Common variation near ROBO2 is associated with expressive vocabulary in infancy

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Twin studies suggest that expressive vocabulary in early childhood is modestly heritable. However, the genes influencing this early linguistic phenotype are unknown. Here we conduct a genome-wide screen and follow-up study of expressive vocabulary in toddlers of European descent from up to four studies of the EArly Genetics and Lifecourse Epidemiology consortium, analysing an early (15–18 months, ‘one-word stage’, \( N_{\text{total}} = 8,889 \)) and a later (24–30 months, ‘two-word stage’, \( N_{\text{total}} = 10,819 \)) phase of language acquisition. For the early phase, one single-nucleotide polymorphism (rs7642482) at 3p12.3 near ROBO2, encoding a conserved axon-binding receptor, reaches the genome-wide significance level (\( P = 1.3 \times 10^{-8} \)) in the combined sample. This association links language-related common genetic variation in the general population to a potential autism susceptibility locus and a linkage region for dyslexia, speech-sound disorder and reading. The contribution of common genetic influences is, although modest, supported by genome-wide complex trait analysis (meta-GCTA \( h^2_{15-18\text{-months}} = 0.13, \) meta-GCTA \( h^2_{24-30\text{-months}} = 0.14 \)) and in concordance with additional twin analysis (5,733 pairs of European descent, \( h^2_{24\text{-months}} = 0.20 \)).

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The number of distinct spoken words is a widely used measure of early language abilities, which manifests during infancy \(^1\). Word comprehension (known as receptive language) in typically developing children starts at the age of about 6–9 months \(^2\), and the spontaneous production of words (known as expressive language) emerges at about 10–15 months \(^3,4\). During the next months the accumulation of words is typically slow, but then followed by an increase in rate, often quite sharp, around 14–22 months of age (‘vocabulary spurt’) \(^1,4\). As development progresses, linguistic proficiency becomes more advanced, with two-word combinations (18–24 months of age) \(^1,3\) and more complex grammatical structures (24–36 months of age) \(^1,3\) arising, accompanied by the steady increase in vocabulary size. Expressive vocabulary is therefore considered to be a rapidly changing phenotype, especially between 12 and 24 months \(^3\), with zero size at birth, \(~50\) words at 15–18 months \(^1,3\), \(~200\) words at 18–30 months \(^1,3\), \(~14,000\) words at 6 years of age \(^3,4\), and \(~50,000\) words in high school graduates \(^5,7\).

Twin analyses of cross-sectional data suggest that expressive vocabulary at \(~24\) months is modestly heritable (\(h^2 = 0.16–0.38\)) \(^5,9\), and longitudinal twin analyses have reported an increase in heritability of language-related factors during development (\(h^2 = 0.47–0.63, \geq 7\) years of age) \(^10\). Large-scale investigations of common genetic variation underlying growth in language skills, however, are challenging owing to the complexity and varying nature of the phenotype. This is coupled with a change in psychological instruments, which are used to assess these abilities with progressing age. Current genome-wide association studies (GWASs) using cross-sectional data on language abilities in childhood and adolescence have failed to identify robust signals of genome-wide association \(^11,12\), and genes influencing earlier, less-complex linguistic phenotypes are currently unknown.

To attempt to understand genetic factors involved in language development during infancy and early childhood, we perform a GWAS and follow-up study of expressive vocabulary scores in independent children of European descent from the general population and analyse an early (‘one-word stage’) and a later (‘two-word stage’) phase of language acquisition. We report a novel locus near ROBO2, encoding a conserved axon-binding receptor, as associated with expressive vocabulary during the early ‘one-word’ phase at the genome-wide significance level, and provide heritability estimates for expressive vocabulary during infancy and early childhood.

**Results**

**Genome-wide association analyses.** We conducted two cross-sectional genome-wide screens corresponding to an early (15–18 months, \(N_{total} = 8,889\)) and a later (24–30 months, \(N_{total} = 10,819\)) phase of language acquisition, respectively, each adopting a two-stage design (Figs 1 and 2; Supplementary Data 1). During these developmental phases, expressive vocabulary was captured with age-specific word lists (adaptations (Supplementary Figs 2 and 3. For the early phase, the strongest wide plots of the association signals are provided in autosomal genotyped or imputed single-nucleotide polymorphisms (SNPs) were studied in 6,851 15-month-old and 6,299 24-month-old English-speaking toddlers, respectively. Genome-wide plots of the association signals are provided in Supplementary Figs 2 and 3. For the early phase, the strongest association signal was observed at rs7642482 on chromosome 3p12.3 near ROBO2 (\(P = 9.5 \times 10^{-7}\), Supplementary Table 1) and for the late phase at rs11742977 on chromosome 5q22.1 within CAMK4 (\(P = 3.5 \times 10^{-7}\), Supplementary Table 2). All independent variants from the discovery analysis (associated \(P \leq 10^{-8}\), Supplementary Tables 1 and 2), including these SNPs, were taken forward to a follow-up study (Methods). This included 2,038 18-month-old Dutch-speaking children for the early phase and 4,520 24–30-month-old Dutch or English-speaking children for the later phase (Supplementary Data 1).

For four independent loci from the early phase GWAS (rs7642482, rs10734234, rs11176749 and rs1654584), but none for the later phase analysis, we found evidence for association within the follow-up cohort (\(P < 0.05\), assuming the same direction of effect as in the discovery sample (Table 1; Supplementary Tables 1–4). In the combined analysis of all available samples (Table 1; Fig. 3a–d) rs7642482 on chromosome 3p12.3 near ROBO2 (the strongest signal in the discovery cohort) reached the genome-wide significance level (\(P = 1.3 \times 10^{-8}\)), and the three other signals approached the suggestive level (rs10734234 on chromosome 11p15.2 near INP, \(P = 1.9 \times 10^{-7}\); rs11176749 on chromosome 12q15 near CAND1; \(P = 7.2 \times 10^{-7}\) and rs1654584 on chromosome 19p13.3 within DAPK3; \(P = 3.4 \times 10^{-7}\)).

Each of these four polymorphisms explained only a small proportion of the phenotypic variance (adjusted regression \(R^2\) for rs7642482 = 0.34–0.35%, rs10734234 = 0.27–0.35%, rs11176749 = 0.25–0.27% and rs1654584 = 0.22–0.49%) in both the discovery and the follow-up cohort, but together the four SNPs accounted for >1% of the variation in early expressive vocabulary scores (joint adjusted regression \(R^2 = 1.10–1.45\%)\). For the SNP reaching genome-wide significance, rs7642482, each increase in the minor G-allele was associated with lower expressive vocabulary, although, due to the rank-transformation, an interpretation of the magnitude of the genetic effect is not informative. An empirical estimate of the genetic effect in the discovery sample, suggested a decrease of 0.098 s.d. in expressive vocabulary scores (95% confidence interval: 0.058; 0.14) per increase in G-allele. We are aware, however, that this signal might be prone to the ‘winner’s curse’ (that is, an overestimation of the effect) and requires further replication within independent samples.

**Characterization of the lead association signals.** rs7642482 is located \(~19\) kb 3’ of ROBO2 (OMIM: 602431), which encodes the human roundabout axon guidance receptor homologue 2 (Drosophila) gene. An in silico search for potentially functional effects using the University of California Santa Cruz Genome Browser provided no evidence that rs7642482 or proxy SNPs \((r^2 > 0.3)\) relate to protein-coding variation within ROBO2. For this, we also confirmed the observed linkage disequilibrium structure within the discovery cohort through local imputation of chromosome 3 using the 1,000 Genomes reference panel (v3.2011123, Supplementary Fig. 4). The sequence at rs7642482 and the flanking genomic interval are, however, highly conserved (rs7642482 Genomic Evolutionary Rate Profiling (GERP) score = 3.49; regional average GERP score near rs7642482 (derived from 100 bases surrounding rs7642482, GWAVA) = 3.06; average GERP score for coding sequences > 2). Encyclopaedia of DNA elements (ENCODE) data indicate that in umbilical vein endothelial cells (HUVEC), rs7642482 overlaps with regulatory chromatin states, such as H3K27ac, which are predicted to be a strong enhancer (Fig. 3e). Additional searches using HaploReg (v2) (ref. 26) identified overlapps with further regulatory DNA features, such as DNase I

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hypersensitive sites and binding sites for transcription factors (lrx, Pou3f2_1). This suggests that variation at rs7642482 might be implicated within regulatory mechanisms in embryonic cell types, consistent with a peak of ROBO2 expression in the human brain during the first trimester (Supplementary Fig. 5). There was no evidence for cis expression quantitative trait loci (eQTL) within ±1 Mb of rs7642482 in postnatally derived cell types or adult brain tissue, based on searches of public eQTL databases (seeQTL)27,28.

Since little is known about the genetic factors affecting language acquisition, the ‘suggestive’ signals at 11p15.2, 12q15 and 19p13.3 may also stimulate future research. rs10734234 resides within the vicinity of INSC (197 kb 3’ of the gene), encoding an adaptor protein for cell polarity proteins (OMIM: 610668). rs11176749 is located near CAND1 (144 kb 3’ of the gene) encoding a F-box protein-exchange factor (OMIM: 607727), which regulates the ubiquitination of target proteins, and rs1654584 is an intronic SNP within DAPK3 encoding the death-associated protein kinase 3, which plays a key role in apoptosis (OMIM: 603289).

Within a further step, we investigated whether the reported association signals are influenced by potential covariates, such as gestational age29 and maternal education30. These have been previously linked to late language emergence in infancy29 and the total number of spoken words in early childhood30, respectively. Studying up to 8,889 15–18-month-old children from the discovery and follow-up cohort, the association signal at rs7642482 increased when gestational age was adjusted for (adjusted $P_{\text{meta}} = 4.0 \times 10^{-5}$, 0.36–0.38% explained variance), while adjustment for maternal education did not affect the

Figure 1 | Study design for the genome-wide screen of early expressive vocabulary. Expressive vocabulary between 15 and 18 months of age was assessed using different forms of the MacArthur Communicative Development Inventories (CDI). Detailed phenotype descriptions are given in Supplementary Data 1.

Figure 2 | Study design for the genome-wide screen of later expressive vocabulary. Expressive vocabulary between 24 and 30 months of age was assessed with different forms of the MacArthur Communicative Development Inventories (CDI) and the Language Development Survey (LDS). Detailed phenotype descriptions are given in Supplementary Data 1.
association (Supplementary Tables 5 and 6). For the remaining SNPs, there was little or no effect on the strength of the genetic association when these covariates were controlled for.

To explore whether the reported association signals influence linguistic skills other than early-phase expressive vocabulary, we also investigated a series of language-related measures during development. We observed no evidence for association between the four SNPs and first single-word utterances in 4,969 12-month-old Finnish children (Supplementary Data 1; Supplementary Table 7). However, this age pertains to a developmental stage where expressive vocabulary is very low, that is, the majority of children speak about one or two words, and pre-linguistic communication skills are still developing31.

All early-phase signals were furthermore attenuated or even abolished when investigated for association with word-production scores during the later phase of language acquisition (24–30 months, Supplementary Fig. 6). This age band spans a phase where growth in linguistic proficiency may relate more to early grammar development including two-word combinations1, and pre-linguistic communication skills are still developing 31. For the remaining SNPs, there was little or no effect on the strength of the genetic association (GCTA)32,33, was mixed (Avon Longitudinal Study of Parents and Children (ALSPAC): $r_g(s.e.) = 0.69(0.20)$, $P = 0.02$, Generation R Study (GenR): $r_g(s.e.) = -0.32(0.97)$, $P = 0.18$). There was also no association between the four reported SNPs and other language-related cognitive outcomes, including verbal intelligence scores, in middle childhood (8–10 years of age) when studying up to 5,540 children from the discovery cohort, apart from nominal associations with reading speed (rs7642482 $P = 0.009$; rs1654584 $P = 0.0035$; Supplementary Tables 8 and 9). Thus, the observed genetic associations, especially at rs7642482, are likely to be time-sensitive and specific to the early phase of language acquisition.

Twin analysis and GCTA. A twin study of 5,733 twin pairs of European descent, including a subset of children from the follow-up cohorts, supported the (modest) influence of additive genetic effects on variability in expressive vocabulary at ~24 months ($\sigma^2(s.e.) = 0.20(0.008)$; Table 2; Supplementary Tables 10 and 11, Methods) and was consistent with previous reports on a smaller sample9. Estimates from twin analysis and GCTA32, performed on the discovery sample, were furthermore in close
concordance (ALSPAC GCTA $h^2\text{(s.e.)}_{15\text{-}18\text{-}\text{months}} = 0.13(0.05)$; GCTA $h^2\text{(s.e.)}_{24\text{-}30\text{-}\text{months}} = 0.17(0.06)$; Table 2). However, in the smaller-sized follow-up samples, GCTA heritability, especially for the later phase, was close to zero (Table 2), and is likely to reflect impaired power during the follow-up. Combining GCTA heritability estimates using meta-analysis techniques (Methods), provided similar estimates as observed for the discovery cohort alone (meta-GCTA $h^2\text{(s.e.)}_{15\text{-}18\text{-}\text{months}} = 0.13(0.05)$, meta-GCTA $h^2\text{(s.e.)}_{24\text{-}30\text{-}\text{months}} = 0.14(0.05)$).

**Table 1 | Lead association signals for early expressive vocabulary (15–18 months of age).**

| SNP                  | E/A | Chr | Pos | Gene* | Discovery (N = 6,851) | Follow-up (N = 2,038) | Meta-analysis (N = 8,889) |
|----------------------|-----|-----|-----|-------|-----------------------|-----------------------|--------------------------|
| rs7642482            | G/A | 3p12.3 | 77,800,446 | ROBO2 | 0.18 | -0.11 (0.022) | 9.5 × 10⁻⁷ | 0.19 | -0.12 (0.040) | 4.4 × 10⁻⁷ | 0.19 | -0.11 (0.019) | 1.3 × 10⁻⁸ | 0.90 |
| rs10734243           | T/C | 15q22.1 | 15,422,436 | INSC | 0.90 | -0.14 (0.032) | 1.1 × 10⁻⁵ | 0.90 | -0.17 (0.059) | 4.5 × 10⁻⁵ | 0.90 | -0.16 (0.028) | 1.9 × 10⁻⁷ | 0.72 |
| rs111716749          | T/A | 1q25 | 66,139,051 | CAND1 | 0.11 | -0.12 (0.027) | 2.1 × 10⁻⁵ | 0.11 | -0.13 (0.050) | 1.0 × 10⁻⁴ | 0.11 | -0.12 (0.024) | 7.2 × 10⁻⁷ | 0.83 |
| rs1654584            | G/T | 19p13.3 | 3,921,683 | DAPK3 | 0.23 | -0.081 (0.020) | 6.2 × 10⁻⁵ | 0.23 | -0.13 (0.038) | 9.2 × 10⁻⁴ | 0.23 | -0.091 (0.018) | 3.4 × 10⁻⁷ | 0.30 |

A, alternative allele; ALSPAC, Avon Longitudinal Study of Parents and Children; CDI, Communicative Development Inventory; Chr, chromosome; E, effect allele; EAF, effect allele frequency; Pos, position; P, $P$-value.

**Discussion**

This study reports a genome-wide screen and follow-up study of expressive vocabulary scores in up to 10,819 toddlers of European origin investigating an early phase (15–18 months) and a later phase (24–30 months) of language acquisition. On the basis of the combined analysis of all available samples, our study identifies a novel locus near ROBO2 as associated with expressive vocabulary during the early phase of language acquisition.

Robo receptors and their Slit ligands (secreted chemorepellent proteins) are highly conserved from fly to human and play a key role in axon guidance and cell migration. In vertebrates, Robo is involved in midline commissural axon guidance, the proliferation of central nervous system progenitors, the spatial positioning of spiral ganglion neurons and the assembly of the trigeminal ganglion, which is the sensory ganglion of the trigeminal nerve. The latter is particularly important for speech production in humans, as the trigeminal nerve provides motor supply to the muscles of mastication, which control the movement of the mandibles, and in addition the nerve transmits sensory information from the face. Thus, genetic variation at ROBO2 may be linked to both speech production abilities and expressive vocabulary size within children of the general population.

Rare recurrent ROBO2 deletions have been discovered in patients with autism spectrum disorder, a severe childhood neuro-developmental condition where core symptoms include deficits in social communication, and decreased ROBO2 expression has been observed in the anterior cingulate cortex and in lymphocytes of individuals with autism. Indeed, the 3p12–p13 region has been linked to dyslexia, and quantitative dyslexia traits, as well as quantitative speech-sound disorder traits and reading. The dyslexia linkage findings have been related to a specific SNP haplotype within ROBO1, a neighbouring gene of ROBO2. In animal models, ROBO1 and ROBO2 are mostly co-expressed and it has been shown that both receptors function cooperatively, for example, with respect to the guidance of most forebrain projections. Thus, it is possible that variation within both ROBO1 and ROBO2 might also contribute to the linkage signals within the reported regions, and our findings highlight ROBO2 as a novel, not yet investigated candidate locus.

Common polymorphisms within ROBO1 have also been associated with reading disability and with performance on tasks of non-word repetition, which is related to phonological short-term memory deficits. However, none of these previously reported ROBO1 variants (rs12495133, rs331142, rs4535189 and rs6803202) were associated with early word production scores within our study (Supplementary Table 12). Vice versa, we also found no association between rs7642482 (ROBO2) and language-related measures, including phonological memory and verbal intelligence in middle childhood, nor was there any association with expressive vocabulary during the later phase of language acquisition (24–30 months of age) or with very first single-word utterances at about 12 months of age. Instead, our findings suggest that the identified ROBO2 signal is specific for an early developmental stage of language acquisition (15–18 months of age), which is characterized by a slow accumulation of single words, followed by an increase in rate that is sometimes related to a ‘vocabulary spurt’. Both in silico analyses and the increase in signal after adjustment for gestational age support the hypothesis that expressive vocabulary during this phase may be affected by perinatal or early postnatal gene regulatory mechanisms. It is furthermore possible that the enhancer effect predicted within HUVEC also relates to a yet uncharacterized embryonic cell type, where expression changes are only detectable on the single-cell level. For example, during the trigeminal ganglion formation placode/neutral crest cells travel as individual cells to the site of ganglion formation, and ROBO2 appears to be expressed in discrete, dispersed regions in the surface ectoderm. This is characteristic of cells, which are about to detach and migrate. Thus, it will require further molecular studies to characterize the biological mechanisms underlying the observed ROBO2 association in more detail.

In line with previous findings, estimates from twin analysis and GCTA (based on large samples) suggest that the proportion of phenotypic variation in early expressive vocabulary, which is attributable to genetic factors, is modest. The concordance of twin and large-sample GCTA heritability estimates indicates, however, that most of this genetic variation is common and that there is little ‘missing heritability’. Thus, a large proportion of phenotypic variation in early expressive vocabulary may be captured by current GWAS designs, given sufficient power.

To conclude, this study describes genome-wide association between rs7642482 near ROBO2 and expressive vocabulary during an early phase of language acquisition where children typically communicate with single words only. The signal is
specific to this developmental stage, strengthened after adjust-
ment for gestational age, and links overall language-related
common genetic variation in the general population to a potential
autism susceptibility locus as well as a linkage region for dyslexia,
speech–sound disorder and reading on chromosome 3p12-p13.

Methods
Phenotype selection and study design. Consistent with the developmental
pattern of language acquisition, the analysis of children’s expressive vocabulary in
infancy was divided between an early phase (15–18 months of age, Fig. 1) and a
later phase (24–30 months of age, Fig. 2) and conducted using independent
individuals of up to four population-based European studies with both quantitative
expressive vocabulary scores and genotypes available (early phase: total N = 8,889;
later phase: total N = 10,819).

Expressive vocabulary scores were measured with age-specific-defined word lists
and either ascertained with adaptations of the MacArthur CDI13-17 or the LDS18
and based on parent-report. The CDIs were developed to assess the typical course
and variability in communicative development in children of the normal
population (8–30 months of age)15. The LDS was designed as a screening tool for
the identification of language delay in 2-year-old children16. Both measures have
sufficient internal consistency, test–retest reliability and validity18,19,20.

Expressive vocabulary during the early phase was captured by an abbreviated
version of the MacArthur CDI (Infant Version13, 8–16 months of age,
Supplementary Data 1) within the discovery cohort (ALSPAC, N = 6,851,
Supplementary Fig. 1a). Note, the Infant CDI has recently become also known as
CDI Words and Gestures24. A Dutch adaptation of the short-form version of the
MacArthur CDI (N-CDI 2A)14,16 was used within the follow-up cohort (GenR,
N = 20,038). Scores in both cohorts comprised both expressive and receptive
language aspects (‘says’ and understands’) and showed a positively skewed data
distribution (1.95 < skewness ≤ 2.39, Supplementary Data 1).

Vocabulary production during the later phase was measured with an abbreviated
version of the MacArthur CDI (Toddler version, 16–30 months of
age)13,15 in the discovery cohort (ALSPAC, N = 6,299, Supplementary Fig. 1b).
Note, the Toddler CDI has recently become also known as CDI Words and
Sentences25. Within the follow-up cohorts, expressive vocabulary was either
assessed with the LDS18 (GenR, N = 1,812; the Raine study N = 981) or an adapted
short form of the MacArthur CDI (MCDI)14,17 (Twins Early Development Study,
Teds, N = 1,727, independent individuals (one twin per pair), N = 5,733 twin pairs
(not all of them have genotype information available)). Later-phase expressive
vocabulary scores measured expressive language only (‘says’) and were either
symmetrically distributed or negatively skewed (–1.68 < skewness ≤ 0.24,
Supplementary Data 1).

In total, three different languages were included in our analyses: English (three
samples: ALSPAC; Teds; Raine), Dutch (one sample: GenR) and Finnish
(sensitivity analysis: Northern Finnish Birth Cohort (NFBC) 1986). The
cross-cultural comparability of the CDI has been explored, and the measures in many
languages, including Dutch and English, show minimal differences in vocabulary
production scores in the early years25. In addition, the standardization within each

sample (see below) would have removed any minor differences between
instruments.

For study characteristics, details on phenotype acquisition and psychological
instruments as well as summary phenotype characteristics (including mean, s.d.,
kurtosis, skewness and age at measurement) are presented for each cohort
and developmental phase in Supplementary Data 1.

For each participating study, ethical approval of the study was obtained by the
local research ethics committee, and written informed consent was provided by all
parents and legal guardians. Detailed information on sample-specific ethical
approval and participant recruitment is provided in Supplementary Note 1.

Genotyping and imputation. Genotypes within each cohort were obtained using
high-density SNP arrays (Supplementary Data 1). Cohort-specific genotyping
information including genotyping platform, quality control (QC) for individuals
and SNPs, the final sample size, the number of SNPs before and after imputation as
well as the imputation procedures are detailed in Supplementary Data 1. Briefly, for
individual sample QC, this included filtering according to call rate, heterozygosity
and ethnic/other outliers, and for SNP QC (prior to imputation) filtering
according to minor allele frequency, call rate and SNPs with deviations from
Hardy–Weinberg equilibrium (detailed exclusion criteria are listed in
Supplementary Data 1). Genotypes were subsequently imputed to HapMap CEU
(phase II and/or III) and/or Wellcome Trust Controls (Supplementary Data 1).
For sensitivity analysis, ALSPAC genotypes on chromosome 3 were also locally
imputed to 1,000 Genomes (v3.20101123, Supplementary Data 1).

Single-variant association analysis. Within each cohort, expressive vocabulary
scores were adjusted for age, age-squared, sex and the most significant ancestry-
informative principal components58 and subsequently rank-transformed to
normality to facilitate comparison of the data across studies and instruments.
The association between SNP and the expressive vocabulary score was assessed
within each cohort using linear regression of the rank-transformed expressive
vocabulary score against allele dosage, assuming an additive genetic model.

In the discovery cohort, the genome-wide association analysis for each phase
was carried out using MACH2QTL57 using 2,449,665 imputed or genotyped SNPs.
SNPs with a minor allele frequency of <0.01 and SNPs with poor imputation
accuracy (MACH R² ≤0.3) were excluded prior to the analysis, and all statistics
were subjected to genomic control correction58 (Supplementary Data 1). All
independent SNPs from the early- and later-phase GWAS below the threshold of
P < 10^-8 (85 and 50 SNPs, respectively) were selected for subsequent follow-up
analysis in additional cohorts. Independent SNPs were identified by linkage
disequilibrium-based clumping using PLINK26 Proxy SNPs within ± 500 kb,
linkage disequilibrium r² ≥0.3 (Hapmap II CEU, Rel 22) were removed. All
analyses within the follow-up samples were carried out in silico using MACH2QTL
or SNPTEST60 software (Supplementary Data 1). For the selected SNPs, estimates
from the discovery (genomic-control corrected) and follow-up cohort(s) were
combined using fixed-effects inverse-variance meta-analysis (R ‘meta’ package).
While testing for overall heterogeneity using Cochran’s Q-test. Signals below a
geno-wide significance threshold of P < 2.5 × 10⁻⁸ (accounting for two GWAS
analyses) were considered to represent robust evidence for association.
An empirical approach (Bootstrapping with 10,000 replicates) was selected to obtain meaningful genetic effects (basic 95% bootstrap confidence interval) of the reported SNPs in the discovery cohort. For this, we utilized a linear model of z-standardized expressive vocabulary scores against allele dosage, adjusted for age, age-squared, sex and the most significant ancestry-informative principal components. The local departmental server of the School of Social and Community Medicine of the University of Bristol was used for data exchange and storage.

Sensitivity analysis in ALSPAC using locally imputed genotypes on chromosome 3 (based on 1,000 Genomes) was performed as linear regression of the rank-transformed expressive vocabulary score against allele dosage, assuming an additive genetic model, using MACH2QTL (Supplementary Data 1).

Direct genotyping of reported SNPs. Reported SNPs with a medium imputation accuracy of MACH $R^2<0.80$ were re-genotyped in the discovery cohort (ALSPAC) to confirm the validity of the observed association signal (rs10734234). Genotyping was undertaken by LGC Genomic Ltd (http://www.lgcgenomics.com/) using a form of competitive allele-specific PCR system (KASPar) for SNP analysis.

Variance explained. To estimate the variation in expressive vocabulary scores explained by each reported SNP and jointly by all reported SNPs together, we calculated the adjusted regression $R^2$ values from ($i$) univariate linear regression of the rank-transformed expressive vocabulary score (see above) against allele dosage and ($ii$) multivariate linear regression of the rank-transformed expressive vocabulary score (see above) against the allele dosage from all reported SNPs. All analyses were performed using R, SPSS or STATA software.

Phenotypic characterization of association signals. To investigate whether there is an association between the first single-word utterances at approximately 12 months and the reported SNPs, we conducted an association analysis in the NFBC 1966. The number of spoken words in the NFBC 1966 (word-list free assessment, ‘words’ are defined) were based on parental response to a questionnaire administered at 12 months of age (Supplementary Data 1). Given the scarcity of categories referring to three or more spoken words, word numbers were dichotomized into ‘1 + words’ (one or more words, 1) versus ‘no words’ (0). The association between early word-production scores and allele dosage of the reported SNPs was studied using logistic regression models, adjusted for sex and the most significant principal components (as exact age at measurement was not available) using SNPTEST.

Pre-school language deficits have been repeatedly associated with later problems in language development, especially reading skills41. To assess whether genetic effects affecting expressive language skills early in life also influence language competencies during later development, we investigated the association between reported SNP signals and a series of language-related cognitive measures previously reported in the ALSPAC cohort (Supplementary Table 8). All outcomes were z-standardized prior to analysis. The association between the transformed outcome and SNP allele dosage was investigated using linear regression adjusted for sex, the most significant principal components and age (except for age-normalized intelligence quotient scores, Supplementary Table 9).

To assess whether gestational age and maternal education influence the association between the reported signals and early expressive vocabulary scores, we ($i$) investigated the association between these potential covariates and the SNPs directly and ($ii$) adjusted the association between genotypes and language measures for potential covariates effects. Gestational age in the relevant ALSPAC cohort was either estimated from medical records or obtained from midwife and hospital registries at birth (Supplementary Data 1), and measured in completed weeks of gestation. Information on maternal education was obtained from antenatal questionnaire data, and dichotomized into lower (1) and higher (0) maternal education (Supplementary Data 1). The association between gestational age and allele dosage for reported SNPs was investigated with linear regression models and adjusted for sex and the most significant principal components in each cohort. The link between maternal education and these SNPs was studied using logistic regression models adjusted for the most significant principal components in each cohort.

We furthermore created new transformations of expressive vocabulary scores, that is, the reported number of words were in addition to the previously described variables (see above) adjusted for gestational age and maternal education, respectively, before they were rank-transformed. Association analysis for reported SNPs was then carried out as described for discovery, follow-up and combined analysis before. All analyses were carried out using R, SPSS or STATA software.

GCTA. The proportion of additive phenotypic variation jointly explained by all genome-wide SNPs together (GCTA heritability) was estimated for all cohorts and analyses windows using GCTA32. In brief, using a sample of independent individuals, the method is based on the comparison of a matrix of pairwise genomic similarity with a matrix of pairwise phenotypic similarity using a random-effects mixed linear model32. Pertinent to this study, GCTA (Supplementary Data 1) was carried out using a rank-transformed expressive vocabulary score (previously adjusted for age, sex and the most significant ancestry-informative principal components in each cohort, see above) and directly genotyped SNPs (ALSPAC, GenK, Raine) or most likely imputed genotypes (TEDS). GCTA estimates from different cohorts were combined using fixed-effects inverse-variance meta-analysis and thereby symmetrically distributed, i.e., while testing for overall heterogeneity using Cochran’s Q-test. The extent to which the same genes contribute to the observed phenotypic correlation between two variables can be furthermore estimated through genetic correlation. To do so, all cohorts with expressive vocabulary measures at two time points (ALSPAC and GenK), genetic correlation ($r_g$) between the rank-transformed scores was estimated using bivariate GCTA analysis33 (based on the genetic covariance between two traits).

Twin analysis. Twin analyses allow the estimation of the relative contributions of genes and environments to individual differences in measured traits. Twin intra-class correlations were calculated34, providing an initial indication of the relative contributions of additive genetic (A), shared environmental (C) and non-shared environmental (E) factors. Additive genetic influence, also commonly known as heritability, is estimated as twice the difference between the identical and fraternal twin correlations. The contribution of the shared environment, which makes members of a family similar, is estimated as the difference between the identical twin correlation and heritability. Non-shared environments, that is, environments specific to individuals, are estimated by the difference between the identical twin correlation and 1, because they are the only source of variance making identical twins different. Estimates of the non-shared environment also include measurement error.

Maximum likelihood structural equation model-fitting analyses allow more complex analyses and formal tests of significance35. Standard twin model-fitting analyses were conducted using Mx36. The model fit is summarized by minus twice the log likelihood ($–2LL$). Differences in $–2LL$ between models provide a goodness of fit statistic. A change in $–2LL$ of 3.84 is significant for a 1 degree of freedom test. Model fit was compared between the full ACE model and the saturated model (where variances are not decomposed into genetic and environmental sources). Reduced models testing CE, AE and E models were compared with the full ACE model and the saturated model. A significant $P$ value indicates a significantly worse fit.

Twin analysis was carried out on rank-transformed expressive vocabulary scores at 24 months (adjusted for age, age-squared and sex), which were assessed in 5,733 twin pairs (monozygotic twins N = 1,969; dizygotic twins (male, female and opposite sex) N = 3,764) from the TEDS39. The URLs for all utilized web pages are given in Supplementary Note 2.

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Northern Finland Birth Cohort 1966 (NFBC 1966)
We thank the late Professor P. Rantakallio (launch of NFBC 1966 and 1986), Ms O. Tornwall and Ms M. Jussila (DNA biobanking). Financial support was received from the Academy of Finland (project grants 104781, 120315, 1114194 and Center of Excellence in Complex Disease Genetics), University Hospital Oulu, Biocenter, University of Oulu, Finland, NHLBI grant R01HL087679-02 through the STAMPEED program (1RL1MH083268-01), ENGAGE project and grant agreement HEALTH-F4-2007-201414, the Medical Research Council (studentship grant G0500539, PrevMetSyn/Salve/MRC), the Wellcome Trust (project grant GR069224), UK. The DNA extractions, sample quality controls, biobank up-keeping and aliquotting was performed in the National Public Health Institute, Biomedicum Helsinki, Finland and supported financially by the Academy of Finland and Biocentrum Helsinki.

Twins Early Development Study (TEDS)
We are enormously grateful to the twins, parents and the twins’ teachers who have supported the Twins Early Development Study (TEDS) for the past 18 years. The TEDS is supported by a program grant from the UK Medical Research Council (G0901245, and previously G0500079), with additional support from the US National Institutes of Health (HD044454, HD059215). We would like to thank the Wellcome Trust Case Control Consortium 2 (WTCCC2) consortium (Supplementary Note 3) for their help with genome-wide genotyping, which was made possible by grants from the WTCCC2 project (085475/B/08/Z, 085475/Z/08/Z). C.M.A.H. was supported by a research fellowship from the British Academy. O.S.P.D. was supported by a Sir Henry Wellcome Fellowship from the Wellcome Trust (WT088984). R.P. was supported by a research professorship from the UK Medical Research Council (G19/2) and a European Research Council Advanced Investigator Award (295366).

Western Australian Pregnancy Cohort study (Raine)
We are grateful to the Raine Foundation, to the Raine Study Families and to the Raine Study research staff. We gratefully acknowledge the assistance of the Western Australian Genetic Epidemiology Resource and the Western Australian DNA Bank (both National Health and Medical Research Council of Australia National Enabling Facilities). We also acknowledge the support of the Healthway Western Australia, the National Health and Medical Research Council of Australia (Grant 572613) and the Canadian Institutes of Health Research (Grant MOP 82893). We gratefully acknowledge the assistance of the Wind Over Water Foundation, the Telethon Institute for Child Health Research and the Raine Medical Research Foundation of the University of Western Australia. A.J.O.W. was supported by a Career Development Fellowship from the NHMRC (Grant number 1004065).

Wuerzburg University research collaboration
We thank T. Haaf, E. Schneider and N. El Hajj (Department of Human Genetics, University of Wuerzburg, Germany) for helpful discussions about the biological role of ROBO2.

EARly Genetics and Lifecourse Epidemiology (EAGLE) consortium
This work was carried out in collaboration with the EAGLE consortium (http://research.lunenfeld.ca/eagle/).

Author contributions
B.S.P., R.A.M.C., A.J.O.W., C.M.A.H., O.S.P.D., P.F.O’R., Q.W.A., F.P.V. and N.M.W. performed study-level data analysis. Study design was by B.S.P., R.A.M.C., A.J.O.W., C.M.A.H., O.S.P.D., J.G., S.R., Y.W., H.T. and G.D.S. B.S.P., R.A.M.C., A.J.O.W., C.M.A.H., O.S.P.D., P.F.O’R., S.R. and Y.W. wrote the paper. Data collection was by S.R., Y.W., L.M., E.L.M., T.S.P., D.P., V.W.V.J., M.-R.J., R.P., C.E.P., H.T. and G.D.S. Genotyping was performed by B.S.P., O.S.P.D., D.M.E., J.P.K., N.M.W., S.M.R., F.R., E.L.M., T.S.P., D.P., Y.W.V.J., M.-R.J., R.P., C.E.P. and G.D.S. B.S.P., R.A.M.C. A.J.O.W., C.M.A.H., O.S.P.D., P.F.O’R., S.R., Y.W., Q.W.A., F.P.V., D.M.E., J.P.K., N.W., L.M., N.J.T., S.M.R., T.C.V., A.H., F.R., E.L.M., T.S.P., P.S.D., D.P., A.R., J.G., V.W.V.J., M.-R.J., R.P., C.E.P., H.T. and G.D.S. revised and reviewed the paper.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: St Pourcain, B. et al. Common variation near ROBO2 is associated with expressive vocabulary in infancy. Nat. Commun. 5:4831 doi: 10.1038/ncomms5831 (2014).

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