Large-scale associations between the leukocyte transcriptome and BOLD responses to speech differ in autism early language outcome subtypes

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Heterogeneity in early language development in autism spectrum disorder (ASD) is clinically important and may reflect neurobiologically distinct subtypes. Here, we identified a large-scale association between multiple coordinated blood leukocyte gene coexpression modules and the multivariate functional neuroimaging (fMRI) response to speech. Gene coexpression modules associated with the multivariate fMRI response to speech were different for all pairwise comparisons between typically developing toddlers and toddlers with ASD and poor versus good early language outcome. Associated coexpression modules were enriched in genes that are broadly expressed in the brain and many other tissues. These coexpression modules were also enriched in ASD-associated, prenatal, human-specific, and language-relevant genes. This work highlights distinctive neurobiology in ASD subtypes with different early language outcomes that is present well before such outcomes are known. Associations between neuroimaging measures and gene expression levels in blood leukocytes may offer a unique in vivo window into identifying brain-relevant molecular mechanisms in ASD.

ASD is heterogeneous at multiple levels (e.g., genetics, cellular and neural systems, cognition, behavior, developmental trajectories, prognosis, and response to treatment)1–3. This multilevel heterogeneity presents a major challenge on the path toward stratified psychiatry and precision medicine4,5. One dimension of heterogeneity with clinical importance for ASD is early language development and outcome. There is a wide spectrum of variability in early language abilities in the population with ASD, from individuals who remain minimally verbal to those who have difficulties similar to specific language impairment, to those who develop near-typical levels of language function6,7. Early language ability is paramount for better understanding a range of clinical phenomena. For example, early language ability is one of the most important predictors of early intervention response and later-life outcomes8–12.

An additional challenge lies in studying the relationships between the macroscale properties of the brain and the molecular mechanisms at play in early development, and how this relationship may be altered in ASD13. fMRI can be used to gain insight into the macroscale, neural systems level of organization and its association with cognitive and behavioral functioning. However, the molecular biological underpinnings of this organization are not well understood. Although blood samples are a practical source for assaying atypical gene expression in early ASD development14,15, a common question is how relevant they are for understanding atypical neural processes in ASD. The evidence for a genetic basis of ASD is strong16,17, and genetic variation is likely to affect gene expression levels in multiple tissues18, including the brain and blood. Thus, identifying associations between the blood leukocyte transcriptome and neuroimaging phenotypes may help shed light on mechanisms affecting early neural systems development in toddlers with ASD compared with typically developing toddlers (TD). Such an in vivo window into the biology of ASD19 may be able to not only advance understanding of the mechanisms underlying atypical brain development in heterogeneous ASD patients but also advance translational work targeted at better monitoring treatment response, predicting prognosis, and evaluating clinical trials.

Here, we asked whether large-scale coordinated gene expression in blood leukocytes might be associated with neural responses to speech, as measured with fMRI, and whether this association might differ between TD and toddlers with ASD and either poor or good early language outcome. A fundamental question in this work was whether differences in early language outcomes might be a biologically relevant basis for stratifying ASD. On the basis of prior work13, suggesting that early language outcome subtypes are underpinned by distinct biology, we predicted that early language outcome ASD subtypes would show different profiles of associations between blood leukocyte gene expression and the functional neural systems response to speech.

Examining large-scale blood leukocyte transcriptome associations with neuroimaging phenotypes in ASD may also identify novel mechanisms involved in ASD. The omnigenic model20, much like other viewpoints on polygenic risk21, predicts that large numbers of genes are relevant to complex traits such as those in ASD. However, the omnigenic model suggests that these genes do not necessarily need to be specific to brain tissue. Genes that are broadly expressed in one or more tissues, including the brain and blood, are

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predicted to contain a large amount of the heritability signal and may contribute more to overall risk than the smaller number of tissue-specific genes implicated in a complex trait\textsuperscript{15}. Applying these ideas to the current study, we predicted that large-scale coordinated transcriptional activity in the blood leukocyte transcriptome might be relevant for explaining neural phenotypes relevant to ASD. The omnigenic model predicts that this large-scale transcriptomic signal would be enriched for genes that are broadly expressed in the brain and many other tissues.

Results
Group differentiation in the superior temporal cortex response to speech and clinical behavioral trajectories over the first 4 years of life. In this study, we compared TD and age-matched toddlers with ASD whose language abilities were assessed around 3–4 years of age. Toddlers with ASD were stratified by poor (ASD Poor) or good (ASD Good) language outcome. ASD Poor was defined by Mullen Expressive Language (EL) and Receptive Language (RL) T scores below 1 s.d. of typical developing age norms (T < 40). In contrast, ASD Good was defined by outcome Mullen EL or RL within 1 s.d. of typical age norms (T ≥ 40) (Methods). In prior work, we have shown that this stratification identifies an ASD subtype with different developmental trajectories and a decreased left-hemisphere superior temporal cortex response to speech, as measured with sleep fMRI, before the diagnosis and outcome are known\textsuperscript{19}. The current dataset included a subset of toddlers from the prior study (n = 41 ASD Poor, n = 24 ASD Good; n = 21 TD) and adds a similar number of new toddlers (n = 22 ASD Poor, n = 16 ASD Good, and n = 16 TD). Therefore, we first ran longitudinal clinical trajectory and fMRI analyses on this combined dataset.

As previously reported\textsuperscript{19}, all longitudinal clinical measures showed evidence of subtype–age interactions (except for Mullen Fine Motor and Autism Diagnostic Observation Schedule (ADOS) total), thus indicating that the groups differed in the slopes of the trajectories (Fig. 1; statistics in Supplementary Table 1). This difference was generally driven by the ASD Poor group, whose downward trajectories were indicative of falling further behind age-appropriate norms. All measures also showed main effects of group and were generally due to all groups differing from one another in a hierarchy of ASD Poor as most severe, ASD Good as intermediate, and TD as least severe. With the fMRI data, we also found that the previously reported\textsuperscript{19} hypoactivation in the left-hemisphere superior temporal cortex remained stable in this combined sample (Fig. 2 and Supplementary Table 2). Whole-brain between-group analyses did not reveal any regions differentiating the groups. However, the lack of effects in this context are likely to be due to low statistical power for whole-brain between-group comparisons\textsuperscript{21}.

Lack of group differentiation within gene expression data alone. The total sample of n = 41 ASD Poor, n = 40 ASD Good, and n = 37 TD individuals was the largest dataset of toddlers for whom both fMRI and gene expression data were available. We next assessed whether differences in blood leukocyte gene expression might reflect different neural systems organization between the two ASD language outcome subtypes. First, we assessed differential expression (DE) between subtypes at the level of individual genes. After correction for multiple comparison, no genes were identified as DE for any pairwise group comparison (Supplementary Table 2). Next, we used weighted gene coexpression network analysis (WGCNA) to decrease redundancy among the 14,313 genes, down to 21 discrete coexpression modules. The coexpression modules were summarized by the first principal component score, also known as the module eigengene (ME)\textsuperscript{21}. Similarly to the DE analysis at the gene level, there were no ME differences between the two ASD subtypes (Supplementary Table 3). Thus, examining blood leukocyte gene expression data in isolation did not significantly differentiate the groups at the current sample sizes. We next turned to examining associations between gene expression and functional neuroimaging phenotypes.

Large-scale blood leukocyte gene coexpression module association with fMRI response to speech. Multivariate analysis of the association between coexpression modules and whole-brain voxel-wise patterns of activation was implemented with partial least-squares (PLS) analyses. Of the 63 total latent variable (LV) coexpression–fMRI pairs, PLS identified only one LV pair with a statistically significant association after multiple-comparison correction (LV1: \(d^2 = 65.47, P = 1.99 \times 10^{-4}, \) false discovery rate (FDR) \(q = 0.0125; \) statistics for all PLS LV pairs in Supplementary Table 4). LV1 accounted for 20.13% of the covariance between gene expression and functional neuroimaging phenotypes.
expression and fMRI data and was spatially distributed across a number of cortical regions that have been found to be highly relevant to speech, language24,25 (e.g., the primary auditory cortex, superior temporal sulcus, inferior frontal gyrus, ventral premotor cortex, and insula), visual and sensorimotor areas (e.g., the primary visual cortex, superior parietal cortex, primary somatomotor cortex, and premotor cortex), cognitive control (dorsolateral prefrontal cortex), and 'social brain' circuitry overlapping with key areas of the default mode network (e.g., the posterior cingulate cortex, medial prefrontal cortex, right temporoparietal junction, and superior temporal sulcus) (Fig. 3a). Subcortical regions such as the striatum and thalamus were also implicated and are highly relevant for language processes such as vocal learning25,26. For example, Area X in songbirds is linked to vocal learning and is homologous to the human dorsal striatum26.

**Extent of nonzero associations across coexpression modules.** To better understand the most important coexpression modules for the PLS LV1 results, we first identified what we termed ‘nonzero’ association modules, which were defined as gene coexpression modules with gene coexpression–fMRI correlations with 95% confidence intervals (CIs) that excluded a correlation of 0. Nonzero modules accounted for approximately half of all modules analyzed (11/21; 52%) (Fig. 3b and Supplementary Table 4). The remaining modules (10/21; 48%) were referred to as ‘zero’ modules, defined as gene coexpression modules for which a correlation of 0 lay within the 95% CIs. Zero modules contributed little to and/or were unreliable in how they contributed to LV1. Nonzero modules covered most (61%) of the transcriptome considered for the WGCNA analysis. This widespread coverage indicated a coordinated and large-scale signal mapping large parts of the blood leukocyte transcriptome associated with macroscale functional neural response to speech, as measured with fMRI.

Most nonzero modules were characterized by a variety of biological processes generally falling within categories such as translation, transcription, cell-cycle, immunological, inflammatory, signal-transduction, and cytoskeleton processes. However, the enrichments differed substantially depending on the module (Fig. 3b; complete description of enrichments for each module in Supplementary Table 5). For instance, nonzero modules M2, M8, and M11 were primarily translation and transcription modules, whereas M1 and M10 were enriched in many of these terms but not translation and transcription. These biological processes have all been implicated in autism. For example, translation processes are affected in many syndromic forms of ASD (such as fragile X syndrome and tuberous sclerosis)27. Many high-confidence ASD-risk genes are known to affect transcription processes (e.g., CHD8)25,29. Cell-cycle processes are involved in aberrant early cell proliferation and increased early brain growth in ASD31,32. Immunological and inflammatory processes have been linked to ASD via various lines of evidence30–32. These results supports the idea that ASD-relevant biological processes can be assayed in blood leukocytes and are associated with early-developing large-scale functional neuroimaging phenotypes.

**Lack of overlap in nonzero modules across ASD subtypes and TD.** Most nonzero modules were present only in one group (9/11; 81%). In fact, only TD and ASD Poor showed evidence of nonzero modules. No nonzero modules were present in the ASD Good subtype. Two (18%) nonzero modules were present in both TD and ASD Poor and were correlated in the same direction. However, the extent of this overlap was not statistically significant (enrichment odds ratio (OR) = 1.67, \(P = 0.65\)) (Fig. 3b). This result suggests that different biological mechanisms within each group may underpin the variability observed in macroscale language-relevant fMRI phenotypes. To further test the importance of the ASD language outcome subtype distinction, we next tested whether a simple case–control distinction might enhance sensitivity in detecting gene coexpression–fMRI relationships. This case–control PLS analysis...
was not able to identify any statistically significant LV pairs at FDR \( q < 0.05 \) with correction for multiple comparisons (Supplementary Table 4). Thus, using early language outcomes as a stratifier in ASD appears to substantially enhance sensitivity for detecting gene coexpression–fMRI relationships.

Nonzero modules are enriched in broadly expressed genes. We next examined what class of genes was likely to strongly contribute to the nonzero modules. On the basis of ideas from the omnigenic model\(^{20}\), genes that are broadly expressed in many tissues including the brain, could also be expressed and measurable in blood leukocytes and therefore may be highly relevant for these nonzero modules associated with a functional neuroimaging phenotype. Remarkably, we found that 81% (9/11) of nonzero modules were enriched in broadly expressed genes (OR = 184.5, \( P = 1.87 \times 10^{-4} \)). All modules enriched in broadly expressed genes were also nonzero modules (Fig. 4b). In contrast, tissue-specific gene lists (for example, brain, whole-blood, and lymphocyte lists) were not heavily enriched in many modules nor were they overrepresented in nonzero modules (brain-specific modules: OR = 0, \( P = 1 \); whole-blood-specific modules: OR = 0.6, \( P = 0.96 \); lymphocyte-specific modules: OR = 4.44, \( P = 0.53 \) (Fig. 4b)). In addition to running these enrichments at the level of overlap among modules, we also ran tests for overlap at the gene level. Approximately 44% of all broadly expressed genes were present in nonzero modules, amounting to a highly significant enrichment (OR = 3.58, \( P = 1.48 \times 10^{-3} \)). Whole-blood and lymphocyte-specific genes also showed evidence of enrichment in nonzero modules (blood OR = 4.79, \( P = 1.57 \times 10^{-18} \);
Nonzero modules are enriched in highly active prenatal coexpression modules associated with ASD. Several lines of evidence suggest that ASD pathophysiology can manifest during prenatal brain development. We therefore examined whether nonzero modules might be enriched in genes that were members of coexpression modules that showed high levels of prenatal expression and that possessed a number of highly penetrant ASD-associated genes. Using lists from two independent studies of the BrainSpan atlas examining either cortical-only or cortical and subcortical regions, we found that approximately 32% of the genes in prenatal and ASD-associated coexpression modules also appeared in nonzero modules (OR > 1.7, P < 0.0056) (Fig. 5a), whereas only 15–17% were present in zero modules (OR < 1.19, P > 0.74). Nonzero modules M13 and M10 drove the enrichment, because no other nonzero modules showed evidence of enrichment in genes in either ASD-associated prenatal-gene list (Fig. 5b). Overall, this evidence supports the idea that some of the genes present in nonzero modules may also be members of prenatally active and ASD-associated coexpression modules.

Nonzero modules are enriched in genes from ASD-downregulated coexpression modules from frontal and temporal cortical tissue. Although we established that nonzero modules overlapped with prenatally relevant coexpression modules bearing ASD-relevant genes, a caveat to this result is that those prenatal, ASD-associated coexpression modules were identified from the BrainSpan dataset, which for obvious reasons does not contain prenatal tissue from donors with ASD. Thus, to more directly connect nonzero modules with cortical gene expression in patients diagnosed with ASD, we used gene expression data from the postmortem frontal and temporal cortical tissue of patients with ASD. Nonzero modules were enriched in genes that were members of ASD-downregulated frontal and temporal cortex coexpression modules (OR = 1.70, P = 0.03). Enrichments at trend levels were also seen for genes from ASD-upregulated coexpression modules (OR = 1.64, P = 0.0502, FDR q = 0.0586) (Fig. 5a). However, no specific nonzero modules seemed to drive this enrichment (Fig. 5b). Although zero modules were not enriched in genes from ASD-downregulated modules (OR = 1.15, P = 0.75), zero modules were enriched in genes from ASD-upregulated modules (OR = 1.79, P = 2.80 × 10⁻⁷) (Fig. 5a). These results further suggest the ASD and brain relevance of genes identified via their nonzero association between expression in blood leukocytes and language-relevant functional neuroimaging phenotypes.

Nonzero modules with preservation of network structure between ASD blood and cortical tissue. Using the same gene expression dataset from postmortem cortical tissue from patients with ASD, we next examined whether the coexpression network structure of nonzero modules identified in blood might be preserved in ASD.
Frontal and temporal cortical tissue. This step is important because it highlights specific modules whose coexpression network connectivity patterns are similar between blood leukocytes and brain tissue. Whereas nonzero modules M8 and M11 showed moderate evidence of preservation ($2 < \text{Zsummary} < 6$), the nonzero M2 module had the highest ranking of all modules with evidence of high-to-moderate preservation ($\text{Zsummary} = 8.1$) (Supplementary Fig. 1). M2 is highly enriched in the term ‘translation in mitochondria’ (Supplementary Table 5), and many M2 hub genes (for example, MRPS12, NDUF3, NDUF8, HINT2, and MRPL14) encode proteins that localize to mitochondria (Supplementary Table 6). This evidence may be relevant in light of possible mitochondrial dysfunction in ASD. Other notable M2 hub genes were DGCR6 and BOLA2. Both are located within prominent ASD-associated copy number variant (CNV) regions of 22q11.21 (DGCR6) and 16p11.2 (BOLA2). Interestingly with regard to evolutionarily accelerated human-specific genes, BOLA2 is known for human-specific duplications and shows upregulated expression in human versus chimpanzee induced pluripotent stem cells. In patients with 16p11.2 CNVs, 96% of breakpoints include human-specific duplications of BOLA2. Deletions and duplications of 16p11.2 are linked to language and its associated neural circuitry. Thus, the evidence may suggest that BOLA2 is an important ASD-relevant 16p11.2 locus but also is more generally relevant for the human-specific ability to develop language and the neural systems supporting that development.

**Nonzero modules are enriched in ASD de novo protein-truncating variants.** We next tested nonzero modules for enrichment in different classes of genetic variants associated with ASD. We first examined enrichment in high-penetrance rare de novo protein truncating variants (dnPTVs). Among the genes highlighted by Kosmicki et al. with two or more dnPTVs in ASD, 43% were also present in nonzero modules, thus resulting in an enrichment at trend-level significance ($OR = 2.58$, $P = 0.08$, FDR $q = 0.0915$). The lack of significant enrichment may have been due to the limited number of known dnPTVs that overlap with the subset of genes considered in our analysis (i.e., 28). When we relaxed the criterion to one or more dnPTVs in ASD but added the constraint that the gene had to have a probability of loss-of-function intolerance ($\text{pLI} \geq 0.9$), we were able to study a larger set of 155 genes with putative ASD-relevant dnPTVs. Under this criterion, we found a significant enrichment of these ASD-risk genes in nonzero modules ($OR = 2.01$, $P = 0.02$) (Fig. 5a), including genes such as ADNP, ANKRD11, DYRK1A, ILF2, KDM5B, KDM6B, MED13L, PHF2, PTEN, SPAST, SUV420H1, TRIP12, WDFY3, and ZC3H4. Of these notable M10 genes, ADNP is within the top 20 hub genes (Supplementary Table 6). In contrast, zero modules were not enriched in these ASD-risk genes, either among the criteria of two or more dnPTVs or one or more dnPTVs and $\text{pLI} \geq 0.9$ ($OR < 1.82$, $P > 0.24$) (Fig. 5a). In addition, and contrary to the enrichment in ASD-associated dnPTVs, we did not find any enrichment among the 543 ASD-associated genes annotated in SFARI Gene for nonzero (OR = 1.36, $P = 0.66$) or zero modules (OR = 1.26, $P = 0.44$) (Fig. 5a). This evidence suggests that some high-penetrance ASD-associated genes are detectable within blood leukocyte gene expression data and show strong association with in vivo functional neuroimaging phenotypes relevant to early language heterogeneity in ASD.

**Nonzero modules are enriched in targets of FMRP and CHD8.** Although nonzero modules do not contain some of the most...
well-known and highly penetrant ASD-associated genes, such as FMR1 and CHD8, these modules may nevertheless overlap with the molecular networks linked to these genes. One way to examine this hypothesis is through testing nonzero modules for enrichment in downstream targets of these highly important genes. Nonzero modules were highly enriched in targets of both FMRP and CHD8 across two different target lists (OR > 1.89, \( P < 0.0269 \)) (Fig. 5a). Numerous modules drive these enrichments, such as M10 and M15 for FMRP targets and M10, M8, M13, and M15 for CHD8 targets (Fig. 5b). In contrast, zero modules were not enriched in target genes of either FMRP or CHD8 (Fig. 5a). These results suggest that nonzero modules also contain genes that are members of FMRP- and CHD8-related networks.

Broadly expressed genes are a prominent source of signal driving enrichments. Finally, given the prominent overlap between broadly expressed genes and nonzero modules, we tested whether many of the other enrichments with nonzero modules might be driven by broadly expressed genes. We first examined the enrichment of broadly expressed genes with all of the gene lists already tested. Notably, we found that nearly all gene lists enriched in nonzero modules were also highly enriched in broadly expressed genes (Fig. 5a). Furthermore, after broadly expressed genes were removed from these lists, the enrichments with nonzero modules largely disappeared (Supplementary Fig. 2). This result suggests that broadly expressed genes drive the enrichments of these lists in nonzero modules.

Discussion

Here, we found one large-scale association between coordinated gene coexpression modules in blood leukocytes and multivariate fMRI response to speech. Highlighting the distinctiveness of ASD language outcome subtypes, we found that blood leukocyte coexpression modules associated with the multivariate fMRI response to speech were different for all pairwise comparisons between groups of TD toddlers and toddlers with ASD plus either poor or good language outcome. Given the early ages at which the blood samples and fMRI data were collected, this association clearly manifests well before stable diagnoses and final language outcomes are known. Coexpression modules of importance in TD but not ASD may signal normative biological processes associated with the development of language-related neural circuitry. These normative processes may be affected in ASD. In addition, modules that diverge among ASD subtypes may indicate risk or protective mechanisms that push different individuals with ASD toward different early developmental language outcomes. Thus, in contrast to the idea that ASD is a uniform condition with similar underlying biological mechanisms in all diagnosed individuals, these results indicate that a behavioral stratifier such as early language outcome holds important information to aid in understanding how the underlying biology may be differentially linked to the way in which macroscale neural systems develop.

These findings may have high translational importance. Both neuroimaging methods and blood sampling to quantify the leukocyte transcriptome with high-throughput techniques are feasible for patients with ASD with different levels of impairment and at early ages. In vivo examination of the molecular mechanisms and their associations with higher-level macroscale neural systems and heterogeneity in clinical phenotypes will be important for furthering progress toward precision medicine\(^{11}\). Endeavors such as evaluating early age treatment response, monitoring clinical trials, and developing prediction tools for diagnosis and prognosis can all be facilitated with this approach to understanding the links among gene expression, macroscale neural systems, and behavioral levels of analysis. Future work will be necessary to determine whether similar associations are present in older children and adults with ASD.

Given the inability to directly and noninvasively assay gene expression from brain tissue in living patients, the current approach offers a novel in vivo window into how molecular mechanisms are associated with ongoing and dynamic macroscale neural systems development across the lifespan in ASD.

Another striking feature of these results is the large-scale nature of the association that covers most of the blood leukocyte transcriptome considered by the coexpression analysis. This feature matches predictions from the omnigenic model\(^{12}\). The omnigenic model suggests that for any complex trait or disorder, most of the heritability signal is spread widely throughout most of the genome. The omnigenic model also suggests that the numerous widespread ‘peripheral’ genes with small effect probably interact within gene regulatory networks with a smaller set of ‘core’ genes with much larger effect. Here, we found evidence that higher-effect rare dnPTVs in ASD that are intolerant to loss-of-function mutations are enriched among nonzero modules. Furthermore, we also found that many targets of FMRP and CHD8 were enriched in nonzero modules. Thus, the massive number of genes present within nonzero modules may indicate a large peripheral background of small-risk common variants that may work en masse and interact in important ways with higher-effect core mechanisms.

The omnigenic model makes another key prediction, namely that such associations may be detectable in many tissue types other than the brain, such as blood leukocytes. The omnigenic model suggests that a large percentage of the genes associated with a complex trait are likely to be broadly expressed genes. Here, we found evidence of large overlap between broadly expressed genes and nonzero modules—approximately 44% of all broadly expressed genes were found within nonzero association modules. In contrast to the observation that nearly all nonzero modules (e.g., 81%) were enriched in broadly expressed genes, only two nonzero modules (e.g., 18%) were enriched in lymphocyte-specific genes. Thus, this large-scale gene coexpression–fMRI association is largely driven by genes broadly expressed in the brain and many other tissues rather than lymphocyte-specific genes. Although we observed enrichments among nonzero modules and genes implicated in vocal learning, human-specific genes, ASD-associated prenatal coexpression modules, cortically ASD-downregulated coexpression modules, ASD dnPTVs, and FMRP and CHD8 targets, most of these enrichments probably emerged because each gene list was heavily enriched in broadly expressed genes. Removing broadly expressed genes from these lists resulted in the elimination of nearly all significant enrichments with nonzero modules. Overall, these results highlight the importance of broadly expressed genes as a novel class of mechanisms for further study in ASD.

This study has some limitations and caveats to bear in mind. First, the genes investigated in the final coexpression and PLS analyses were a subset of all genes in the entire genome that could be considered. Therefore, although nonzero modules did cover a large proportion of the genes examined in the analysis, they did not cover most of the entire genome. The extent of coverage of nonzero modules was certainly compatible with ideas about polygenic architecture underlying complex neural phenotypes\(^{11}\). However, the coverage of nonzero modules cannot be interpreted with respect to the omnigenic model in terms of sheer size. The current study did, however, evaluate predictions from the omnigenic model, particularly with respect to the importance of broadly expressed genes. However, this result can also be consistent with polygenic viewpoints, particularly if most of the polygenic associations reside within broadly expressed genes. Second, because the expression data were measured from a non-neural tissue, many brain-specific genes were not considered in the analyses. Thus, the current dataset cannot indicate the importance or lack thereof with regard to brain-specific genes, nor can it allow for comparisons of the relative importance of broadly expressed genes versus brain-specific genes.
In summary, we identified a large-scale association between multiple coordinated blood leucocyte gene coexpression modules and the multivariate fMRI response to speech. The associated coexpression modules were different for all pairwise comparisons between TD toddlers and toddlers with ASD plus good versus poor early language outcome. The associated coexpression modules were highly enriched in broadly expressed genes as well as ASD, prenatal, human-specific, and language-relevant genes. These results are congruent with predictions from polygenic and omnigenic models and suggest that gene expression in peripheral cells such as blood leucocytes is associated with the in vivo functional neural response to language that differentiates toddlers with ASD and poor versus good early language outcomes. The study showcases a novel in vivo approach that may be used in future work toward precision medicine goals.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41593-018-0281-3.

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Author contributions
E.C., K.P., L.E., M.V.L., and T.P. conceived the idea and designed the study. M.V.L. conceived and performed all analyses. T.P., V.G., V.W., R.A.I.B., and N.E.L. aided in data analyses. E.C., K.P., L.E., L.L., and C.C.B. collected data. E.C., K.P., L.E., N.E.L., T.P., and M.V.L. obtained grant funding. M.V.L. and E.C. wrote the manuscript. All authors contributed to editing the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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Methods

Participants. This study was approved by the Institutional Review Board at the University of California, San Diego. Parents provided written informed consent according to the Declaration of Helsinki and were paid for their participation. Identification to the procedures used in our earlier studies19,59,63, toddlers were recruited through two mechanisms: community referrals (for example, website) or a general population-based screening method called the 1-Year Well-Baby Check-Up Approach27 (now called the Get S.E.T. Early model) that allowed for the prospective study of ASD beginning as young as ages 12 to 24 months, on the basis of a toddler’s failure of the CSBS-DP Infant-Toddler Checklist27. All toddlers, including control subjects, participated in a series of tests collected longitudinally across all visits, including the ADOS (Module T, 1, or 2),74 Mullen Scales of Early Learning27, and Vineland Adaptive Behavior Scales48. All testing occurred at the University of California, San Diego Autism Center of Excellence (ACE). Toddlers who received their first diagnostic evaluation at younger than 36 months of age were included to reflect the best diagnostic testing once every 12 months until age 3–4 years, when a final diagnosis was given. No randomization procedures were implemented as part of the data-collection process. Data collection and analyses were not performed blind to the conditions of the experiment.

A total of n = 118 toddlers were scanned with fMRI and had available gene expression data. No statistical methods were used to predetermine sample sizes, but our sample sizes are currently among the largest of any fMRI study to date on ASD at very early ages in toddlerhood. From these 118 toddlers, n = 81 individuals with ASD were examined and were split into two language-outcome subtypes. n = 41 individuals with ASD (34 males and 7 females) were classified as having ‘poor’ language outcome (ASD Poor), on the basis of having both Mullen EL or RL T scores more than 1.5 s.d. below the norm of 50 (that is, T < 40) at the final testing time point (mean age at fMRI scan = 29.53 months, s.d. at fMRI scan = 8.04, range = 12–46 months). Another n = 40 individuals with ASD (30 males and 10 females) were classified as having ‘good’ language outcome (ASD Good), on the basis of having either Mullen EL or RL T scores greater than or equal to 40 (that is, T ≥ 40) at the final testing time point (mean age at fMRI scan = 29.73 months, s.d. at fMRI scan = 8.51, range = 12–45 months). The usage of the term ‘Good’ here is not used to refer to ability level in absolute terms but instead reflects ability relative to the ASD Poor subgroup. These ASD subtypes were compared to n = 37 typically developing toddlers (21 males and 16 females; mean age at MRI scan = 26.19 months, s.d. at MRI scan = 10.20, range = 12–45 months). ASD subtypes and TD did not statistically differ in age at the time of scanning (t(211) = 1.87, P = 0.15). Additional demographic and phenotypic information can be found in Supplementary Table 7.

Blood sample collection, RNA extraction, quality control, and sample preparation. Four to six milliliters of blood was collected into EDTA-coated tubes from toddlers on visits when they had no fever, cold, flu, infections, or other illnesses, or use of medications for illnesses 72 h before the blood draw. Blood samples were passed over a LeukoLOCK filter (Ambion) to capture and stabilize leukocytes and immediately placed in a −20 °C freezer. Total RNA was extracted according to standard procedures and manufacturer’s instructions (Ambion, LeukoLOCK disks (Ambion cat. no. 1933) were freed from RNA later, and Tri- reagent (Ambion cat. no. 9738) was used to flush out the captured lymphocytes and lyse the cells. RNA was subsequently precipitated with ethanol and purified though washing and cartridge-based steps. The quality of the mRNA samples was quantified according to the RNA Integrity Number (RIN): values ≥ 6.0 were considered acceptable27, and all processed RNA samples passed RIN quality control. Quantification of RNA was performed with a NanoDrop spectrophotometer (Thermo Scientific). Samples were prepared in 96-well plates at a concentration of 25 ng/μl.

Gene expression and data processing. RNA was assayed at Scripps Genomic Medicine for labeling, hybridization, and scanning with the Illumina BeadChip pipeline (Illumina) according to the manufacturer’s instructions. All arrays were scanned with the Illumina BeadArray Reader and read into Illumina GenomeStudio software (version 1.1.1). Raw data were exported from Illumina GenomeStudio, and data preprocessing was based on the criteria of having both (for R (http://www.R-project.org/) and Bioconductor (http://www.bioconductor.org/)) Raw and normalized data are part of larger sets deposited in the Gene Expression Omnibus database (GSE42133 and GSE111175).

A larger primary dataset of blood leukocyte gene expression was available from 363 samples from 314 toddlers 1–4 years old. The samples were assayed with the illumina microarray platform in three batches. The datasets were combined by matching the Illumina Probe ID and probe nucleotide sequences. The final set included a total of 20,194 gene probes. Quality-control analysis was performed to identify and remove 23 outlier samples from the dataset. Samples were marked as outliers if they showed low signal intensity (average signal two s.d. lower than the overall mean). After removing any pairwise correlation that was greater than 0.9, we performed principal component analysis to filter out batch effects, sex, and RIN. The batch, sex, and RIN-adjusted data were used in all further downstream analyses. We also checked for differences in the proportion estimates of different leukocyte cell types (neutrophils, B cells, T cells, NK cells, and monocytes) with the CellCODE deconvolution method66, but we found no evidence of differences across groups in terms of any type (Supplementary Table 8).

In addition to the primary analyses with WGCNA, differential expression analysis at the level of individual genes was also conducted with limma and DE genes were identified if they passed Storey FDR q < 0.05 (ref. 57). Data distributions were assumed to be normal, but normality was not formally tested for each gene.

Weighted gene coexpression-network analysis. We decreased the number of features in the gene expression dataset from 14,313 genes down to 21 modules of tightly coexpressed genes. This data-reduction step was achieved with WGCNA, implemented within the WGCNA library in R2. Correlation matrices estimated with the robust correlation measure of biweight midcorrelation were computed and then converted into adjacency matrices that retained the sign of the correlation. These adjacency matrices were then raised to a soft power of 2 (Supplementary Fig. 3a). This soft power was chosen by finding the first soft power for which a measure of R2 scale-free topology-model fit saturated at least above R2 > 0.8 (ref. 58), and the slope was between −1 and 2 (ref. 59). The soft-power-thresholded adjacency matrix was then converted into a topological overlap matrix (TOM) and then a TOM dissimilarity matrix (1 − TOM). The TOM dissimilarity matrix was then input into agglomerative hierarchical clustering with the average linkage method. Gene modules were defined from the resulting clustering tree, and branches were cut with a hybrid dynamic tree-cutting algorithm (deepSplit parameter = 4) (Supplementary Fig. 3b). Modules were merged at a cut height of 0.2, and the minimum module size was set to 10. Only genes with a module membership of r > 0.3 were retained within modules. For each gene module, a summary measure called the ME was computed as the first principal component of the scaled (standardized) module expression profiles. We also computed module membership for each gene and module. Module membership indicated the correlation between each gene and the ME (Supplementary Table 6). Genes that could not be clustered into any specific module were left within the M0 module, and this module was not considered in any further analyses. Analysis of group differences in MEs was also conducted with linear models and correction for multiple comparisons at FDR q < 0.05 (Supplementary Table 3; Supplementary Fig. 4). Data distributions were assumed to be normal, but normality was not formally tested for each module or each module by condition. One contrast of interest was analyzed in this study: all speech conditions versus rest. At early language learning ages, when neonates, infants, and toddlers are not yet experts at language, forward and backward speech both activate language-sensitive temporal areas; consequently, specific comparisons between them tend to be nonsignificant20. Therefore, forward and backward speech stimuli both appear to be effective in stimulating language-sensitive cortices, by perhaps both being treated as potentially languagerelevant by the language-experienced infant and toddler, and by the language-related caveat for forward versus backward speech, our main contrast of interest was all speech versus rest.

Imaging data acquisition and task design. The fMRI task was identical to that used in our previously published studies22,60,61 and consisted of three types of speech stimuli (complex forward speech, simple forward speech, and backward speech) as well as rest blocks interspersed between task blocks to forestall possible habituation across blocks. Blocks were 20 s in duration. All speech conditions were created with the same female speaker. One contrast of interest was analyzed in this study: all speech conditions versus rest. At early language learning ages, when neonates, infants, and toddlers are not yet experts at language, forward and backward speech both activate language-relevant temporal areas; consequently, specific comparisons between them tend to be nonsignificant20. Therefore, forward and backward speech stimuli both appear to be effective in stimulating language-sensitive cortices, by perhaps both being treated as potentially languagerelevant by the language-experienced infant and toddler, and by the language-related caveat for forward versus backward speech, our main contrast of interest was all speech versus rest.

Imaging data were collected on a 1.5 Tesla General Electric MRI scanner during natural sleep at night; no sedation was used. High-resolution T1-weighted anatomical scans were collected for warping fMRI data to a standard atlas space. Blood oxygenation level-dependent (BOLD) signal was measured across the whole brain with echoplanar imaging during the language paradigm (echo time = 30 ms, repetition time = 2,500 ms, flip angle = 90°, bandwidth = 70 kHz, field of view = 25.6 cm, in-plane resolution = 4 × 4 mm, slice thickness = 4 mm, 31 slices). The paradigm was designed such that the presentation of head motion was minimal (mean FD < 0.25) for nearly all subjects in all groups (ASD Good, mean = 0.11 mm, s.d. = 0.23; ASD Poor, mean = 0.07 mm, s.d. = 0.08; TD, mean = 0.07 mm, s.d. = 0.03) and that groups did not differ in either mean FD (F(2,115) = 1.12, P = 0.33) or mean DVARS.
DMRI data analyses. Preprocessing of functional imaging data was implemented within the Analysis of Functional NeuroImages (AFNI) software package. The preprocessing pipeline comprised motion correction, normalization to Talairach space, and smoothing (Gaussian kernel with 8-mm full width at half maximum). First-level and second-level mass-univariate whole-brain activation analyses were modeled with the general linear model (GLM) to SPM8 (https://www.fil.ion.ucl.ac.uk/spm/). Events in first-level models were modeled with the canonical hemodynamic response function and its temporal derivative. All first-level GLMs included motion parameters as covariates of no interest. High-pass temporal filtering was applied with a cutoff of 0.0078 Hz (1/128 s) to remove low-frequency drift in the time series. For whole-brain analyses, the distributions were assumed to be normal, but normals were not formally tested. Group-level analyses were implemented with the general linear model in SPM8. We ran whole-brain analyses for the contrast of all speech versus rest within and between-groups and thresholded at a voxelwise FDR $q < 0.05$ (ref. 72). For between-group ROI analysis, we used meta-analytic ROIs from the Neurosynth term ‘language’ of frontal and temporal cortical areas in both hemispheres, which were identical to those used in a prior paper19. We computed the difference in reported from Boyle et al.20. These classes were (i) broadly expressed genes, (ii) expressed genes in corticospinal brain areas, (iii) brain-specific genes, (iv) whole-blood-specific genes, and (v) lymphocyte-specific genes. The background pool number for these hypergeometric tests was 14,313. Next, we tested whether nonzero modules were heavily enriched in modules from one or more of these gene classes. The background total for these tests was set to the total number of coexpression modules (e.g., 21).

Further enrichment tests were done across a wider range of gene lists of theoretical importance. Songbirds are often used as animal models relevant to the vocal-learning component of language21. We investigated enrichments with differentially expressed genes taken from a microarray dataset of Area X in songbirds22. To identify DE genes between singing versus nonsinging birds, we reanalyzed this dataset (GEO GSE38419) with limma,23 and DE genes were identified if they passed Storey FDR $q < 0.05$ (ref. 24). Given the uniquely human nature of language, we also tested hypotheses regarding enrichments among genes that were transcriptionally different in the cortical tissue between humans and chimpanzees (that is, human–specific genes). These tests were performed across gene lists from two independent investigations on human–specific gene expression differences, in which the common overlap among the two lists was small (4.38%)25-27. Ample evidence suggests that prenatal brain developmental periods are critical for ASD13, 36–38. To test for enrichment in prenatal ASD-associated coexpression modules for every voxel, we identified fMRI–gene expression LV pairs, a permutation test was run with 10,000 permutations. To identify reliably contributing voxels for fMRI–gene expression pairwise group comparisons we used Welch’s t test.

DMRI–gene expression association analysis. To assess multivariate DMRI–gene expression relationships, we used PLS analysis28,29. PLS is widely used in the neuroimaging literature, particularly to explain multivariate neural responses in terms of multivariate behavioral patterns of variation or a design matrix. Given that the current dataset was massively multivariable in terms of both DMRI and gene expression datasets, we used PLS to elucidate how variation in neural response to speech across large-scale neural systems covaried with gene expression, as measured by ME values of coexpression modules. PLS allows for identification of such relationships by finding latent DMRI–gene expression variable pairs (LV) that maximally explained covariation in the dataset and were uncorrelated with other latent DMRI–gene expression variable pairs. The strength of such covariation was denoted by the singular value ($d_i$) for each brain–behavior LV, and hypothesis tests were made via permutation tests on the singular values. Furthermore, identifying brain regions that most strongly contributed to each LV pair was made via bootstrapping, whereby a bootstrap ratio was created for each voxel and represented the reliability of that voxel for contributing strongly to the LV pattern identified. The bootstrap ratio was roughly equivalent to a Z statistic and could be used to threshold data to find voxels that reliably contribute to an LV pair. The PLS analyses reported here were implemented within the pls toolbox (http://www.rotan-baycrest.on.ca/pls/). Here we input first-level speech versus rest contrast images into the PLS. For gene expression data, we input ME values for all 21 coexpression modules. For statistical inference on identified DMRI–gene expression LV pairs, a permutation test was run with 10,000 permutations. To identify reliably contributing voxels for DMRI–gene expression LVs and to compute 95% CIs on DMRI–gene expression correlations, bootstrapping was used with 10,000 resamples. To show voxels that most reliably contributed to significant DMRI–gene expression LVs, we thresholded data for visualization at a BSR of 1.96 and –1.96. The strength of DMRI–gene expression correlations for significant LVs was displayed as a bar graph with 95% bootstrap CIs as error bars. Gene coexpression modules in which the 95% CIs did not encompass 0 were denoted nonzero association modules. All other modules in which the 95% CIs included 0 were denoted zero modules.

From the PLS results, we tested whether the nonzero associations across modules were comparable across ASD subtypes and between different data sets. We performed gene-set enrichment tests across the 21 ASD subtypes and TD. To test for this question, we counted the overlap among nonzero association modules in each group and ran hypergeometric tests that explicitly tested for statistically significant overlap or commonality of nonzero associations across groups.

Enrichment tests. Tests for functional (process-level) enrichment across all modules were implemented with the MetaCore GeneGO software platform. Further gene-set enrichment tests (hypergeometric tests and enrichment OR) were done on tissue-specific gene lists. First, we annotated each coexpression module by enrichment in four types of gene classes of relevance, as defined by GTEx data reported by Boyle et al.30. These classes were (i) broadly expressed genes, (ii) brain–specific genes, (iii) whole-blood–specific genes, and (iv) lymphocyte–specific genes. The background pool number for these hypergeometric tests was 14,313. Next, we tested whether nonzero modules were heavily enriched in modules from one or more of these gene classes. The background total for these tests was set to the total number of coexpression modules (e.g., 21).

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Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection No software was used for data collection.

Data analysis Software used for data analysis included Illumina GenomeStudio software (version 1.1.1), R version 3.4.2, RStudio (version 1.1.383), various R libraries available on CRAN, GitHub, and Bioconductor (e.g., easypackages, ggplot2, psych, here, patchwork, CellCODE, limma, qvalue, WGCNA, gplots), MATLAB R2017b, plsgui MATLAB toolbox, SPM8 MATLAB Toolbox, AFNI version 17.3.00, MetaCore GeneGO software version 5.0. Custom code for implementing all analyses can be found at https://github.com/mlombardo/asdlangoutcomebloodgexfmripls

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The raw data that support the findings from this study are publicly available from the NIH National Database for Autism Research (NDAR). Raw blood leukocyte gene expression data is publicly available via Gene Expression Omnibus (GEO) (GSE42133; GSE111175). Song bird area X gene expression data is publicly available on GEO (GSE34819). GTEx data is publicly available at https://gtexportal.org. ASD post-mortem cortical gene expression can be found at https://github.com/dhglab/Genome-wide-changes-in-IncRNA-alternative-splicing-and-cortical-patterning-in-autism.

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Sample size
No statistical methods were used to pre-determine sample sizes, but our sample sizes are currently amongst the largest of any fMRI study to date on ASD at very early ages in toddlerhood. Furthermore, for the primary hypothesis test of the manuscript (i.e. partial least squares analysis), statistical power analyses have not been formally developed for such analyses and thus a priori power calculations could not be done.

Data exclusions
Gene expression data were taken from a larger superset of data available on ASD and TD toddlers. Quality control analysis of this superset was performed to identify and remove 23 outlier samples. Samples were marked as outlier if they showed low signal intensity (average signal two standard deviations lower than the overall mean), deviant pairwise correlations, deviant cumulative distributions, deviant multi-dimensional scaling plots, or poor hierarchical clustering, as described elsewhere (Pramparo et al., 2015, Molecular Systems Biology, 11, 841). From this high-quality superset, we utilized the maximum number of ASD and TD subjects whom also had fMRI data available.

Replication
A replication dataset for the primary PLS analyses was not available. However, for enrichment analyses, when possible, we utilized two independent gene lists from different studies (e.g., human-specific genes, ASD prenatal genes, FMRP or CHD8 targets) in to identify replicable enrichments.

Randomization
Random allocation of participants to groups is not applicable because ASD and TD labels belong to specific groups of participants. No other randomization procedures were implemented as part of the data collection process.

Blinding
Data collection and analyses were not performed blind to the conditions of the experiment.

Reporting for specific materials, systems and methods

Materials & experimental systems
n/a  |  Involved in the study
✓  |  Unique biological materials
✓  |  Antibodies
✓  |  Eukaryotic cell lines
✓  |  Palaeontology
✓  |  Animals and other organisms
✓  |  Human research participants

Methods
n/a  |  Involved in the study
✓  |  ChIP-seq
✓  |  Flow cytometry
✓  |  MRI-based neuroimaging
### Human research participants

**Population characteristics**  
A total of n=118 toddlers were scanned with fMRI and had available gene expression data. From these 118 toddlers, n=81 ASD individuals were examined and were split into 2 language outcome subtypes. n=41 individuals with ASD (34 male, 7 female) were classified as ‘poor’ language outcome (ASD Poor), based on the criteria of having both Mullen EL and RL T-scores more than 1 standard deviation below the norm of 50 (i.e. T<40) at the final testing time-point (mean age at fMRI scan = 29.53 months, SD at fMRI scan = 8.04, range = 12-46 months). Another n=40 individuals with ASD (30 male, 10 female) were classified as ‘good’ language outcome (ASD Good), based on having either Mullen EL or RL T-scores greater than or equal to 40 (i.e. T ≥ 40) at the final testing time-point (mean age at fMRI scan = 29.73 months, SD at fMRI scan = 8.51, range = 12-45 months). The usage of the term ‘Good’ here is not used to refer to ability level in absolute terms, but more reflects ability relative to the ASD Poor subgroup. These ASD subtypes were compared to n=37 typically-developing toddlers (21 male, 16 female; mean age at fMRI scan = 26.19 months, SD at fMRI scan = 10.20, range = 12-45 months). ASD subtypes and TD did not statistically differ in age at the time of scanning (F(2,115) = 1.87, p = 0.15).

**Recruitment**  
Identical to the approach used in our earlier studies, toddlers were recruited through two mechanisms: community referrals (e.g., website) or a general population-based screening method called the 1-Year Well-Baby Check-Up Approach that allowed for the prospective study of ASD beginning at 12 months based on a toddler’s failure of the CSBS-DP Infant-Toddler Checklist. All toddlers were tracked from an intake assessment around 12 months and followed roughly every 12 months until 3–4 years of age. All toddlers, including normal control subjects, participated in a series of tests collected longitudinally across all visits, including the Autism Diagnostic Observation Schedule (ADOS; Module T, 1, or 2), the Mullen Scales of Early Learning, and the Vineland Adaptive Behavior Scales. All testing occurred at the University of California, San Diego Autism Center of Excellence (ACE). We are not aware of any self-selection or other biases likely to impact the recruitment of our cohort.

### Magnetic resonance imaging

**Experimental design**  
**Design type**  
Block design

**Design specifications**  
- Number of speech blocks = 9
- Length of each block = 20 secs
- Interval between blocks = 20 secs

**Behavioral performance measures**  
Since toddlers were scanned during natural sleep, no behavioral measures were collected during scanning.

**Acquisition**  
**Imaging type(s)**  
functional

**Field strength**  
1.5T

**Sequence & imaging parameters**  
Imaging data were collected on a 1.5 Tesla General Electric MRI scanner during natural sleep at night; no sedation was used. High-resolution T1-weighted anatomical scans were collected for warping fMRI data into standard atlas space. Blood oxygenation level-dependent (BOLD) signal was measured across the whole brain with echoplanar imaging during the language paradigm (echo time = 30 ms, repetition time = 2,500 ms, flip angle = 90 degrees, bandwidth = 70 kHz, field of view = 25.6 cm, in-plane resolution = 4 x 4 mm, slice thickness = 4 mm, 31 slices).

**Area of acquisition**  
whole-brain coverage

**Diffusion MRI**  
☑️ Used

**Preprocessing**  
**Preprocessing software**  
Preprocessing of functional imaging data was implemented within the Analysis of Functional Neuroimages (AFNI) software package. The preprocessing pipeline was comprised of motion correction, normalization to Talairach space, and smoothing (8mm full-width at half-maximum (FWHM) Gaussian kernel).

**Normalization**  
linear normalization to Talairach space

**Normalization template**  
Talairach

**Noise and artifact removal**  
Motion parameters were included as regressors in the GLMs

**Volume censoring**  
No volume censoring was done

**Statistical modeling & inference**  
**Model type and settings**  
First-level and second-level mass-univariate whole-brain activation analyses were modeled with the general linear model (GLM) in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/). Events in first-level models were modeled using the canonical hemodynamic response function and its temporal derivative. All first-level GLMs included motion parameters as
covariates of no interest. High-pass temporal filtering was applied with a cutoff of 0.0078 Hz (1/128 seconds) in order to remove low frequency drift in the time series.

Effect(s) tested
Speech vs Rest

Specify type of analysis:

✓ Whole brain
☐ ROI-based
☐ Both

Statistic type for inference
Voxel-wise

Correction
Voxel-wise FDR

Models & analysis

Gene co-expression modules were summarized by the module eigengene (first principal component of genes from a module). Module eigengene values were inserted into the PLS analysis as the gene expression dataset, while whole-brain t-maps from the Speech vs Rest contrast were inserted into the PLS analysis as the neuroimaging dataset. A permutation test (10,000 permutations) was done for hypothesis testing of latent-variable pairs and bootstrapping was conducted to compute bootstrap ratios for brain and gene expression variables.

Multivariate modeling and predictive analysis

✓ Multivariate modeling or predictive analysis

n/a

Involved in the study

✓ Functional and/or effective connectivity
✓ Graph analysis