord et al., 2012).

The Autism Diagnostic Interview-Revised (ADI-R) (Rutter et al., 2003b) is a semi-structured interview containing questions about the three areas of behaviors associated with an autism spectrum disorder (social, communication, and restricted behavior). Most items are scored from zero (no abnormality) to three (abnormality that interferes with daily life). As with the ADOS, a select subset of items is included in the diagnostic algorithm, with separate cut-off scores for each domain. Most ADI-R diagnostic algorithm items are coded based on past behavior for adult participants; however, current functioning can also be coded and assessed with a current algorithm scoring system.

The Social Communication Questionnaire (SCQ) (Rutter et al., 2003a) is a 40-item, parent-completed, screening questionnaire, based on the initial mandatory probes from the original ADI (LeCouteur et al., 1989) covering the areas of communication, reciprocal social interactions, and restricted and repetitive behaviors and interests. As such, it is sometimes used as proxy for the ADI-R, and is highly correlated with ADI-R scores (Rutter et al., 2003a). Each item is checked as "yes" or "no", and assigned a point rating of "1" (presence of abnormal behavior) or "0" (absence of abnormal behavior). The points are summed and yield a total possible score of 0 – 39. Totals are compared to a cut-off of ≥15 for an autism spectrum disorder. There are two different versions of the SCQ: 1) a "current" version designed for children under the age of five years and 2) a "lifetime" version designed for children five years of age or older, with all questions based on lifetime or past behavior. The lifetime version was used for all participants in this study.

The Social Responsiveness Scale-2 (SRS-2) (Constantino, 2012) is a questionnaire focusing on social difficulties present in individuals with autism consisting of 65 behaviors organized into five domains. The SRS-2 Adult Form (Self-Report) was completed by our participants and the SRS-2 Adult Form (Relative/Other Report) was completed by a parent or significant other.

Other Psychological Measures: The Benton Facial Recognition task measures the ability to discriminate identity of faces, thereby assessing basic visuoperceptual ability in relation to face processing; results are reported as standardized T-scores (Benton et al., 1983).

The Reading the Mind in the Eyes (revised version) (Baron-Cohen et al., 2001) task is widely used as a measure of social cognition. It asks participants to make social judgments from the eye region of faces and is known to yield impaired performance in people with autism. Results were scored relative to normative data for the revised version gathered from a sample of 122 adults recruited from the community (Baron-Cohen et al., 2001).

The Empathizing Quotient (Baron-Cohen and Wheelwright, 2004) and Systemizing Quotient - Revised (Baron-Cohen et al., 2003) questionnaires are self-report instruments that assess the drive to identify others' thoughts or emotions (empathizing) and the drive to understand and construct lawful systems for governing behavior (systemizing). Higher systemizing/empathizing ratios are associated with autism spectrum diagnoses (Wheelwright et al., 2006).

The Liebowitz Social Anxiety Scale (LSAS) (Liebowitz, 1987) is a self-report questionnaire that contains 24 items, 13 concerning performance anxiety and 11 concerning social situations. Each item is rated separately for fear and avoidance behavior, resulting in overall social anxiety severity rating, and scores on four subscales: 1) performance fear, 2) performance avoidance, 3) social fear, and 4) social avoidance. For the total LSAS score, a diagnostic cut-off of 30 points provides the best balance of sensitivity and specificity for diagnosis (Mennin et al., 2002),

Procedures

The ADOS was administered to all AgCC participants by one of four administrators (three of the authors: LKP, DK, CC; and Deborah Childress, University of North Carolina) who attended training on the measure and achieved inter-rater reliability of at least 80% (M=88.25%) on the

protocol administration and algorithm scoring. ADI-R was administered by two of the authors of this paper (LP & DK) who had achieved reliability of 90% on the protocol and algorithm. All other tasks were administered by trained Caltech research staff according to standardized instructions.

For the ASD group, approximately 30% of the ADOS administrations were double-coded to ensure consistency in scoring (scoring disagreements were addressed through discussion and video review to reach a consensus) and individuals who did not meet autism spectrum criteria on the ADOS were excluded. Since all ASD participants had a prior diagnosis, parent-report criteria were not required for inclusion in this group. When possible, SCQ and/or ADI-R were acquired from parents and used for group comparisons but not for diagnostic classification (SCQ n = 17, ADI-R n =17; no parent report available n = 7).

For AgCC participants, a formal best-estimate diagnosis was determined by meeting autism or autism spectrum criteria on Module 4 of the ADOS, together with clinical diagnosis based on interviews with participants and parents, and meeting autism spectrum criteria on the SCQ and/or ADI-R. SCQ was completed by parents of 22 participants with AgCC, 14 of whom were additionally interviewed using the ADI-R. Full ASD criteria could not be assessed in four participants with AgCC because parent reports were unavailable.

In all AgCC cases, ADOS was double-coded by at least two of the authors using the aforementioned consensus procedures. In addition to ensuring consistency in scoring, double coding of AgCC cases reduced potential influence of examiner bias. ADOS administrators were

not blind to neurological diagnosis; however, only L.K.P. had prior exposure to individuals with AgCC. Therefore all AgCC cases were scored by at least one examiner who was blind to research on AgCC and to any hypothesis regarding its relationship to ASD.

Participant Groups and Data Analysis

Based on the criteria above, to address our first aim we identified participants with AgCC who met full criteria for an ASD diagnosis and compared them to participants with AgCC who met ASD criteria on the ADOS and in recent clinical history but did not meet ASD criteria on parent report (i.e. did not have evidence of ASD during early childhood). A two (group) by three (communication, social, and restricted behavior scales) ANOVA was used to examine ADOS scores and ADI-R scores across the two groups. Student's t-tests and Fischer's exact test were used to compare these groups on all other measures.

To address our second aim regarding expression of the autism behavioral phenotype and other social impairments in adults with AgCC, participants with AgCC were classified into one of two broad categories based on ADOS scores and recent clinical history: a) AgCC$^+$ (AgCC 'plus' exhibition of current behavior consistent with an autism spectrum disorder) or b) AgCC$^-$ (AgCC without current evidence of ASD). Demographic variables for the two AgCC subgroups (AgCC$^+$ and AgCC$^-$) were compared with the ASD group using ANOVA, followed by post-hoc comparison of groups using Tukey's HSD test with significance set at $\alpha = .05$. For data analysis, the three groups (AgCC$^+$, AgCC$^-$, and ASD) were compared using ANCOVA to control for variations in full-scale IQ. Mauchly's test of sphericity was conducted for each ANCOVA and in cases where sphericity was violated at $p < .05$, the Huynh-Feldt correction

was used if epsilon was greater than or equal to .75 and Greenhouse-Geisser was used if epsilon was less than .75. Significant findings from ANCOVA were followed by post-hoc between-group contrasts controlling for full-scale IQ with significance set at **α** = .05 and Bonferroni corrected for multiple comparisons (**α** = .0167 unless noted otherwise). Finally, individual performance on each measure was examined relative to published norms and/or clinical cut-off scores. Mehta and Patel's extension of the Fischer's exact test was used to compare clinically categorized scores across the three groups. Confidence intervals (CI) for proportions were calculated using 1000 sample bootstrapping.

Results

Formal Autism Diagnosis in AgCC

Three out of 22 participants with AgCC met full criteria for an autism spectrum diagnosis (13.6%, 95% CI [0, 31.8]). Three more met ADOS criteria for an autism spectrum disorder and had a clinical diagnosis on the autism spectrum but did not meet ASD criteria on parent report. These six individuals all had complete AgCC. As expected, comparison of the three who met full ASD criteria with the three who did not meet criteria on parent report confirmed the former group had significantly higher scores on SCQ $t(4) = 4.01$, $p = .02$. The group difference on ADI-R almost met significance (3 ADI-R scales repeated-measures ANOVA p = .056, η^2_p = .75).

The AgCC group who met full ASD criteria also exhibited higher ADOS scores than those who only met ASD criteria on ADOS (repeated-measures ANOVA with three ADOS scores) $F(1,4)$ = 8.32, $p = .045$, $\eta^2_p = .68$, but the interaction of group by ADOS scale was not significant, $\eta^2_p =$

.36, nor was the overall effect of ADOS scales, η^2_p = .67. Post-hoc comparisons corrected for multiple comparisons (three ADOS scales) confirmed significantly greater autism symptomatology on the ADOS communication scale in the group who met full autism criteria, $t(4)$ = 4.03, p = .016, d = 4.03, with no group differences on the social scale, d = 1.11, or the restricted behaviors scale, d = .44. These groups also did not differ on age, d = .28, full scale IQ, d = .88, verbal IQ, d = 1.03, performance IQ, d = .35, gender, p = .40, handedness, p = 1.00, or education, p = 1.00 and did not differ on any additional other experimental measures (see Supplementary Table 1).

Autism Spectrum Behaviors in Adults with AgCC

Eight out of 26 individuals in the AgCC group met criteria for an autism spectrum disorder on the ADOS and in recent clinical history (AgCC$^+$ = 30.8%, 95% CI [11.5, 50]). This group includes the six participants from the previous analyses, as well as two individuals for whom parent report was unavailable. All participants in the AgCC$^+$ subgroup had complete AgCC (Table 2); however, type of AgCC diagnosis (complete vs. partial) was not a significant predictor of ADOS scores across all participants with AgCC (point biserial correlation with non-parametric Wilcoxon rank sum test, p = .313). The AgCC$^+$ and AgCC$^-$ subgroups did not differ significantly on gender, p = .35, handedness, p = .11, education p = .92, age, d = -.04, full scale IQ, d = .63, verbal IQ, d = .60, or performance IQ, d = .40.

Variations in the behavioral phenotype of AgCC were examined by comparing those who displayed autism symptoms (AgCC$^+$), those who did not (AgCC$^-$), and a separately recruited sample of adults with ASD but no neurological abnormality (see Methods for details). The three

groups differed significantly on IQ scores (full-scale IQ, $F(2,51) = 9.51$, $p < .001$, $\eta^2_p = .27$; performance IQ, $F(2,51) = 6.84$, $p = .002$, $\eta^2_p = .21$; verbal IQ $F(2,51) = 5.51$, $p = .007$, $\eta^2_p = .18$), but not age, $F(2,51) = 0.04$, $p = .96$ $\eta^2_p = .002$. Post-hoc Tukey's tests revealed significantly lower full-scale IQ and performance IQ in both AgCC groups than in the ASD group. Verbal IQ was significantly lower in AgCC$^+$ than ASD, but AgCC$^-$ and ASD groups did not differ. Full-scale IQ was introduced as a covariate in all subsequent 3-group comparisons. In addition, analyses reported below were repeated with a small subsample of participants selected so as to equate groups with respect to IQ (see Supplement 1 and Supplementary Table 2).

On measures of mood and affect, the three groups did not vary significantly on either subtest of the Positive and Negative Affect Schedule (Negative $\eta^2_p = .06$; Positive $\eta^2_p = .03$), or on either subtest of the State Trait Anxiety Inventory (State $\eta^2_p = .06$; Trait $\eta^2_p = .01$). Although the three groups varied significantly on BDI-II, $F(2,49) = 3.44$, $p = .04$, $\eta^2_p = .12$, post-hoc contrasts between groups were not significant.

Since ADOS algorithm scores were used for diagnostic categorization, significant between-group differences were expected (Figure 2A plots η^2_p for group contrasts; Figure 3 plots individual scores for ADOS subscales for all participants in each group). Repeated measures ANCOVA comparing the three groups by three ADOS scales (communication, social, and restricted behavior) confirmed a significant group difference, $F(2,50) = 45.38$, $p < .001$, $\eta_p^2 = .65$, in addition to a significant interaction of ADOS by group, $F(2.75,68.65) = 11.39$, $p < .001$, $\eta_p^2 = .31$, but not a significant effect of ADOS score, $\eta_p^2 = .07$. Post-hoc comparisons confirmed that the AgCC$^-$ subgroup exhibited significantly less autism symptomatology than both the

AgCC⁺, $F(1,50) = 22.73$, $p < .001$, $\eta_p^2 = .31$, and ASD groups, $F(1,50) = 86.65$, $p < .001$, $\eta_p^2 = .63$, while the AgCC⁺ and ASD groups did not differ ($\eta_p^2 = .08$).

Significant group differences were also found for each ADOS scale (Figure 3; communication, $F(2,50) = 29.33$, $p < .001$, $\eta_p^2 = .54$; social, $F(2,50) = 28.21$, $p < .001$, $\eta_p^2 = .53$; total, $F(2,50) = 34.60$, $p < .001$, $\eta_p^2 = .58$; restricted behavior, $F(2,50) = 11.41$, $p < .001$, $\eta_p^2 = .31$). Post-hoc comparisons (Figure 2A) confirmed that the AgCC⁻ subgroup exhibited significantly less autism symptomatology than both the AgCC⁺ (communication $F(1,50) = 20.775$, $p < .001$, $\eta_p^2 = .29$; social $F(1,50) = 10.89$, $p = .002$, $\eta_p^2 = .18$; total $F(1,50) = 16.78$, $p < .001$, $\eta_p^2 = .25$; restricted $F(1,50) = 6.88$, $p = .012$, $\eta_p^2 = .12$) and ASD groups (communication $F(1,50) = 52.582$, $p < .001$, $\eta_p^2 = .51$; social $F(1,50) = 55.24$, $p < .001$, $\eta_p^2 = .52$; total $F(1,50) = 66.32$, $p < .001$, $\eta_p^2 = .57$; restricted $F(1,50) = 21.18$, $p < .001$, $\eta_p^2 = .30$), but the AgCC⁺ and ASD groups did not differ (communication $\eta_p^2 = .01$; social $\eta_p^2 = .08$; total $\eta_p^2 = .07$; restricted $\eta_p^2 = .01$). The ADOS analyses reported above were repeated using the total scores for each section (not just the algorithm items), yielding an identical pattern of results. Table 2 presents the number of participants in each subgroup who met autism or ASD criteria.

The three groups differed significantly on SCQ scores, $F(2,35) = 5.39$, $p = .009$, $\eta^2 = .25$ (Table 3, Figures 2A,4). In post-hoc analyses, the ASD group scored significantly higher than the AgCC⁻ group, $F(1,35) = 10.62$, $p = .002$, $\eta_p^2 = .24$, but scores in the AgCC⁺ group did not differ from either the AgCC⁻, $\eta_p^2 = .03$, or ASD group, $\eta_p^2 = .05$. The ratio of participants who met ASD criteria on the SCQ differed significantly across three groups (2x3 Fischer's exact test, $p = .003$), but in post-hoc comparisons only the AgCC- and ASD groups differed significantly, $p = $

.001. Fifteen out of 17 individuals in the ASD group met SCQ criteria for autism spectrum diagnosis (88%, 95% CI [70.6, 100]), but only five out of 16 in the AgCC$^-$ group (31.3%, 95% CI [12.5, 56.3]) and three out of six in the AgCC$^+$ group (50%, 95% CI [16.7, 83.3]).

ADI-R raw scores on three scales (communication, social, and restricted behaviors) were compared across the three groups using ANCOVA. We found a significant difference between groups, $F(2,27) = 6.98$, $p = .004$, $\eta_p^2 = .34$, and interaction of ADI-R by group, $F(3.50,47.19) = 3.03$, $p = .03$, $\eta_p^2 = .18$, but not a significant effect of ADI-R scale, $\eta_p^2 = .09$. In post-hoc contrasts ADI-R scores indicated significantly less autism symptomatology in the AgCC$^-$ group than ASD, $F(1,27) = 12.93$, $p = .001$, $\eta_p^2 = .32$, but AgCC$^+$ did not differ from either ASD, $\eta_p^2 = .19$, or AgCC$^-$ groups, $\eta_p^2 = .00$.

In separate analyses with raw scores from each ADI-R scale, the three subgroups differed significantly on ADI-R communication, $F(2,27) = 9.51$, $p = .001$, $\eta_p^2 = .41$ and social, $F(2,27) = 4.53$, $p = .02$, $\eta_p^2 = .25$, but not on restricted behaviors, $\eta_p^2 = .14$ (Table 3, Figure 4). In post-hoc between-group contrasts, ADI-R scores indicated significantly less autism symptomatology in AgCC$^-$ than the ASD group on communication, $F(1,27) = 17.55$, $p < .001$, $\eta_p^2 = .39$ and social scales, $F(1,27) = 8.47$, $p = .007$, $\eta_p^2 = .24$. Although AgCC$^+$ and ASD groups did not differ on ADI-R scores overall (see post-hoc contrasts from three group by three ADI-R scale ANCOVA in previous paragraph), post-hoc contrasts of raw scores from individual ADI-R scales revealed significantly lower communication scores in AgCC$^+$ than in ASD $F(1,27) = 8.95$, $p = .006$, $\eta_p^2 = .25$, but no difference between these groups on social scores, $\eta_p^2 = .13$ (Figure 2A, 4B). The AgCC groups did not differ from one another on communication, $\eta_p^2 =$

.001, or social scores, $\eta_p^2 = .000$, and no group contrasts were significant for restricted behavior scores (η_p^2 range from .004 - .01).

ADI-R scores were also analyzed with respect to diagnostic cut-offs. Two out of five individuals in the AgCC[+] group (40%, 95% CI [0, 80]) and three out of nine in the AgCC[-] group (33%, 95% CI [0, 66.7]) met ADI-R criteria for an ASD diagnosis, while as expected all 17 ASD participants met ADI-R criteria. Three-group comparisons of diagnostic ratios using 2x3 Fischer's exact test were significant for overall ADI-R diagnosis, $p < .001$, as well as for ADI-R communication, $p = .001$, and social, $p < .001$ scales, but groups did not differ on rate of meeting the restricted behaviors cut-off, $p = .055$. Post-hoc between-group comparisons confirmed ADI-R diagnosis of ASD was significantly more likely in the ASD group than in both AgCC groups (AgCC[+], $p = .006$; AgCC[-], $p < .001$). Likewise, social scores in the ASD range were significantly more likely in the ASD group than in either AgCC group (AgCC[+], $p = .006$, AgCC[-], $p = .002$), and communication scores in the ASD range were more likely in the ASD group than the AgCC[-] group, $p = .002$. In contrast, the ASD and AgCC[+] groups did not differ on the ratio of participants with communication scores in the ASD range ($p = .043$). Table 3 presents the number of participants in each subgroup who met ASD criteria on the ADI-R.

In summary, during the ADOS approximately a third of our AgCC sample exhibited behaviors consistent with an ASD diagnosis (classified in this study as AgCC[+]), and ADOS scores in the remainder of the AgCC sample (the AgCC[-] group) were significantly below both the AgCC[+] and ASD groups. According to SCQ and ADI-R parent report measures, the AgCC[-] group exhibited significantly less autism symptomatology during early childhood than the ASD group,

with the AgCC[+] group scoring in between these groups. On SCQ raw scores and diagnostic ratios, the AgCC[+] group did not differ significantly from either the AgCC[-] or ASD groups. However, on the ADI-R, the AgCC[+] diagnostic outcome was similar to the ACC[-] group, both being significantly less likely than ASD group to meet overall diagnostic criteria. (For more information on within-subject consistency across parent report scores, see Supplement 2.)

Self-Awareness in AgCC and ASD

SRS-2 Adult Form (Self-Report) scores and SRS-2 Adult Form (Relative/Other Report) scores did not differ significantly across the three groups ($\eta_p^2 = .10$, $\eta_p^2 = .09$ respectively, Figure 2B). Using published cut-offs, SRS-2 Adult Form scores were converted to an ordinal scale indicating symptom severity (none, mild, moderate, or severe, Table 4). As with the ANCOVA results using raw scores, severity categorization of the three groups did not differ significantly on SRS-2 Adult Form (Self-Report), $p = .13$ or on SRS-2 Adult Form (Relative/Other Report), $p = .45$. However, when severity categories were combined into an 'impaired' category and the ratio of impaired to non-impaired there was a significant effect of group on the self-report form, $p = .04$, but not on other-report, $p = .51$. Post-hoc comparisons of self-report scores were not significant after correction for repeated measures (AgCC[+] versus ASD, $p = .04$; AgCC[+] versus AgCC[-] $p = .36$, AgCC[-] versus ASD $p = .06$).

If we presume that other-report scores provide a fairly accurate assessment of participants' social functioning, then comparison of self-report and other-report scores may offer insight regarding participants' self-awareness about these behaviors. To assess self-awareness using this hypothesis, SRS-2 Adult Form (Relative/Other Report) and (Self-Report) scores were first

correlated across the entire sample, while controlling for full scale IQ. Although there was a weak but significant positive correlation across the entire sample, $R^2 = .16$, t(38) = 2.64, $p < .02$, $d = .86$, when examined within groups, this correlation was significant only for AgCC+, $R^2 = .63$, $t(5) = 2.93$, $p < .05$, $d = 2.62$ (AgCC- $d = 1.04$; ASD $d = .16$).

To assess self-report accuracy at a group-level, we examined the proportion of elevated scores on self- and other-report. Over half of the participants in each group had elevated scores on other-report (AgCC+ 57%, 95% CI [14.3, 85.7]; AgCC- 64.7%, 95% CI [41.2, 88.2]; ASD 77.8%, 95% CI [55.6, 94.4]), but only in the ASD group did over half of the participants also endorse elevations on self-report (AgCC+ 12.5%, 95% CI [0, 37.5]; AgCC- 35.3%, 95% CI [11.8, 58.8]; ASD 62.5%, 95% CI [45.8, 79.2]. Overall, the ASD group exhibited the smallest difference between proportion of elevated scores on self-report and other-report (19.9, 95% CI [9.4, 46.2]), with the largest difference in the AgCC+ group (44.6, 95% CI [-7.1, 85.7]), followed by the AgCC- group (29.4, 95% CI [5.9, 58.8]). This suggests a general pattern of greater self-awareness in ASD and lesser awareness in AgCC+.

Finally, on a subject-by-subject basis we identified over- and under-reporting on self-report in comparison to other-report. Participants in the three groups were equally likely to over-endorse symptoms on self-report (i.e. some degree of impairment acknowledged on self-report and normal range score on other report was found in 3 out of 18 individuals with ASD, 2 out of 17 in the AgCC- group and none in the AgCC+ group; 2x3 Fisher exact test $p = .83$). Likewise across groups, participants were equally likely to under-endorse on self-report (normal range scores on self report and elevated score on other report in three out of seven individuals in the

AgCC[+] group, seven out of 17 participants in the ACC[-] group, and 6 out of 18 in the ASD group; 2x3 Fisher exact test $p = .84$).

Characterization of Additional Social Deficits In AgCC and ASD

We carried out further exploratory analyses to identify other aspects of social functioning that may distinguish between the AgCC subgroups and the ASD group (results for the following tests are presented in Table 4 and Figure 2B).

Facial perception scores (Benton Facial Recognition Test) and emotion identification from eyes (Reading the Mind in the Eyes) did not differ across the three subgroups ($\eta_p^2 = .03$, $\eta_p^2 = .04$ respectively). Clinically significant impairment in facial recognition was equally likely in all three groups (2x3 Fischer's exact test, $p = .08$),

The three groups also did not differ on the Systemizing Quotient-Revised, $\eta^2 = .008$, but exhibited significant variability on Empathizing Quotient $F(2,47) = 4.86$, $p = .012$, $\eta_p^2 = .17$. In post-hoc contrasts, both AgCC subgroups endorsed greater empathizing than the ASD group (AgCC[+] $F(1,47) = 6.60$, $p = .013$, $\eta_p^2 = .12$; AgCC[-] $F(1,47) = 7.27$, $p = .01$, $\eta_p^2 = .13$) but did not differ from one another, $\eta_p^2 = .009$. In one-sample t-tests comparing empathizing scores with published norms (Wheelwright et al., 2006), the AgCC[+] group did not differ from expected, $d = .42$, but scores were significantly below normal in AgCC[-], $t(16) = -2.40$, $p = .029$, $d = .61$; and ASD groups, $t(25) = -9.44$, $p < .001$, $d = 1.61$. On systemizing scores, neither AgCC group differed from norms, (AgCC[+] $d = .09$; AgCC[-], $d = .14$) but the ASD group's scores were significantly elevated, $t(25) = 2.40$, $p = .02$, $d = .51$.

Individual Empathizing Quotient and Systemizing Quotient-Revised scores were compared to gender-specific normative data acquired from an adult community-based sample (Wheelwright et al., 2006), with scores greater than 1.5 standard deviations from the mean identified as clinically significant (Table 4). Scores categorized by clinical range (elevated, average, lowered) did not differ across the three groups for Empathizing Quotient, $p = .15$ or Systemizing Quotient-Revised, $p = .19$. Finally, Empathizing Quotient and Systemizing Quotient-Revised scores were used to calculate 'brain-types' for each individual (Goldenfeld et al., 2005) as predicted by the E-S theory (Baron-Cohen et al., 2003). The three groups did not differ significantly on 'brain-type' score $\eta_p^2 = .11$.

The three subgroups differed significantly on a global measure of social anxiety (Total LSAS $F(2,44) = 4.04$, $p = .025$, $\eta_p^2 = .16$). Post-hoc contrasts revealed significantly less anxiety in the AgCC$^+$ group than the ASD group, $F(1,44) = 6.75$, $p = .013$, $\eta_p^2 = .13$, with AgCC$^-$ scoring similarly to both other groups (AgCC$^+$ $\eta_p^2 = .02$; ASD $\eta_p^2 = .11$). Despite these group differences in raw score, likelihood of endorsing clinically significant symptoms of social anxiety disorder did not differ across groups, $p = .11$ (Table 4).

A 3-group by 4-LSAS-subscale ANCOVA confirmed a significant difference across groups as was found on Total LSAS score $F(2,44) = 4.04$, $p = .025$, $\eta^2 = .16$. ANCOVA also revealed a significant main effect of subscale, $F(2.58, 113.39) = 4.11$, $p = .012$, $\eta^2 = .10$, but not a group by subscale interaction, $\eta^2 = .08$. Post-hoc contrasts found no significant group differences across

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LSAS subscale scores (AgCC$^+$ and AgCC$^-$ η^2 = .02; AgCC$^+$ and ASD, η^2 = .13; AgCC$^-$ and ASD, η^2 = .11).

Group differences on the LSAS ANCOVA were further explored by applying ANCOVA separately to each LSAS subscale while controlling for multiple comparisons (α = .0125). The three groups varied significantly on performance avoidance, $F(2,44)$ = 4.98. p = .011, η^2 = .19, but not on the other scales (social avoidance, p = .02, η^2 = .16; performance fear, p = .04, η^2 = .13; social fear, p = .10, η^2 = .10). However, since group differences would be significant for social avoidance and performance fear if Bonferroni correction was not used, we examined post-hoc contrasts for these measures in addition to performance avoidance.

Post hoc analyses of LSAS performance avoidance, performance fear, and social avoidance found no differences between the AgCC$^-$ and AgCC$^+$ groups (η_p^2 range from .003 - .08). In contrast, the AgCC$^+$ group endorsed significantly less performance-related anxiety than the ASD group (performance fear, $F(1,44)$ = 6.73, p = .013, η_p^2 = .13, performance avoidance, $F(1,44)$ = 8.31, p = .006, η_p^2 = .16), and the AgCC$^-$ group endorsed significantly less avoidance across situations than the ASD group (social avoidance, $F(1,44)$ = 7.07, p = .011, η_p^2 = .14, performance avoidance, $F(1,44)$ = 6.50, p = .014, η_p^2 = .13).

Exploratory correlations across all measures are reported in Supplement 3 and Supplementary Figure 1.

Discussion

We found that approximately a third of adults with AgCC exhibited current behaviors consistent an autism spectrum disorder, whereas considerably fewer met full formal autism diagnostic criteria once parent report of childhood behaviors was included. This indicates that congenital disruption of the corpus callosum constitutes a specific and major risk factor for developing autism symptomatology in adulthood, and furthermore that the developmental course in such individuals may differ from what is typically observed in autism spectrum disorders. The findings provide a highly quantitative and detailed assessment of autism in AgCC, additional description of social cognition more broadly, and motivate important hypotheses for future studies.

Autism Incidence in AgCC

Identifying the subset of AgCC for whom autism develops in a manner typical of ASD, and who meet full formal criteria for an autism diagnosis, was the first aim of this study. Only three out of 22 individuals in our AgCC sample fit into this group. Additionally, we discovered that this group exhibited more significant communication impairments in adulthood than the group of people with AgCC whose onset of autism symptoms presented only later in life. These two subgroups of AgCC did not differ on any demographic variables, other autism diagnostic instruments, or other social skill measures, and all participants in these groups had complete AgCC. We conclude that approximately a third of adults with AgCC and full scale IQ greater than or equal to 70 exhibit a typical autism profile in adulthood, even though only very few meet full diagnostic criteria once developmental history is included. Furthermore, our findings suggest that a pervasive communication impairment may distinguish these two varieties of autism presentation in AgCC.

Autism Symptomatology Subgroups in AgCC

Our second aim was to characterize autism symptoms and other social impairments in adults with AgCC. In addition to the group described in aim one (i.e. people with AgCC who exhibit the full autism syndrome including childhood onset), five additional participants with AgCC exhibited clinically significant symptoms of autism during direct observation (ADOS) totaling 8 out of 26 of our AgCC sample. This incidence is consistent with previous autism screening in adolescents and adults with AgCC (Lau et al., 2013). Unlike autism progression described above for the 3 individuals meeting full ASD criteria, for the majority of individuals with AgCC, emergence of autism symptoms may occur in differing sequence and at different ages. For example, whereas autism features social and communication abnormalities by around age 3, in people with AgCC, social and other behavioral disabilities are more likely to reach a clinically relevant threshold between ages six and 11 (Badaruddin et al., 2007). Consistent with this developmental pattern, at least three participants with AgCC in our study displayed delayed onset of social autism symptoms (point of onset could not be determined for two additional individuals with AgCC and autism symptoms in adulthood).

Importantly, the individuals with AgCC and autism symptomatology on ADOS all had complete callosal agenesis; however they were not distinguished from the remaining AgCC subjects by presence or absence of residual callosum, nor by age or IQ. This pattern of findings is consistent with the idea that specific developmental trajectories can give rise to autistic symptoms, independently of the effects of IQ. It also indicates that congenital disruption of the corpus callosum constitutes a specific and major risk factor for developing autism, along with

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other impairments in the social-communication domain. Among the remaining two-thirds of our AgCC sample, approximately half met autism criteria on either social or communication scales of the ADOS (seven out of 18) or ASD diagnosis on the ADI-R (two out of 18), even though they did not reach full criteria for a diagnosis on the ADOS.

This brings us to one additional set of questions: What characteristics, if any, distinguish between individuals with AgCC who develop autistic behaviors and those who will not? Likewise, what characteristics, if any, distinguish between the individuals with significant autism symptoms on ADOS who also have AgCC and those who do not have AgCC? The pattern of similarities and differences across these three groups may provide insights about the relationship between callosal disconnection and autism.

ADOS scores were used to define the three groups and therefore clearly distinguish between them. Likewise, because the ASD group was defined in part by parent-reports of autism symptoms during childhood, that group's SCQ and ADI-R scores were significantly higher than the AgCC$^-$ group's scores (with the exception of ADI-R restricted behaviors which did not differ across groups). However for the AgCC$^+$ group, all diagnostic parent-report scores were similar to the AgCC$^-$ group, and they were significantly less likely than the ASD to meet diagnostic criteria on the ADI-R. Taken together, this pattern supports our description of delayed onset of autistic social symptoms in AgCC and early onset of restrictive behaviors, suggesting that limitations of interhemispheric connectivity may contribute specifically to disrupted development of more complex social skills beginning in later childhood, as well as

possibly facilitating the generation of restricted and repetitive behavior patterns characteristic of autism.

Social Cognition across Groups

In addition to autism diagnostic criteria described above, the three groups exhibited very similar impairments on a broader array of social processing skills (Figure 2B). Discrepancies between self- and other-report scores on the SRS-2 indicated impoverished self-awareness in all three groups, but this limitation was more pronounced in AgCC+ than in ASD (with AgCC- showing an intermediate pattern). Apart from apparently worse self-awareness in AgCC$^+$ than AgCC$^-$, the two AgCC groups did not differ from one another on any other social measures. Compared with the ASD group, both AgCC groups scored significantly higher on the Empathizing Quotient, suggesting that emotional responsiveness and theory of mind may be somewhat more fully developed in AgCC than in ASD (albeit still below normal range). Previous studies have reported a similar pattern of mild theory of mind deficits in AgCC (Symington et al., 2010), as well as limited but intact psychophysiological arousal in response to emotional images (Paul et al., 2006), but a recent case study of AgCC with autism reported lower empathizing performance than in autism without AgCC (Lombardo et al., 2012). Finally, although social anxiety scores were generally elevated in all groups, subtle variations offer additional insights. Relative to ASD, the AgCC$^+$ group endorsed lower levels of social anxiety, primarily due to lower performance anxiety. In contrast, the AgCC$^-$ group endorsed less avoidance than ASD, but equivalent amounts of social and performance-related fear. As reported in Supplement 3, exploratory analyses revealed distinct patterns of correlation between ADOS and LSAS scores across groups. Although these correlations did not reach significance, they suggest that social

avoidance and social fear can be decoupled in relation to autism symptomatology, a provocative suggestion which merits further exploration. In sum, ADOS scores provided the only clear and consistent differences between the individuals who displayed autism symptoms (AgCC$^+$ and ASD) and those who did not (AgCC$^-$). All three groups shared similar degrees of impairment on a range of social measures, with subtle variations indicating that relative to ASD, the social dysfunction in AgCC involves less reduction in empathizing and lower levels of social anxiety, regardless of whether or not the AgCC is comorbid with ASD.

Deficits across a range of tasks that tap social cognition in the current study (Figure 2B), as well as deficits evident in prior work (see also Introduction), confirm a profound dysfunction in the social domain in AgCC. Although functional connectivity (Tyszka et al., 2011) and interhemispheric transfer studies (Paul et al., 2007) illustrate the remarkable compensatory development of the acallosal brain, our assessment of adults confirms that social impairments in AgCC remain even after the majority of cortical development is complete. In fact, as we noted, some social impairments appear to emerge later in the course of development. Diversity in the pattern of findings is in line with the known heterogeneity of autism, and points to the need for a more detailed assessment of developmental courses in AgCC involving not only larger samples but also longitudinal studies in infants and children.

Caveats and Future Directions

It is important to emphasize that our study, while in many respects descriptive, is not circular. For instance, one might reason that since we pre-selected an AgCC subgroup who met autism criteria (i.e., the AgCC$^+$ subgroup), of course these would show social impairments normally

found in autism. But the measures used to diagnose autism, and the experimental tasks, were independent. Once autism had been diagnosed, we used a different, and quite wide, range of assessments leaving open—and indeed finding—some patterns also found in autism in general, and some not. It is of course true that, by selection, the AgCC$^+$ group would have higher overall scores on the ADOS than would the AgCC$^-$ group—but this says little about the detailed pattern on subscales, and nothing about their similarities or differences on the Empathizing Quotient, Systemizing Quotient-Revised, SRS-2, Benton Faces, or the other tasks which were not used for diagnostic purposes.

Perhaps the strongest conclusion from our study regarding the neurological basis for autism is that developmental absence of the corpus callosum is associated with a higher risk of developing autism, a hypothesis consistent with the current view that disruption of long-range brain connectivity underlies autism (Geschwind and Levitt, 2007). In terms of more microstructural causes, this hypothesis fits with what is known about the array of genetic mutations and copy number variations identified to contribute to autism: many involve genes coding for pathfinding, signaling, or structural proteins known to be involved in synaptogenesis, neuronal migration, and synapse maintenance (Geschwind and Levitt, 2007, Parikshak et al., 2013). At the systems level, this hypothesis fits with deficits in integration of information processing that is spatially distributed (Frith, 1989). In the case of AgCC, this would specifically involve functions that are hemispherically lateralized to either the left (e.g., language) or right (e.g., emotion, visuospatial processing) cerebral hemisphere. In a developmental context, it is likely that normal interaction and competition between hemispheres

would be abolished in AgCC, conceivably resulting in more distributed cognitive functions in the adult brain.

It is important to note that the causal relationship between callosal agenesis and autism remains unclear. For instance, the two syndromes could result from mutations in shared set of genetic and environmental distal causes. It is also possible that absence of the corpus callosum, by itself, contributes causally to the development of autism, conferring simply an additive risk. Some evidence to begin to distinguish these possibilities is now emerging: a recent study found overlap in the de novo mutations that contribute AgCC, and those linked to autism (Sajan et al., 2013). Further studies that combine genetic analyses with ASD assessment in people with AgCC will be required to gain insight into this complex issue.

There are several important future directions from our results. First, as we noted above it will be important to begin to link some of the genes known to contribute to AgCC also to aspects of autism through larger family studies. Several such projects are now beginning; while obviously limited by small sample sizes, accrual over time is likely to yield important confirmations, as well as discoveries, of genes that contribute to autism. A second important direction lies in further validating and describing neurological models of autism. While inbred mice with callosal agenesis (the BTBR strain) have been studied as mouse models of autism for some time (Silverman et al., 2010), the present findings may provide a human neurological model that could inform, and be used better to translate between, both the mouse model and humans with autism. Finally, a particularly exciting future direction leverages off the fact that AgCC, like autism, is a developmental disorder but that it, unlike autism, can now be diagnosed before

birth. High-resolution ultrasound has made possible the routine discovery of AgCC in utero (Santo et al., 2012). Not only does this suggest the possibility of identifying a much larger sample of individuals with AgCC, but it also opens the door for beginning to study their brains and their behavior at birth (or even before), with the potential to give us valuable insights into the earliest aspects of social development and its dysfunction. The ideal comparison between ASD and AgCC would constitute a longitudinal study in such a population, mapping their developmental trajectory across multiple domains—and continuing across the lifespan.

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Table 1: Demographics and background neuropsychological measures

	AgCC (*n*=26)	ASD (*n*=28)
Age		
mean ± sd	28.77 ± 11.25	29.61 ± 11.11
range	16 - 54	19 - 56
FSIQ**		
mean ± sd	95.46 ± 14.15	109.54 ± 11.86
range	78 - 129	93 - 133
PIQ**		
mean ± sd	93.92 ± 15.45	107.43 ± 12.58
range	69 - 117	84 - 128
VIQ *		
mean ± sd	97.35 ± 15.66	110.21 ± 16.14
range	76 - 135	80 - 139
Gender	10F: 16M	5F: 23M
Handedness	4A: 7L: 15R	5A: 1L: 22R
Education	6 in high school 15 high school grad 2 bachelors degree 3 masters degree	1 in high school 16 high school grad 8 bachelors degree 3 masters degree
PANAS Positive		
mean ± sd	32.62 ± 9.14	31.74 ± 9.99
range	17 -50	10 - 50
PANAS Negative		
mean ± sd	13.88 ± 6.54	14.00 ± 4.57
range	10 - 38	10 - 31
STAI State		
mean ± sd	31.88 ± 10.06	37.38 ± 9.55
range	20 - 60	20 - 53
STAI Trait		
mean ± sd	37.96 ± 12.31	41.5 ± 10.30
range	21 - 67	23 - 60
BDI-II		
mean ± sd	7.85 ± 9.38	6.04 ± 5.99
range	0 - 36	0 – 21

* p < .005; ** p < .001. sd = Standard Deviation; FSIQ = Full Scale Intelligence Quotient; PIQ = Performance Intelligence Quotient; VIQ = Verbal Intelligence Quotient; PANAS = Positive and Negative Affect Scale; STAI = State Trait Anxiety Inventory; BDI – II = Beck Depression Inventory-II.

Table 2: Demographics by diagnostic category

	AgCC[+] (*n*=8)	AgCC[-] (*n*=18)	ASD (*n*=28)
AgCC Type	8 complete	13 complete 5 partial	NA
Age			
mean ± sd	28.38 ± 12.03	28.94 ± 11.25	29.61 ± 11.11
range	16 - 47	16 - 54	19 -56
FSIQ [a]			
mean ± sd	89.25 ± 13.08 +++	98.22 ± 14.07 +++	109.54 ± 11.86 + ++
range	79 - 113	78 - 129	93- 133
PIQ [b]			
mean ± sd	89.5 ± 15.66 +++	95.89 ± 15.38 +++	107.43 ± 12.58 + ++
range	69 - 117	73 - 117	84 - 128
VIQ [b]			
mean ± sd	90.75 ± 15.11 +++	100.28 ± 15.39	110.21 ± 16.14 +
range	76 - 115	78 - 135	80 - 139
Gender	2F: 6M	8F: 10M	5F: 23M
Handedness	1L: 7R ++	4A: 6L: 8R +	5A: 1L: 22R
Education	2 in high school 4 high school grad 1 bachelors degree 1 masters degree	4 in high school 11 high school grad 1 bachelors degree 2 masters degree	1 in high school 16 high school grad 8 bachelors degree 3 masters degree

Significant difference in 3-group ANCOVA at [a] p < .001, [b] p < .01. On Tukey's HSD at p < .05 corrected for multiple comparisons + differed from AgCC[+], ++ differed from AgCC[-], +++

differed from ASD. sd = Standard Deviation; FSIQ = Full Scale Intelligence Quotient; PIQ =

Performance Intelligence Quotient; VIQ = Verbal Intelligence Quotient.

Table 3: Autism diagnostic measures

	AgCC[+]	AgCC[-]	ASD
ADOS Communication [a d]	n = 8	n = 18	n = 28
mean ± sd	4.00 ± 1.6 ++	1.06 ± .87 *	4.14 ± 1.60 ++
range	2 - 6	0 - 3	2 - 7
>= autism	6	1	24
>= ASD & < autism	2	4	4
ADOS Social [a d]			
mean ± sd	6.13 ± 3.00 ++	1.72 ± 1.81 *	8.5 ± 3.38 ++
range	4 - 13	0 - 7	4 -17
>= autism	4	1	23
>= ASD & < autism	4	1	5
ADOS Total [a d]			
mean ± sd	10.13 ± 4.16 ++	2.78 ± 2.13 *	12.64 ± 4.62 ++
range	7 - 19	0 - 8	7 - 23
>= autism	3	0	18
>= ASD & < autism	5	1	10
ADOS Restricted [a]			
mean ± sd	1.38 ± 1.30 ++	.06 ± .24 *	1.64 ± 1.34 ++
range	0 - 3	0 - 1	0 - 4
SCQ [b e]	n = 6	n = 16	n = 17
mean ± sd	12.33 ± 6.12	10.31 ± 7.35 +++	21.00 ± 6.46 ++
range	5 - 19	1 - 25	7 - 31
>= ASD	3	5 +++	15 ++
ADI-R Social [c d]	n = 5	n = 9	n = 17
mean ± sd	10.60 ± 9.74	11.22 ± 8.66 +++	21.12 ± 5.12 ++
range	2 - 26	2 - 24	12 - 29
>= ASD	2 +++	4 +++	17 *
ADI-R Communication [a d]			
mean ± sd	10.40 ± 5.64 +++	9.44 ± 4.69 +++	16.24 ± 3.40 *
range	4 - 19	4 - 18	11 - 22
>= ASD	3	4 +++	17 ++
ADI-R Restricted			
mean ± sd	2.60 ± 0.89	3.22 ± 2.39	5.47 ± 2.24
range	2 - 4	0 - 7	2 - 10
>= ASD	2	5	15

Table 4: Mean scores on psychological measures by diagnostic classification

	AgCC[+]	AgCC[-]	ASD
SRS-2 Adult (Relative/Other)	n = 7	n = 17	n = 18
mean ± sd	78.14 ± 16.41	78.65 ± 37.68	95.78 ± 31.47
clinically elevated	1 mild 3 moderate	4 mild 2 moderate 5 severe	3 mild 6 moderate 5 severe
SRS-2 Adult (Self Report)	n = 8	n = 17	n = 24
mean ± sd	45.63 ± 22.87	61.59 ± 28.40	82.04 ± 27.56
clinically elevated [d]	1 moderate	4 mild 1 moderate 1 severe	4 mild 8 moderate 3 severe
Benton Facial Recognition	n = 6	n = 16	n = 26
mean ± sd	41.67 ± 7.42	44.88 ± 4.98	45.85 ± 4.10
clinically impaired	3	4	3
RMiE	n = 5	n = 13	n = 22
mean ± sd	22.00 ± 4.47	24.85 ± 3.34	24.64 ± 4.50
below normal limits	2	0	2
EQ [a]	n = 8	n = 17	n = 26
mean ± sd	39.63 ± 10.01 +++	36.59 ± 13.26 +++	26.62 ± 9.55 *
outside normal limits	1 below	2 below, 2 above	9 below
SQ-R	n = 8	n = 17	n = 26
mean ± sd	57.88 ± 30.78	58.00 ± 14.34	66.69 ± 23.55
outside normal limits	1 below, 1 above	1 below	2 below, 6 above
LSAS	n = 8	n = 18	n = 22
LSAS Total [a]	32.38 ± 20.25 +++	42.00 ± 25.14	59.82 ± 30.18 +
LSAS Performance Fear	6.50 ± 3.85 +++	11.78 ± 6.57	13.64 ± 8.35 +

LSAS Performance Avoidance [a]	8.50 ± 5.81 +++	10.44 ± 7.63 +++	14.41 ± 7.63 *
LSAS Social Fear	8.38 ± 5.45	10.11 ± 7.35	15.73 ± 8.39
LSAS Social Avoidance	9.00 ± 9.38	9.67 ± 6.07 +++	16.05 ±7.98 ++
clinically elevated	4	13	19

[a] $p < .05$ in 3-group ANCOVA. [d] $p < .05$ on 3-group x 2-level Fischer's exact test (comparing impaired with non-impaired). Post-hoc contrasts controlling for full scale IQ and multiple comparisons at $p < .05$ * differed from all other subgroups, + differed from AgCC[+], ++ differed from AgCC[-], and +++ differed from ASD. SRS-2 = Social Responsiveness Scale – 2; sd = Standard Deviation; RMiE = Reading the Mind in the Eyes; EQ = Empathizing Quotient; SQ-R = Systemizing Quotient; LSAS = Liebowitz Social Anxiety Scale; 'Normal limits' is defined as within 1.5 standard deviations of the mean.

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Figure Captions

Figure 1: Structural MRI of participants with agenesis of the corpus callosum. Here we divided the entire AgCC group, based on neurological criteria, into those with complete agenesis and those with partial agenesis, and for each constructed a midspace template by iterative nonlinear registration to an initial MNI-space target template of all T1-weighted structural images from that group (Tyszka et al., 2011): (a) midspace template for complete AgCC (N=21), (b) representative anatomy in a participant with complete AgCC, (c) midspace template for partial AgCC (N=5), and (d) representative anatomy in a participant with partial AgCC. Diffusion studies indicate that structural connectivity of the remnant corpus callosum in partial AgCC can be highly variable (Wahl et al., 2009). Therefore, the location of residual callosum cannot be assumed to represent the same connectivity as that region within an intact corpus callosum.

Figure 2: Effect sizes (partial eta squared) from between-group comparisons on (a) diagnostic measures and (b) measures of related social skills. Non-significant findings are shown in dark blue. In (a) measures on the y-axis are listed in order of greatest to smallest effect size in comparisons of AgCC⁻ and ASD. *** $p < .001$, ** $p < .01$, * $p < .05$ in 3-subgroup univariate ANCOVA.

Figure 3: ADOS algorithm scores. Individual participants' data are shown on the (a) communication, (b) social, (c) total (communication + social) and (d) restricted behaviors scales. Higher scores indicate greater symptomatology, with red dotted-line denoting the

cutoff for autism diagnosis criteria and blue line the cutoff for autism spectrum criteria. Circles represent individual participants, with subgroup indicated by color ($AgCC^+$ = blue, $AgCC^-$ = red, ASD-autism = green, ASD-PDD = aqua) and x-axis location (jittered to show individual circles more clearly).

Figure 4: Parent ratings on autism diagnostic scales. Individual scores for the parent rating scales including (a) SCQ raw score (b) ADI-R communication, (c) ADI-R social, and (d) ADI-R restricted interests and restricted behaviors algorithms. Higher scores indicate greater symptomatology. On ADI-R scales, the red dotted line indicates lower-limit of autism spectrum criteria.

Supplementary Figure 1: Correlations between measures. Pearson r correlations were calculated between all measures and Fisher z-transformed within the (a) ASD, (b) $AgCC^+$ and (c) $AgCC^-$ groups. Z-values of differences between matrices were calculated using Fisher's formula for comparing independent groups (Fisher, 1921). Figures d – f display comparisons between (d) $AgCC^-$ and ASD groups, (e) $AgCC^+$ and ASD groups and (f) $AgCC^-$ and $AgCC^+$ groups. For all figures, X's mark the correlations that remained significant following FDR correction ($q = .05$). To facilitate visual comparisons and capture all data, figures for individual groups (a-c) are scaled from -2 to 2, while the difference matrices (d-f) are scaled from -6 to 6.