Associations of Prenatal Nicotine Exposure and the Dopamine Related Genes ANKK1 and DRD2 to Verbal Language

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Abstract

Language impairment (LI) and reading disability (RD) are common pediatric neurobehavioral disorders that frequently co-occur, suggesting they share etiological determinants. Recently, our group identified prenatal nicotine exposure as a factor for RD and poor reading performance. Using smoking questionnaire and language data from the Avon Longitudinal Study of Parents and Children, we first determined if this risk could be expanded to other communication disorders by evaluating whether prenatal nicotine exposure increases risk for LI and poor performance on language tasks. Prenatal nicotine exposure increased LI risk (OR = 1.60; p = 0.0305) in a dose-response fashion with low (OR = 1.25; p = 0.1202) and high (OR = 3.84; p = 0.0002) exposures. Next, hypothesizing that the effects of prenatal nicotine may also implicate genes that function in nicotine related pathways, we determined whether known nicotine dependence (ND) genes associate with performance on language tasks. We assessed the association of 33 variants previously implicated in ND with LI and language abilities, finding association between ANKK1/DRD2 and performance on language tasks (p<0.0003). The associations of markers within ANKK1 were replicated in a separate LI case-control cohort (p<0.05). Our results show that smoking during pregnancy increases the risk for LI and poor performance on language tasks and that ANKK1/DRD2 contributes to language performance. More precisely, these findings suggest that prenatal environmental factors influence in utero development of neural circuits vital to language. Our association of ANKK1/DRD2 further implicates the role of nicotine-related pathways and dopamine signaling in language processing, particularly in comprehension and phonological memory.

Introduction

Language impairment (LI) and reading disability (RD) have prevalences of 5–8% and 5–17%, respectively, in schoolchildren [1–2], and together comprise the majority of learning disabilities. LI and RD are characterized by difficulty in the understanding and/or utilization of verbal and written language, respectively, despite normal development and adequate educational opportunity [1–2]. LI and RD are related disorders, as both involve deficits in the integration and utilization of communicative tools. Impaired phonological skills have been implicated in both LI and RD [1–7]. LI and RD are frequently comorbid; as children with LI are at higher risk of developing RD than their typically developing peers [1–2,8]. The degree of relatedness and the frequent comorbidity of LI and RD indicate they may share risk factors. Twin and family studies have shown that both LI and RD have a significant genetic component, with heritability estimates of 45–73% and 54–64%, respectively [8–10]. However, specific environmental and genetic risk factors for LI and RD, and the extent to which they are shared between the two disorders, remain largely unknown.

One possible environmental risk factor for LI and RD is exposure of the developing fetus to toxins and substances in utero via the maternal environment and behavior, specifically smoking or nicotine exposure. The harm of prenatal nicotine exposure has been well-documented [11–13]. Despite this, studies estimate 14–37% of women smoke during pregnancy [14]. Prenatal nicotine exposure is a risk factor for several neurobehavioral conditions such as Attention Deficit-Hyperactivity Disorder (ADHD), learning disabilities, and substance abuse [15–17]. Some studies have
expanded these findings to RD, LI, and neurocognition [18–
19,21]; while others have argued that nicotine variables may
capture factors not adequately controlled for in statistical models,
such as socioeconomic status [20–21]. Our recent work showed an
association between prenatal nicotine exposure and poor reading
performance in schoolchildren, after adjustment for a wide range of
confounders, including socioeconomic status, type of school
attended, birth weight, and gestational age [22]. However, further
study is necessary to determine whether prenatal nicotine exposure
also influences language abilities and LI.

The implication of prenatal nicotine exposure in communica-
tion performance raises the question of how this exposure exerts its
effects. One possibility is that genetic variants previously associated
with nicotine dependence (ND) and nicotine-related pathways
may have pleiotropic effects. That is, genetic variants that
disposition individuals to ND may also contribute to communi-
cation processes. Studies of ND have revealed that it has a
significant genetic component and identified several candidate
genes, including DRD2, ANKK1, CHRNA4, and CHRNA5. Many
of these genes are involved in neuronal signaling pathways, including
the cholinergic and dopaminergic neurotransmitter pathways. The
implication of various signaling pathways further suggests that
variation in these genes may affect multiple cerebral processes,
such as addiction, language, and reading. Several of these ND
genes, including BDNF, DRD2, and ANKK1, have been associated
with neurobehavioral phenotypes [23–24]. ANKK1 and DRD2
have been associated with autism, executive functioning, and
verbal ability [25–26]. However, these reports are few in number,
and replication in larger cohorts is needed.

The present study expands on our previous work to examine
prenatal nicotine exposure and its related pathways with regard to
LI and its associated language domains. First, we analyze the
relationship of prenatal nicotine exposure with performance on
language tasks and LI. Due to nicotine’s detrimental effects on
brain function, we hypothesize that prenatal smoking exposure will
also be a risk factor for poor language performance and LI.
Second, we assess whether known ND variants contribute to
language abilities. ND genes have known neurological functions,
particularly in neuronal signaling; therefore, we also hypothesize
ND variants associate with language performance and LI.

Materials and Methods

Subjects

The Avon Longitudinal Study of Parents and Children
(ALSPAC) is a population-based, birth cohort in Avon, United
Kingdom. Subjects were recruited before birth, resulting in a total
of 15,436 fetuses, of whom 14,701 were alive at 1 year of age.
Recruitment, participants, and study methodologies are described
in detail elsewhere [http://www.bristol.ac.uk/alspac] [27–28].
7170 subjects completed language measures at age 8 years.
Subjects with IQ ≤75 on the Wechsler Intelligence Scale for
Children (WISC-III) Total IQ were excluded from the present
study [29]. To prevent population stratification in genetic analyses,
subjects of non-European descent were also removed. Addition-
ally, samples with genotyping call rate <0.80 were excluding from
analyses, leaving a final sample size of 3579 individuals. Ethical
approval was obtained from ALSPAC Ethics and Law Committee,
Local UK Research Ethics Committees, and Yale Human
Investigation Committee.

ALSPAC Language Measures

Language measures were collected during clinical interviews at
age 8 years. An adaptation of the Nonword Repetition Task
(NWR), in which subjects repeated recordings of nonwords, was
used to assess short-term phonological memory and processing
abilities [30]. Children also completed the Wechsler Objective
Language Dimensions (WOLD) verbal comprehension task at age
8 years [31], where they answered questions about a paragraph
read aloud by an examiner describing a presented picture. We
focused on these measures because individuals with LI are known
to consistently perform poorly on NWR and WOLD comprehen-
sion tasks, and these tasks are commonly used in genetic and
epidemiologic studies of LI [32–33]. Z-scores were calculated for
each subject on each individual measure, and to capture deficits in
two of the primary domains of LI, the average z-score of NWR
and WOLD comprehension tasks was calculated. To assess the
risk imparted to severe LI, we defined LI cases as scoring ≥2.0
standard deviations below sample means on either task.

Exposure and Covariate Variables

Questionnaires for smoking frequency and cigarette brand were
completed by mothers at gestational age 8, 18, and 32 weeks and
at 8 weeks following birth. Although cigarettes contain thousands
of compounds, nicotine is the most prevalent, pharmacologically
active ingredient that is likely responsible for smoking’s deleterious
effects. Therefore, we calculated the level of nicotine exposure for
each time point based upon the nicotine content of the cigarette
brands smoked. Because of limited power to divide nicotine
exposure into trimesters, we used the maximum nicotine exposure
to derive prenatal nicotine exposure [22,34–35]. First, prenatal
nicotine exposure was dichotomized into exposed and non-
exposed groups. To examine dose-response, prenatal nicotine
exposure was further categorized into three groups: no exposure
(0 mg*day$^{-1}$), low exposure (≤17 mg*day$^{-1}$), and high exposure
(>17 mg*day$^{-1}$) [36]. 17 mg was chosen as it is the average
amount of nicotine in one pack of cigarettes.

Due to the interdependence between overall cognition and
communication, subjects with WISC-III Total IQ scores ≤75
were excluded from analysis [37]. To further control for the effects
of IQ, WISC-III Performance IQ scores were included as a
covariate in analyses [29]. Performance IQ was chosen to prevent
controlling for language abilities captured by Verbal and Total IQ
scores. In addition to Performance IQ, we adjusted for the
following 11 covariates to control for known confounding
relationships with language: mother’s age at delivery, maternal
prenatal alcohol consumption [38], maternal social class, child-
parent interaction time, mother’s attendance at antenatal classes,
sex, ADHD status, school type, gestational age, birthweight,
and resuscitation status [39] (Table S1).

Statistical and Genetic Analyses

First, SAS 9.2 was used to statistically analyze the association of
prenatal nicotine exposure with language performance in the
ALSPAC cohort. Dichotomized prenatal nicotine exposure status
was examined first, followed by dosage categories. For quantitative
measures, we fitted crude linear regression models, with prenatal
nicotine exposure as the predictor for each language outcome.
Next, multivariable regression models adjusted for covariates were
used to identify specific effects of prenatal nicotine exposure. We
used logistic regression models to fit prenatal nicotine exposure
and covariates for each dichotomized language measure. Odds
ratios (OR) were calculated for exposed/non-exposed, then for the
low and high dosage categories.

Next, 33 single nucleotide polymorphisms (SNPs) in 12 genes,
previously implicated in ND, nicotine pathways, and/or substance
dependencies, were genotyped on the Sequenom platform (San
Diego, CA), following the manufacturers guidelines at the Yale
Center for Genome Analysis (Orange, CT) (Table 1). 32 of the 33 ND variants had call rates $\geq 90\%$, were biallelic, had minor allele frequencies $\leq 0.01$, and were in Hardy-Weinberg equilibrium ($p < 0.001$). To correct for the 32 genetic association tests performed in the ALSPAC cohort, Bonferroni correction was applied to adjust for multiple testing ($\alpha = 0.05/32 = 1.56 \times 10^{-2}$).

Since these ND variants have a prior relationship with nicotine and/or addiction, we created a subsample of subjects not exposed to nicotine and repeated associations to avoid possible confounding.

Associated variants were then examined in the Iowa LI cohort. The Iowa LI cohort is comprised of 219 LI cases and 209 sex- and age-matched, unrelated controls collected at the University of Iowa. Subjects completed various language measures, including the Peabody Picture Vocabulary Test (PPVT) and NWR, which were used to derive a composite language score, which was dichotomized into case-control status at 2 median standard deviations [40]. Single marker analysis in both cohorts was performed with linear and logistic regression under additive models using SNP & Variation Suite (SVS) v7.6.4 (Golden Helix, Bozeman, MT). Haplotype regions were constructed following the 4-gamete rule using HaploView v4.2, and haplotype association tests were performed using PLINK v1.07.

### Results

**Prenatal Nicotine Exposure and Language**

In the ALSPAC sample, subjects exposed to prenatal nicotine performed on average 4.75–5.39% worse on language measures compared to non-exposed subjects (Table 2). When separated into nicotine dosage categories, those exposed to high levels of prenatal nicotine performed on average the worst on all measures compared to low (ranging from 6.20–7.95% worse) and no exposure (ranging from 9.63–11.58%) groups (Table 2).

Crude linear regression analyses comparing groups exposed to prenatal nicotine to the non-exposed groups showed that prenatal nicotine exposure is associated with performance on NWR and comprehension tasks ($p < 0.0002$) (Table 3). After adjusting for covariates, the association with average performance on the NWR/comprehension tasks persisted ($p = 0.0262$), while there was a trend with the NWR task ($p = 0.0799$). Crude analyses for exposure dosage showed a deleterious effect of prenatal nicotine exposure on NWR and comprehension tasks ($p < 0.0002$) (Table 4). After covariate adjustment, there was a negative effect of high dose of prenatal nicotine exposure on comprehension ($p = 0.0011$) and average performance on NWR/comprehension ($p = 0.0011$), with trend toward a negative effect of high exposure for the NWR task alone ($p = 0.0729$).

### Table 1. Nicotine dependence (ND) markers genotyped in the ALSPAC sample.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Gene</th>
<th>Location</th>
<th>MAF</th>
<th>Variant</th>
<th>Gene</th>
<th>Location</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2072660</td>
<td>CHRNB2</td>
<td>1q21.3</td>
<td>0.240</td>
<td>rs10893365</td>
<td>PKNOX2</td>
<td>11q24.2</td>
<td>0.171</td>
</tr>
<tr>
<td>rs2072661</td>
<td>CHRNB2</td>
<td>1q21.3</td>
<td>0.244</td>
<td>rs10893366</td>
<td>PKNOX2</td>
<td>11q24.2</td>
<td>0.168</td>
</tr>
<tr>
<td>rs1246358</td>
<td>CHRND</td>
<td>2q31</td>
<td>0.253</td>
<td>rs11220015</td>
<td>PKNOX2</td>
<td>11q24.2</td>
<td>0.174</td>
</tr>
<tr>
<td>rs13277254</td>
<td>CHRNB3</td>
<td>8p21</td>
<td>0.212</td>
<td>rs11602925</td>
<td>PKNOX2</td>
<td>11q24.2</td>
<td>0.176</td>
</tr>
<tr>
<td>rs4950</td>
<td>CHRNB3</td>
<td>8p21</td>
<td>0.214</td>
<td>rs12284594</td>
<td>PKNOX2</td>
<td>11q24.2</td>
<td>0.170</td>
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<tr>
<td>rs6474413</td>
<td>CHRNB3</td>
<td>8p21</td>
<td>0.214</td>
<td>rs1426153</td>
<td>PKNOX2</td>
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<td>0.174</td>
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<tr>
<td>rs4075274</td>
<td>NTRK2</td>
<td>9q21.33</td>
<td>0.434</td>
<td>rs750338</td>
<td>PKNOX2</td>
<td>11q24.2</td>
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<tr>
<td>rs2030324</td>
<td>BDNF</td>
<td>11p14.1</td>
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<td>rs1051730</td>
<td>CHRNA3</td>
<td>15q25</td>
<td>0.329</td>
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<tr>
<td>rs4274224</td>
<td>DRD2</td>
<td>11q23.1</td>
<td>0.493</td>
<td>rs1317266</td>
<td>CHRNA3</td>
<td>15q25</td>
<td>0.226</td>
</tr>
<tr>
<td>rs4648318</td>
<td>DRD2</td>
<td>11q23.1</td>
<td>0.239</td>
<td>rs578776</td>
<td>CHRNA3</td>
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<td>rs6495308</td>
<td>CHRNA3</td>
<td>15q25</td>
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<td>rs6278</td>
<td>DRD2</td>
<td>11q23.1</td>
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<td>rs8034191</td>
<td>LOC123688</td>
<td>15q25</td>
<td>0.331</td>
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<td>rs11604671</td>
<td>ANKK1</td>
<td>11q23.1</td>
<td>0.488</td>
<td>rs16969968</td>
<td>CHRNA5</td>
<td>15q25</td>
<td>REMOVED</td>
</tr>
<tr>
<td>rs1800497</td>
<td>ANKK1</td>
<td>11q23.1</td>
<td>0.197</td>
<td>rs2229959</td>
<td>CHRNA4</td>
<td>20q13.33</td>
<td>0.113</td>
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<tr>
<td>rs2734849</td>
<td>ANKK1</td>
<td>11q23.1</td>
<td>0.485</td>
<td>rs2236196</td>
<td>CHRNA4</td>
<td>20q13.33</td>
<td>0.252</td>
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<tr>
<td>rs4938013</td>
<td>ANKK1</td>
<td>11q23.1</td>
<td>0.321</td>
<td>rs2273504</td>
<td>CHRNA4</td>
<td>20q13.33</td>
<td>0.162</td>
</tr>
<tr>
<td>rs7118900</td>
<td>ANKK1</td>
<td>11q23.1</td>
<td>0.185</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ND, nicotine dependence; MAF, minor allele frequency.
doi:10.1371/journal.pone.0063762.t001

### Table 2. Descriptive statistics of language scores among exposure groups.

<table>
<thead>
<tr>
<th></th>
<th>Non-smoking</th>
<th>Any Exposure</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N Mean(SD)</td>
<td>N Mean(SD)</td>
<td>N Mean(SD)</td>
<td>N Mean(SD)</td>
</tr>
<tr>
<td>NWR</td>
<td>4720 7.37(2.43)</td>
<td>758 7.02(2.48)</td>
<td>615 7.10(2.45)</td>
<td>143 6.66(2.56)</td>
</tr>
<tr>
<td>Comprehension</td>
<td>4724 7.60(1.91)</td>
<td>760 7.19(1.93)</td>
<td>617 7.30(1.93)</td>
<td>143 6.72(1.88)</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; NWR, nonword repetition.
doi:10.1371/journal.pone.0063762.t002
In ALSPAC, LI had a prevalence of 4.90%, which is consistent with estimates in the general population [1–2]. Exposure to prenatal nicotine increased risk for LI, after controlling for covariates (OR = 1.60 [1.04–2.45]; p = 0.0305) (Table 3). Risk of developing LI occurred in a dose response fashion with low (OR = 1.25 [0.76–2.04]; p = 0.1202) and high (OR = 3.85 [1.87–7.94]; p = 0.0009) prenatal nicotine exposure levels (Table 6).

### Association of ND Markers to Language

Single-marker analysis revealed associations between SNPs within **ANKK1** and language performance as measured by the average z-score on NWR/comprehension tasks (p≤1.9×10−5) (Table 7). Haplotype associations were similar, showing association between a haplotype containing **ANKK1** and **DRD2** markers and language performance (Table 8). This haplotype included a majority of the significant SNPs from single marker analysis, suggesting these markers captured the same variability in the locus. Interestingly, the **ANKK1** haplotype block contained a marker in the **DRD2** gene located adjacent to **ANKK1** (rs6278) (Figure 1). These associations persisted when examined in ALSPAC subjects not exposed to prenatal nicotine (Table 7). There was no evidence of interaction between prenatal nicotine exposure and ND variants in the ALSPAC sample. Associations of SNPs within **ANKK1** (rs2734849 and rs11604671) were from epigenetic modifications due to the nicotine exposure [41].

### Discussion

Our investigation examined the effects of prenatal nicotine exposure and nicotine-related genetic variants on LI and performance on language tasks. We found increased risk of LI and poor performance on language tasks in subjects exposed to prenatal nicotine. In addition, there was a genetic association between single markers within **ANKK1** and a haplotype spanning **ANKK1**/**DRD2** and language performance, further implicating nicotine-related and dopamine pathways in language. These findings show the importance of the prenatal environment and dopamine to language and cognitive development.

### Prenatal Nicotine Exposure and Language

We found an association of prenatal nicotine exposure on language performance and LI, after adjusting for known covariates, such as socioeconomic status, type of school attended, and parent interaction. This relationship appears to be specific to language skills and independent of overall cognitive skills, as Performance IQ was accounted for in all final models. These results expand upon our previous findings, showing the detrimental effects of prenatal nicotine exposure on phonology, reading fluency, reading comprehension, and reading accuracy. These components are foundational to the development of reading and language skills in children. Our previous study found that deficits in reading comprehension similar to the ones we found in verbal comprehension, suggesting prenatal nicotine exposure exerts an effect on how children ascertain meaning in verbal and written language.

The negative effects of prenatal nicotine exposure on reading and language may reflect changes in gene expression resulting from epigenetic modifications due to the nicotine exposure [41]. Future studies should examine how nicotine exposure interacts with genes associated with communication, such as **DCDC2**, **KIAA0319**, and **FOXP2**, and their epigenetic regulation. One investigation demonstrated the contribution of 5’ regions marked by acetylated H3 histones in **KIAA0319** to RD, suggesting the importance of epigenetic regulation to language [42]. Epigenetic studies in combination with neurotoxicological studies should be explored to determine whether and how nicotine exposure alters gene expression and cellular function.

### Table 3. Effects of any prenatal nicotine exposure on language performance.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Crude Model</th>
<th>Adjusted Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed</td>
<td>Overall</td>
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<tr>
<td>NWR</td>
<td>Beta</td>
<td>p-value</td>
</tr>
<tr>
<td>Comprehension</td>
<td>−0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Avg NWR Comp</td>
<td>−0.18</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Table 4. Effects of prenatal nicotine dosage on language performance.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Crude Model</th>
<th>Adjusted Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>NWR</td>
<td>Beta</td>
<td>p-value</td>
</tr>
<tr>
<td>Comprehension</td>
<td>−0.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Avg NWR Comp</td>
<td>−0.13</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: NWR, nonword repetition; Avg NWR Comp. average of z-scores of nonword repetition and verbal comprehension tasks.

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In addition to possible changes directly to gene regulation, mouse and rat models have shown that prenatal nicotine exposure permanently affects neurochemical signaling pathways, including dopaminergic pathways, and alter their developmental trajectories over the lifespan [43–45]. Animals exposed to gestational nicotine have higher dopamine turnover in the frontal cortex [44,46]. These results from animal models, in conjunction with our findings implicating prenatal nicotine and dopamine signaling in LI, suggest that deficits and permanent changes in dopamine activity resulting from exposure and genetic variants have a substantial influence upon language skills and development. These implications, however, still must be explored and confirmed in human studies, possibly through magnetic resonance spectroscopy (MRS). MRS would permit in vivo monitoring of dopamine signaling in the human brain. MRS could specifically interrogate the influence of prenatal nicotine exposure on dopaminergic signaling, gene expression, and language in the same subjects.

**ANKK1/DRD2 and Language**

Single marker and haplotype analyses showed association between language performance and ANKK1/DRD2. In addition to past associations with ND, ANKK1 and DRD2 have been associated with other neurobehavioral traits including alcohol dependence, reinforcement learning, working memory, and executive function [47–51]. Dopamine is a key neurotransmitter in the corticostriatal system that subserves procedural and reinforcement learning. Animal and human studies, using dopamine agonists and/or antagonists, show that alterations in dopamine receptor function change reinforcement learning [52]. Recently, reinforcement learning was shown to be associated with individual differences in language in a task influenced by dopamine signaling [53–54]. Additionally, past studies have associated ANKK1/DRD2 to working memory. Working memory is directly associated with language skills in children, and in fact, impairments in working memory have been proposed to play a direct role in the development of language deficits seen in children with LI [55–56]. Changes in dopaminergic function, whether from genetic predisposition (ANKK1/DRD2) or environmental exposure (prenatal nicotine), yield alterations in working memory and reinforcement learning. These changes, which arise via permanent alterations in dopamine function, appear to influence language development as well as other neurobehavioral domains, including nicotine and substance use.

Despite the wide range of literature examining ANKK1/DRD2 and neurobehavioral traits, there have been limited reports examining the role of ANKK1 and DRD2 specifically in language and language-related domains. Beaver et al. reported an association between DRD2 and performance on an abbreviated form of the PPVT [26]. The PPVT is a standardized measure of expressive and receptive vocabulary, which may be analogous to deficits measured in our verbal and reading comprehension tasks, although the tasks in this study measure higher order cognitive processing. Our findings expand the role of ANKK1 and DRD2 from known effects on working memory, reinforcement learning, and predisposition to nicotine use to now include verbal language. Additionally, these findings point to a role for dopamine as a mechanism in processes involved in language development. In this regard, these findings and the implications of prenatal nicotine exposure on brain neurochemistry support the notion that procedural learning, rooted in the dopamine rich basal ganglia, plays an important role in language development [57–59].

The relationship between the neighboring genes ANKK1 and DRD2 has been a source of controversy. In our study, we found association between language and a haplotype block stretching across ANKK1 and DRD2, suggesting that we, like most studies, are unable to refine our associations to a single gene. However, previous work has shown that the rs1800497 polymorphism is associated with the number of D2 dopamine binding sites and glucose metabolism in the central nervous system [60–62]. Reduced dopamine signaling and glucose metabolism in the central nervous system may adversely affect high order cognitive functioning including verbal language processing. Functionally, there is limited evidence on how ANKK1 and DRD2 may interact. Huang et al. suggested ANKK1 may influence DRD2 expression via NF-kB signaling [63]. However, evidence supporting this hypothesis is limited and in vivo analyses are needed to discern any functional relationship between ANKK1 and DRD2. Additionally, associations of ANKK1 and DRD2

<table>
<thead>
<tr>
<th>Table 5. LI risk based on prenatal nicotine exposure.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crude Model</strong></td>
</tr>
<tr>
<td>1.77 (1.31–2.40)</td>
</tr>
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</table>

Abbreviation: OR, odds ratio. doi:10.1371/journal.pone.0063762.t005

<table>
<thead>
<tr>
<th>Table 6. LI risk based on prenatal nicotine dosage.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crude Model</strong></td>
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<td>1.43 (1.00–2.05)</td>
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Abbreviation: OR, odds ratio. doi:10.1371/journal.pone.0063762.t006

<table>
<thead>
<tr>
<th>Table 7. Single marker genetic associations with average of Nonword Repetition and Verbal Comprehension tasks.</th>
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<tr>
<td><strong>Variant</strong></td>
</tr>
<tr>
<td>rs2734849</td>
</tr>
<tr>
<td>rs11604671</td>
</tr>
<tr>
<td>rs4938013</td>
</tr>
<tr>
<td>rs7118900</td>
</tr>
<tr>
<td>rs1800497</td>
</tr>
<tr>
<td>rs6278</td>
</tr>
</tbody>
</table>

*No SMK p-value refer to associations in cohort of subjects not exposed to prenatal nicotine. doi:10.1371/journal.pone.0063762.t007
may reflect linkage disequilibrium in the locus, and may be capturing the signal from a single, unidentified causative variant.

Limitations
This investigation is subject to several limitations. First, the use of maximum amount of prenatal nicotine exposure may be an overestimation due to possible reductions and cessations of smoking during the prenatal period. However, the smoking data obtained accurately reflects the exposure since the information was collected in the pre/perinatal period. Second, although we controlled for many factors associated with language, this study cannot control for all possible, unmeasured factors that may confound associations. However, our models encompass a broad range of covariates relative to other previous studies. Third, due to the design of the ALSPAC cohort and amount of time following subjects, missing data are to be expected. The subsample used to complete association analyses has various demographic and environmental differences compared to the overall sample, which is more representative of the general population in the Avon region of the United Kingdom (Table S2). These factors were controlled for in the analysis of prenatal nicotine exposure, but our findings must be replicated in a more diverse, representative sample before being expanded to the general population. Fourth, there are inherent differences between our discovery cohort, ALSPAC, and our replication cohort, Iowa LI. Subjects in ALSPAC were recruited during the prenatal period, and investigators aimed to collect a sample that reflected the general population in the Avon region of the United Kingdom. Iowa LI is a case-control cohort that recruited cases with LI and matched controls. Therefore, genetic associations of \textit{ANKK1}/\textit{DRD2} in the two cohorts are not identical. However, the initial and replicated associations do suggest that \textit{ANKK1}/\textit{DRD2} and dopamine signaling modulate language skills in children.

![Figure 1. Linkage disequilibrium between ANKK1 and DRD2.](image)

**Table 8.** Haplotype Association of \textit{ANKK1}/\textit{DRD2} with average of Nonword Repetition and Verbal Comprehension tasks.

<table>
<thead>
<tr>
<th>Variants</th>
<th>Genes</th>
<th>Haplotype</th>
<th>Beta</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11604671, rs2734849, rs1800497, rs6278</td>
<td>ANKK1/DRD2</td>
<td>ACCG</td>
<td>0.053</td>
<td>3.2×10^{-4}</td>
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</tbody>
</table>

doi:10.1371/journal.pone.0063762.t008
Conclusions

Prenatal nicotine exposure has a negative effect on language abilities in schoolchildren. These results support the growing body of evidence that the development of communication skills begins during fetal development. Future studies should determine the effects of exposure to first-hand nicotine exposure and other prenatal and postnatal toxins. The genetic associations of \textit{ANKK1} and \textit{DRD2} with language performance further suggest that nicotine-related pathways modulate verbal language processing. More specifically, we implicate dopamine signaling in the comprehension and processing of verbal language. Other factors in dopamine and other major neurotransmitter signaling pathways should be examined.

References


Supporting Information

Table S1 Distribution of covariates among smoking groups. Values are either percentages or means (SD). *Indicates \( \chi^2 \) two-tailed p-value <0.05 from univariate analyses of each covariate and prenatal nicotine exposure outcome. **Indicates ANOVA p-value <0.05 from comparison of each covariate and prenatal nicotine exposure outcome. (DOC)

Table S2 Comparison of those included in analyses and the overall ALSPAC cohort. Data are presented as either percentages or mean (SD). (DOC)

Acknowledgments

We thank all the families and participants who took part in these studies. We also wish to acknowledge the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. We thank Dr. David E. Odd for useful clarifications on the reusistation data. We are also grateful to the Yale Center for Genome Analysis, specifically Irina Tikhonova and Anna Rogers, for genotyping services.

Author Contributions

Conceived and designed the experiments: JDE NRP KC JRG. Performed the experiments: JDE NRP KC JRG. Analyzed the data: JDE. Contributed reagents/materials/analysis tools: LLM KM SMR JBT. Wrote the paper: JDE.

Table 9. Replication of genetic associations in Iowa LI cohort.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Gene</th>
<th>Trait</th>
<th>p-value</th>
<th>OR</th>
</tr>
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<tbody>
<tr>
<td>rs11604671</td>
<td>ANKK1</td>
<td>Case-Control</td>
<td>3.84 x 10^{-3}</td>
<td>1.4 (1.1–2.0)</td>
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<tr>
<td>rs2734849</td>
<td>ANKK1</td>
<td>Case-Control</td>
<td>7.41 x 10^{-3}</td>
<td>1.4 (1.1–2.0)</td>
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<tr>
<td>rs1800497</td>
<td>ANKK1</td>
<td>Case-Control</td>
<td>1.9 x 10^{-2}</td>
<td>1.5 (1.1–2.1)</td>
</tr>
<tr>
<td>rs11604671</td>
<td>ANKK1</td>
<td>GORT Comp</td>
<td>3.3 x 10^{-2}</td>
<td>N/A</td>
</tr>
<tr>
<td>rs11604671</td>
<td>ANKK1</td>
<td>PPVT</td>
<td>2.5 x 10^{-2}</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; GORT Comp, Gray Oral Reading Test Comprehension; PPVT, Peabody Picture Vocabulary Test. doi:10.1371/journal.pone.0063762.t009
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