AWARD NUMBER: W81XWH-14-1-0318

TITLE: Genetic and Diagnostic Biomarker Development in ASD Toddlers Using Resting-State Functional MRI

PRINCIPAL INVESTIGATOR: David C. Glahn

CONTRACTING ORGANIZATION: Yale University
New Haven, CT 06520-8047

REPORT DATE: September 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
September 2016	Annual	1 Sep 2015 - 31 Aug 2016

4. TITLE AND SUBTITLE

Genetic and Diagnostic Biomarker Development in ASD Toddlers Using Resting-State Functional MRI

5a. CONTRACT NUMBER
W81XWH-14-1-0318

5b. GRANT NUMBER
W81XWH-14-1-0318

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Glahn, David C.

email: david.glahn@yale.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Yale University
New Haven, CT 06511-6614

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our overarching strategy is to exploit three large neuroimaging/neurobehavioral datasets to identify brain-imaging based biomarkers for Autism Spectrum Disorders (ASD). At Yale, we focus on determining if brain networks are influenced by genetic factors and if these genetic factors also influence other traits associated with ASD. Specifically, we will provide heritability estimates and test for pleitropy between putative ASD functional and structural networks and cognitive and behavioral traits. To demonstrate the feasibility of this analytic approach, we conducted an initial analysis with over 250 seed regions simultaneously to provide measures of brain connectivity within 14 previously derived functional networks. Next, we estimated heritability for these structural and functional networks, examined the co-heritability between different modalities (e.g. function and structure) and searched the genome for chromosomal loci influencing these networks. We demonstrated that many of these intrinsic brain networks are heritable, that different genetic factors influence brain function and structure and localized a number of chromosomal regions that harbor genes influencing brain connectivity. These findings, which were presented at the annual meeting of the Organization for Human Brain Mapping, clearly demonstrate our ability to conduct similar analyses with ASD specific networks, one these are identified in the BrainMap and ACE datasets.

15. SUBJECT TERMS

Autism spectrum disorder (ASD); biomarker; early brain development; intrinsic functional brain networks; functional MRI (fMRI); clinical outcome; genomic; heritability; genetic control; pleitropy

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	11	19b. TELEPHONE NUMBER *(Include area code)*

TABLE OF CONTENTS

1. INTRODUCTION

Our strategy is to exploit three large neuroimaging/neurobehavioral datasets in order to identify brain-imaging based biomarkers for Autism Spectrum Disorders (ASD), including 1) BrainMap, developed and maintained by Peter Fox at the University of Texas Health Science Center at San Antonio (UTHSCSA); 2) the Autism Center of Excellence (ACE) neuroimaging archive, developed and maintained by Eric Courchesne at the University of California at San Diego (UCSD); and 3) the Genetics of Brain Structure (GOBS) neuroimaging genetics archive, developed and maintained by David Glahn at Yale University. To develop ASD biomarkers, we aim to (1) develop multi-regional functional-connectivity models of networks implicated in ASD by iterative and hierarchical meta-analyses of the BrainMap database; (2) test the ability of the neural-system functional-connectivity models to differentiate between ASD and TYP children in a cohort previously acquired ACE cohort; and assess the heritability and pleitropy of the these functional networks, in a previously imaged and previously genotyped cohort of families with extended pedigrees. At Yale, we focus on the final aim, estimating heritability of putative ASD networks and testing for pleitropy between these networks and cognitive and behavioral measures.

Given delays associated with generating whole genome sequence data on the GOBS cohort, we requested and received a 1 year no cost extension. Thus, a number of key analyses are still ongoing.

2. KEYWORDS

Autism spectrum disorder (ASD); biomarker; early brain development; intrinsic functional brain networks; functional MRI (fMRI); clinical outcome; genomic; heritability; genetic control; pleitropy

3. ACCOMPLISHMENTS

a. What were the major goals of the Yale site?

At the Yale site, we focus estimating heritability of putative ASD functional and structural networks, testing for pleitropy between these networks and cognitive and behavioral measures and training post-doctoral associates and others to conduct the needed analyses. Below we outline the major tasks identified in the original Scope of Work (SOW) relevant for this reporting period.

Administrative Tasks (Prior to Aims)

Major Task 1 *was to submit and obtain ethics approval from our local ethical review board*. We accomplished this goal (Milestone #1) ahead of schedule: Yale's Human Research Protection Program board approved the project on April 15, 2014 (HIC 1403013622).

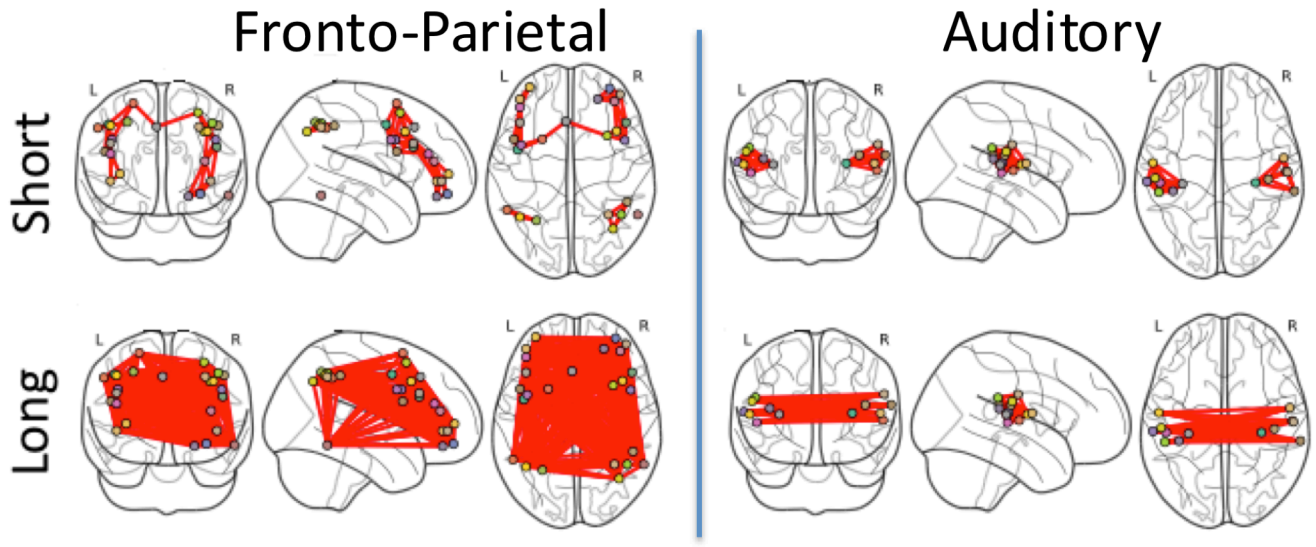
Major Task 2 *was to advertise, interview, hire and train staff dedicated for the project*. After several rounds of interviews and advertising in national and international scientific meetings, we offered a post-doctoral fellowship to Dr. Karen Hodgson (see section 7. **Participants**, below). Dr. Hodgson accepted the offer and joined our group in February of 2015. She is currently classified as a Postdoctoral Associate at Yale University, Department of Psychiatry, who is 100% dedicated to this project. Dr. Hodgson has under gone an extensive training program and has mastered the methods necessary for the genetic analyses to be conducted for this project (Milestone #2).

Tasks In support of Specific Aim 3 (Yale University Site)

Major Task 1 *involved the pre-processing of structural and functional data for subjects from the GOBS cohort.* Pre-processing involved a number of quality control and analytic steps. Quality control, preprocessing and neuroanatomic parcellation were performed on

~1500 scans from individuals in randomly ascertained extended pedigrees by April/May of 2015 (Subtask 1). In total, 1004 images were found to be of adequate quality and were reliably parcellated using FreeSurfer 5.1. Similarly, quality control, preprocessing and functional parcellation was conducted on ~900 scans from individuals in randomly ascertained extended pedigrees by May of 2015 (Subtask 2). In total, 783 images were found to be of adequate quality and were reliably parcellated into functional networks using ICA tools. Thus, Milestone #1 was accomplished for the GOBS cohort by May of 2015.

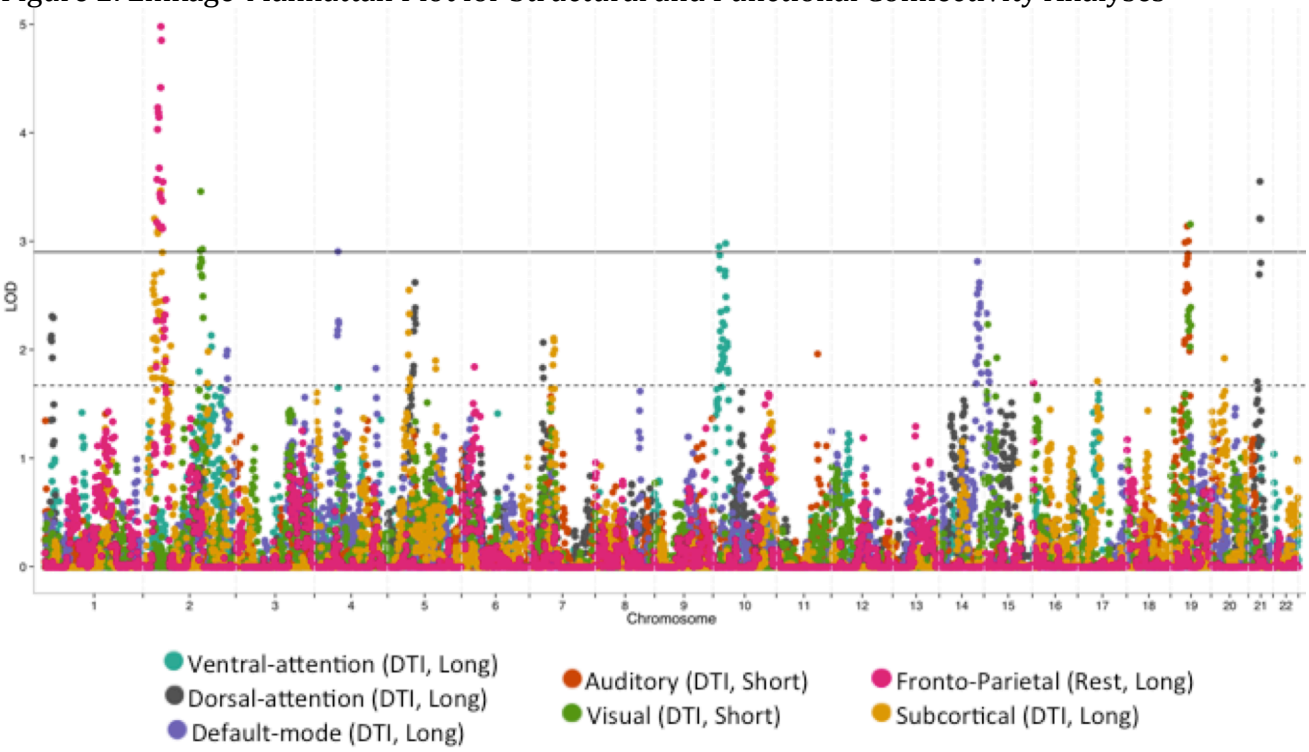
Figure 1. Example Functional Connectivity Networks based upon methods by Power et al (2013)



Major Task 2 *involved conducting intrinsic connectivity analyses from functional networks derived from the BrainMap and ACE datasets.* Two "agnostic" intrinsic connectivity analyses were conducted by August of 2015. The first, utilized an extension of the methods initially published by Power and colleagues (Power JD, Schlaggar BL, Lessov-Schlaggar CN, Petersen SE. Evidence for hubs in human functional brain networks. Neuron. 2013 Aug 21;79(4):798-813. PMID: 23972601). This approach uses over 250 seed regions simultaneously to provide regional and network-level measures of brain connectivity. Using this analytic approach, we estimated heritability for a set of structural and functional networks, examined the co-heritability between these different modalities and searched the genome for chromosomal loci influencing these networks. In **Figure 1**, we provide examples of two of the 14 derived networks, based on the network configuration determined in the Power et al., work. For our current experiment, we defined network connections as either short or long (greater or less than 40mm). In **Figure 2**, we indicate the number and chromosomal locations of loci that influenced network-based connectivity measures found to be significantly heritable. Findings from these analyses were presented at the 21st annual meeting of the Organization for Human Brain Mapping in Honolulu, HI, entitled "Shared and Unique Genetic Influences on Structural and Functional Connectivity."

The second analytic approach involved the application of surface based analytic techniques developed by the Human Connectome Project (http://www.humanconnectome.org), a NIH roadmap initiative designed to map normal variation in brain connectivity. This computationally demanding analytic strategy derives dense connectivity maps for each subject based upon a surfaced based parelation and then combined these connectivity maps using a combined function-structure alignment strategy. This method provides similar heritability estimates as those derived using the Power et al method.

Figure 2. Linkage-Manhattan Plot for Structural and Functional Connectivity Analyses



In addition to the work with intrinsic connectivity traits described above, we have conducted a number of analyses relevant to brain-behavior changes in autism. For example, we recently examined the relationship between cortical gyrification and intelligence in our large, genetically informative population. Gyrification is the process of forming the characteristic folds of the human cerebral cortex and there are several articles indicating aberrant gyrification patterns in children and adults with autism (e.g. Wallace et al., *Brain*, 2013 PMID= 23715094; Hardan et al., *Psychiatry Res*, 2004 PMID= 15465295; Jou et al., J Child Neurol, 2010 PMID= 20413799). Furthermore, it appears that decreased gyrification in prefrontal cortex is associated with deficit functional connectivity in children with autism (Schaer et al., *Front Hum Neurosci*, 2013; PMID = 24265612). Finally, there is some evidence that gyrification patterns, particularly as they associate with IQ, may be an autism endophenotype, a trait sensitive to genetic liability for the illness (Kates et al., *Autism Res*, 2009 PMID= 19890876). Thus, examining the genetic influences the relationship between cortical gyrification and intelligence appears to be an interesting new way to dissect genetic liability for autism.

To date, our analyses have focused on replicating prior findings of the relationship between local gyrification patterns and intelligence (Gregory et al., Cur Biol, 2016 PMID=27133866). In two large cohorts, Gregory and colleagues found that general cognitive ability was significantly associated with increasing gyrification in a network of neocortical regions, including large portions of the prefrontal cortex, inferior parietal lobule, and temporoparietal junction, as well as the insula, cingulate cortex,

Figure 3. Association between Local Gyrification and General Cognitive Ability (n~1000)

and fusiform gyrus. This pattern of results is consistent with the Parieto-Frontal Integration Theory of intelligence (Jung and Haier, *Behave Brain Sci*, 2007 PMID= 17655784) and with brain regions implicated in autism. Using data from 1004 individuals from the "Genetics of Brain Structure and Function" study, we generally replicate these findings. Further, we demonstrate that common genetic factors appear to influence local gyrification and an general cognitive ability index. Next, we plan to determine the common genetic influences on gyrification/intelligence and risk for autism using a rare-variant based enrichment score developed in our laboratory. The goal of this analysis is to determine if genes (variants) associated with autism risk also influence the relationship between cortical gyrification and intelligence. If such a relationship can be established, then it is possible to enumerate some of the biological pathways through which risk genes give rise to autism risk. Such information is invaluable for the development of treatment or remediation strategies.

These analyses fulfill Milestone #2.

Once we receive the functional networks derived from BrainMap (Specific Aim 1) and the ACE cohort (Specific Aim 2), we will conduct similar analyses with these data.

b. What was accomplished under these goals at the Yale University site?

All of the work described above was conducted at the Yale site. In addition, a conceptually similar analyses was conducted using neuroanatomic networks disrupted in schizophrenia. Specifically, we used source-based morphometry, a multivariate technique optimized for structural MRI, in a large sample of randomly ascertained pedigrees (N = 887) to derive an insula-medial prefrontal cortex (mPFC) component and to investigate its genetic determinants. First, we replicated the insula-mPFC grey matter component as an independent source of grey matter variation in the general population, and verified its relevance to schizophrenia in an independent case-control

sample. Secondly, we showed that the neuroanatomical variation defined by this component is largely determined by additive genetic variation (h^2 = 0.59), and genome-wide linkage analysis resulted in a significant linkage peak at 12q24 (*LOD* = 3.76). This region has been of significant interest to psychiatric genetics as it contains the Darier's disease locus and other proposed susceptibility genes (e.g. *DAO*, *NOS1*), and it has been linked to affective disorders and schizophrenia in multiple populations. Thus, in conjunction with previous clinical studies, our data imply that one or more psychiatric risk variants at 12q24 are co-inherited with reductions in mPFC and insula grey matter concentration. The results of these analyses were reported in a manuscript recently accepted for publication in a peer-reviewed journal. This article was published: Sprooten E, Gupta CN, Knowles EE, McKay DR, Mathias SR, Curran JE, Kent JW Jr, Carless MA, Almeida MA, Dyer TD, Göring HH, Olvera RL, Kochunov P, Fox PT, Duggirala R, Almasy L, Calhoun VD, Blangero J, Turner JA, Glahn DC. Genome-wide significant linkage of schizophrenia-related neuroanatomical trait to 12q24., Am J Med Genet B Neuropsychiatr Genet. 2015 Dec;168(8):678-86. PMID: 26440917. The success of this similar project speaks to the feasibility and potential for success of the ASD project.

c. What opportunities for training and professional development has the project provided at the Yale University site?

Although Dr. Karen Hodgson joined the team with considerable molecular genetics experience, she did not have formal training in quantitative or statistical genetics. Thus, in order for Dr. Hodgson to perform the analyses needed for the current project, she learned a new skill set involving the use of complex analytic methods in the service of furthering our understanding of human brain connectivity in general and how connectivity is disrupted in ASD.

d. How were the results disseminated to communities of interest?

We have presented preliminary analyses at the 2015 annual meeting for the Organization for Human Brain Mapping. Additional analyses will be present at the 2017 Society for Biological Psychiatry meeting.

e. What do you plan to do during the next reporting period to accomplish the goals?

In the coming months we anticipate receiving the final set of the initial intrinsic network results in the Autism Spectrum Disorder sample from Dr. Courchesne. We additionally expect to receive analysis from Dr. Courchesne comparing and contrasting his initial ASD intrinsic network results from Dr. Fox's multi-stage MACM analyses. We intend to utilize these results in addition to the findings described above from the Yale site to use resting-state connectivity alterations and behavioral characterizations of ASD as quantitative traits, computing heritability and pleitropy estimates.

Further, we anticipate receiving whole genome sequence data for the complete GOBS sample in September of 2016. Once we receive the sequence data, a rather labor intensive quality control must be conducted. Prior experience suggests that this quality control process requires two and a half months (completed by mid-December 2016).

4. IMACT

a. What is the impact on understanding ASD brain development of the project?

As outlined above, we have a number of preliminary results that are directly relevant to the

proposed project, further demonstrate the plausibility of the proposed analyses and improve our understanding of the neurogenetics of human brain connectivity. However, as described above, the exact functional and structural connectivity models that typify ASD have yet to be created (e.g. Aims 1 & 2) and, thus, we have yet to estimate their genetic effects.

b. What was the impact of the project results on other disciplines, technology transfer, or society beyond science and technology?

Other Disciplines: *Neurogenetics*. Thus far, our project has estimated the genetic control over functional and structural connectivity measures, documented that independent genetic factors appear to influence these traits, and localized chromosomal loci influencing either functional, structural or both structural and functional connectivity. These results will be directly relevant for ASD when the exact networks (biomarkers) associated with ASD are identified.

Technology Transfer: Our initial findings were reported in an international scientific meeting in May 2015. The article documenting these findings is in development and will be submitted to a peer-review journal for publication in the fall.

Society: Nothing to Report

5. CHANGES/PROBLEMS

No scientific, design, or experiment problems have occurred and thus no significant changes to the project are proposed. As described above, we completed pre-processing of functional and structural connectivity measures using the originally proposed methods. However, newer surface-based methods developed by the Human Connectome Project have become available to the scientific community. Thus, we have implemented these methods as well and will conduct all analyses in parallel.

6. PRODUCTS

The products resulting from the project during the reporting period include the following conference paper:

- Glahn et al., "Shared and Unique Genetic Influences on Structural and Functional Connectivity," 21st annual meeting of the Organization for Human Brain Mapping, Honolulu, HI
- Sprooten E, Gupta CN, Knowles EE, McKay DR, Mathias SR, Curran JE, Kent JW Jr, Carless MA, Almeida MA, Dyer TD, Göring HH, Olvera RL, Kochunov P, Fox PT, Duggirala R, Almasy L, Calhoun VD, Blangero J, Turner JA, Glahn DC. Genome-wide significant linkage of schizophrenia-related neuroanatomical trait to 12q24., Am J Med Genet B Neuropsychiatr Genet. 2015 Dec;168(8):678-86. PMID: 26440917.

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

a. **What individuals have worked on the project?**

Work on this project has been limited to David C Glahn, PhD, the PD/PI, and Dr. Karen Hodgson, post-doctoral associate.

David C. Glahn, Ph.D. (0.6 calendar months), years 1-2. Partnering Principal Investigator is an expert in the application of neurocognitive and neuroimaging phenotypes in large-scale behavioral and molecular genetic studies of psychiatric illnesses. He is a Professor in the Department of Psychiatry, Yale University School of Medicine, and an Olin Neuropsychiatric Research Center Scholar where he directs the Imaging Genomics laboratory. As outlined in the Scope of Work, Dr. Glahn has ultimate responsibility for conducting neurocognitive, neuroimaging and behavioral genetic analyses in support of Specific Aim 3.

Karen Hodgson (7.2 calendar months, or 60% effort), years 1-2. Under the supervision of Dr. Glahn, this Dr. Hodgson has conduct neurocognitive, neuroimaging and behavioral genetic analyses in support of Specific Aim 3. In addition, she liaisons with other investigators involved in the project.

b. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report

c. **What other organizations were involved as partners?**

As per the original application, the other organizations involved as partners are the University of Texas Health Science Center San Antonio (Dr. Fox, the overall project P.I.) and the University of California San Diego site (Dr. Courchesne, P.I. at that site).

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARD: This project is part of a Collaborative Award and this Progress Report is from the Yale University site (Glahn). Comparable progress reports from Dr. Peter Fox at the University of Texas Health Science Center San Antonio and Dr. Eric Courchesne at University of California San Diego will be submitted separately.

9. APPENDICES

Attached is the published abstract for Glahn et al., "Shared and Unique Genetic Influences on Structural and Functional Connectivity," 21st annual meeting of the Organization for Human Brain Mapping, Honolulu, HI

Glahn et al., "Shared and Unique Genetic Influences on Structural and Functional Connectivity," 21st annual meeting of the Organization for Human Brain Mapping, Honolulu, HI

Abstract: The relationship between in vivo measures of structural connectivity, often indexed with diffusion-weighted imagining, and functional connectivity, typically measured with resting-state functional MRI, appears to be complex. While structural connections appear to facilitate some aspects of functional connectivity, functional relationship may include multiple structural pathways. However, most systems neuroscience models of brain connectivity suggest that anatomical and physiological processes are dependent, in part, upon common neurobiological mechanisms. While there is growing evidence that measures of functional and structural connectivity are influenced by genetic factors, little is known about potential pleiotropy (e.g. the same genes influencing both structural and functional connectivity). Using 1606 individuals from extended pedigrees with both resting-state and diffusion weighted scans, we (1) establish the heritability of structural and functional connectivity in previously defined brain networks, (2) use genetic correlations to show statistical evidence that common genes influence both types of measures, and (3) show that specific chromosomal loci influence both structural and functional connectivity.