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

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(Article begins on next page)
Introduction

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 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.

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 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.

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Factors. Twin and family studies have shown that both LI and RD have a significant genetic component, with heritability estimates of 45–73% and 54–84%, 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 communication 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 predispose individuals to ND may also contribute to communication processes. Studies of ND have revealed that it has a significant genetic component and identified several candidate genes, including DRD2, ANKK1, CHRNA4, and CHRN2. 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 15,438 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 scores ≤75 were excluded from the present study [29]. To prevent population stratification in genetic analyses, subjects of non-European descent were also removed. Additionally, samples with genotyping call rate <0.80 were excluded 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 comprehension 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
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 ($p = 0.0729$).
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 [1.04–2.43]; p = 0.0305) (Table 5). 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 replicated in the Iowa LI cohort with LI case-control status of SNPs within exposure and ND variants in the ALSPAC sample. Associations between single markers within ANKK1 and 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 alters 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.

### 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.

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

<table>
<thead>
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<th>Overall</th>
<th>Exposed</th>
<th>Overall</th>
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<td>Adjusted Model</td>
<td>Crude Model</td>
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<td></td>
<td>Beta</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Beta</td>
<td>p-value</td>
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<tr>
<td>Nonword Repetition (NWR)</td>
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<td>0.0799</td>
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<tr>
<td>Comprehension</td>
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<td>&lt;0.0001</td>
<td>−0.08</td>
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<td>Avg NWR Comp</td>
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Abbreviations: NWR, nonword repetition; Avg NWR Comp. average of z-scores of nonword repetition and verbal comprehension tasks.

doi:10.1371/journal.pone.0063762.t003

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

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<th>Measure</th>
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<td>Crude Model</td>
<td>Adjusted Model</td>
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<td>Beta</td>
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<tr>
<td>Nonword Repetition (NWR)</td>
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<td>−0.29</td>
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<td>0.0002</td>
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<tr>
<td>Comprehension</td>
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<td>&lt;0.0002</td>
<td>−0.46</td>
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<td></td>
<td></td>
<td>&lt;0.0001</td>
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<td></td>
<td></td>
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<tr>
<td>Avg NWR Comp</td>
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<td>&lt;0.0001</td>
<td>−0.37</td>
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<td></td>
<td></td>
<td>&lt;0.0001</td>
<td></td>
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doi:10.1371/journal.pone.0063762.t004
ALTERATIONS IN DOPAMINE FUNCTION, APPEAR TO THEN INFLUENCE REINFORCEMENT LEARNING. THESE CHANGES, WHICH ARISE VIA PERMANENT (PRENATAL NICOTINE), YIELD ALTERATIONS IN WORKING MEMORY AND GENETIC PREDISPOSITION (WITH LI [55–56]. CHANGES IN DOPAMINERGIC FUNCTION, WHETHER FROM IMPAIRMENTS IN WORKING MEMORY HAVE BEEN PROPOSED TO PLAY A ROLE IN DOPAMINE SIGNALING [53–54]. ADDITIONALLY, PAST STUDIES HAVE FOUND ANKK1 ASSOCIATION BETWEEN LANGUAGE PERFORMANCE AND DOPAMINERGIC SIGNALS [53].

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 NEUROTHERMATOR 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 THEN 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 RECEPITIVE VOCABULARY, WHICH MAY BE ANALOGOUS TO DEFICITS MEASURED IN OUR VERBAL AND READING COMPREHENSION TASKS. 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, ROOED 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 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 5: LI risk based on prenatal nicotine exposure.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Gene</th>
<th>p-value</th>
<th>No SMK p-value</th>
</tr>
</thead>
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<tr>
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<td>ANKK1</td>
<td>2.0×10^{-4}</td>
<td>1.9×10^{-4}</td>
</tr>
<tr>
<td>rs11604671</td>
<td>ANKK1</td>
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<td>2.2×10^{-4}</td>
</tr>
<tr>
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<td>ANKK1</td>
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</table>

*No SMK p-value refer to associations in cohort of subjects not exposed to prenatal nicotine.*

### Table 6: LI risk based on prenatal nicotine dosage.

<table>
<thead>
<tr>
<th>OR</th>
<th>p-value</th>
<th>OR</th>
<th>p-value</th>
</tr>
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<tr>
<td>1.77 (1.31–2.40)</td>
<td>0.0002</td>
<td>1.60 (1.04–2.45)</td>
<td>0.0305</td>
</tr>
</tbody>
</table>

**Abbreviation:** OR, odds ratio.

**Note:** 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.

### Table 7: Single marker genetic associations with average of Nonword Repetition and Verbal Comprehension tasks.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Gene</th>
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<td>ANKK1</td>
<td>1.2×10^{-3}</td>
<td>1.2×10^{-3}</td>
</tr>
<tr>
<td>rs1800497</td>
<td>ANKK1</td>
<td>1.9×10^{-3}</td>
<td>2.6×10^{-4}</td>
</tr>
<tr>
<td>rs6278</td>
<td>DRD2</td>
<td>8.8×10^{-3}</td>
<td>1.7×10^{-2}</td>
</tr>
</tbody>
</table>

*No SMK p-value refer to associations in cohort of subjects not exposed to prenatal nicotine.*

**Abbreviation:** OR, odds ratio.
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.

**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>3.2x10^{-4}</td>
</tr>
</tbody>
</table>

**Figure 1.** Linkage disequilibrium between \textit{ANKK1} and \textit{DRD2}. Linkage disequilibrium (LD), as measured by $D'$, among markers in the \textit{ANKK1} and \textit{DRD2} genes. There is a 12kb haplotype block spanning the two genes (markers: rs11604671, rs2734849, rs1800497, and rs6278).

doi:10.1371/journal.pone.0063762.g001
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>
</thead>
<tbody>
<tr>
<td>rs11604671</td>
<td>ANKK1</td>
<td>Case-Control</td>
<td>3.84 x 10^{-3}</td>
<td>1.4</td>
</tr>
<tr>
<td>rs23734849</td>
<td>ANKK1</td>
<td>Case-Control</td>
<td>7.41 x 10^{-3}</td>
<td>1.4</td>
</tr>
<tr>
<td>rs1800497</td>
<td>ANKK1</td>
<td>Case-Control</td>
<td>1.9 x 10^{-2}</td>
<td>1.5</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

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 ANKK1 and 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 $X^2$ 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 reusacitation 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 JRG.


