

# Delving into the Genetic Causes of Language Impairment in a Case of Partial Deletion of *NRXN1*

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## Keywords

2p16.3 deletion · *NRXN1* · Cognitive impairment · Language deficits · Pragmatic impairment · Language-related gene networks

## Abstract

**Introduction:** Copy-number variations (CNVs) impacting on small DNA stretches and associated with language deficits provide a unique window to the role played by specific genes in language function. **Methods:** We report in detail on the cognitive, language, and genetic features of a girl bearing a small deletion (0.186 Mb) in the 2p16.3 region, arr[hg19] 2p16.3(50761778\_50947729)×1, affecting exons 3–7 of *NRXN1*, a neurexin-coding gene previously related to schizophrenia, autism (ASD), attention deficit hyperactivity disorder (ADHD), mood disorder, and intellectual disability (ID). **Results:** The proband exhibits many of the features commonly found in subjects with deletions of *NRXN1*, like ASD-like traits (including ritualized behaviors, disordered sensory aspects, social disturbances, and impaired theory of mind), ADHD symptoms, moderate ID, and impaired speech and language. Regarding this latter aspect, we observed altered speech production, underdeveloped phonological aware-

ness, minimal syntax, serious shortage of active vocabulary, impaired receptive language, and inappropriate pragmatic behavior (including lack of metapragmatic awareness and communicative use of gaze). Microarray analyses point to the dysregulation of several genes important for language function in the girl compared to her healthy parents. **Discussion:** Although some basic cognitive deficit – such as the impairment of executive function – might contribute to the language problems exhibited by the proband, molecular evidence suggests that they might result, to a great extent, from the abnormal expression of genes directly related to language.

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## Introduction

Copy number variants (CNVs) found in individuals with language and pragmatic deficits provide a unique window to the genetic foundations of the human ability to learn and use languages. This is particularly true for CNVs affecting a reduced number of genes and resulting in a common phenotypic profile. In this paper, we report on a girl with a microdeletion in the 2p16.3 region affect-

ing the *NRXN1* gene. This gene encodes a neurexin [Rowen et al., 2002]. Neurexins are polymorphic cell surface proteins implicated in synapse development and maintenance [Missler and Südhof, 1998]. The *NRXN1* protein has been shown to regulate synaptic activity, neuritogenesis, and neuronal network assembly during neocortical development [Südhof, 2008; Gjølund et al., 2012; Jenkins et al., 2016]. Genomic losses involving *NRXN1* have been associated with different neurocognitive conditions, including schizophrenia (SZ), autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), seizures, mood disorder, or intellectual disability (ID) [for recent reviews, see Lowther et al., 2017; Hu et al., 2019; Castronovo et al., 2020; Tromp et al., 2021]. Whereas, as noted, the clinical profile of patients with deletions in this gene is pretty variable, language and communication deficits are commonly reported in most of them [Béna et al., 2013; Curran et al., 2013; Al Shehhi et al., 2019]. To date, the most detailed report on language and speech problems in subjects with deletions in *NRXN1* is that of Brignell et al. [2018], who observed that children (aged 1.8–17 years) with *NRXN1* deletions affecting exons 1–3 or 1–5 of the gene exhibited a pretty variable phenotype with regards to language deficits. Speech sound problems were frequent, but not severe, whereas many children also had difficulties with receptive language. Language use in social settings was impaired too.

The reported heterogeneity in the severity and the type of speech and language problems associated with deletions in this gene urges to find and characterize in detail the clinical presentation of new CNVs affecting the gene. Moreover, as genes work in a coordinated way, it is well possible that changes in the dosage of *NRXN1* resulting from CNVs affect downstream genes with a more direct/profound impact on speech and language. Having all this in mind, in this paper we provide a detailed characterization of the speech, language, and communication deficits observed in our patient. Additionally, we build on our current knowledge of the genetics of language development and language evolution, as well as on the observed changes in the expression pattern of selected genes in the blood of our proband, for hypothesizing about the molecular causes of language dysfunction associated with *NRXN1* deletions.

## Materials and Methods

### *Linguistic, Cognitive, and Behavioral Assessment*

The global developmental profile of the proband was assessed with the Spanish version of the Battelle Developmental Inventory [de la Cruz and González, 2011], which consists of 341 items aimed

to evaluate the subject's personal/social development, adaptive capabilities, gross and fine motor abilities, receptive and expressive communication skills, and cognitive development. In addition, the proband's language features and communication skills were examined in detail through the analysis of a 10-min sample of an interaction between the child and her sister while playing in a room at her speech language therapist's place under her mother's surveillance. The conversation was video-recorded and then transcribed and coded using the CHAT (Codes for the Human Analysis of Transcripts) software, a tool of the CHILDES Project [MacWhinney, 2000]. CHAT allows to code for speech production phenomena on the main lines of the transcript, and for phonological, morphological, syntactic, and pragmatic phenomena on dependent lines. Information about prosody, dialectal articulation, communicative gestures, and communicative use of gaze can be included within square brackets in the main lines. Pauses are also coded in the main lines following CHAT conventions for estimated duration. We tagged every language error or anomaly with an indication of the structural components of language affected. We then evaluated their impact on communication and labelled them according to PREP-CORP (PRagmatic Evaluation Protocol for the analysis of oral CORPpora) [Fernández-Urquiza et al., 2017a]. This protocol has been previously used for outlining the pragmatic profiles of several neurodevelopmental disorders [e.g., Fernández-Urquiza et al., 2015, 2016, 2017b; Shiro et al., 2016, 2019; Diez-Itza et al., 2018, 2022].

### *Molecular Analyses*

#### Microarrays for Whole-Genome CNV Search and Chromosome Aberration Analysis

Peripheral venous blood lymphocytes were grown following standard protocols and collected after 72 h. DNA from the patient and her parents was extracted from 100 µL of EDTA-anticoagulated whole blood using MagNA Pure (Roche Diagnostics, West Sussex, UK). The DNA was then hybridized on a CGH platform (Agilent Technologies). The derivative log ratio spread value was 0.13. The platform included 60,000 probes. Data were analyzed with the Agilent Genomic Workbench 7.0, and the ADM-2 algorithm (threshold = 6.0; aberrant regions had more than 5 consecutive probes).

#### Microarrays for Whole-Genome Expression Analysis

In order to determine the genes that could be differentially expressed in the proband compared to her healthy parents, microarray analyses of blood samples from the 3 of them were performed. Total RNA was extracted with the PAXgene Blood RNA Kit IVD (Cat. No./ID: 762164). RNA quality and integrity were confirmed with a Bioanalyzer RNA 6000 Nano. All samples had RNA integrity number values >9. An Affymetrix® Scanner 3000 7G was then used for analyzing transcriptome changes. The resulting raw data were processed with the Affymetrix® GeneChip® Command Console® 2.0 program. Next, \*.CEL files were checked to certify the RNA integrity and the suitability of the labeling and the hybridization processes. Finally, the raw data from the different arrays were normalized with the SST (Signal Space Transformation)-RMA (Robust Microarray Analysis) tool [Irizarry et al., 2003]. Normalized data (\*.CHP files) were subsequently used to search for differentially expressed genes in the proband compared to her parents. Statistical analyses were conducted with the LIMMA (Linear Models for Microarray Analysis) package of BioConductor, using the TAC 4.0 software.

### *In silico Analyses*

In order to delve into the molecular causes of the speech and language problems exhibited by the girl, we used String 11.0 ([www.string-db.org](http://www.string-db.org)) for finding putative functional links between NRXN1 and the products of genes important for language. We focused on 2 types of genes. A first group of genes encompasses candidates for highly prevalent (specific) language disorders, in particular, developmental dyslexia (DD) and specific language impairment (SLI). For DD, we relied on the list provided by Paracchini et al. [2016], which includes genes resulting from candidate association studies, genome-wide association analyses (GWAs), quantitative GWAs, CNV studies, and next-generation sequencing (NGS) analyses; but we also surveyed the literature via PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), looking for additional candidates. For SLI, we made use of the lists published by Pettigrew et al. [2016] and Chen et al. [2017], which include candidates resulting from linkage analyses, GWA studies, and NGS analyses; but we also surveyed the literature via PubMed for finding other strong candidates for SLI. A second group of genes encompasses candidates for language evolution, as discussed by Boeckx and Benítez-Burraco [2014a, b] and Benítez-Burraco and Boeckx [2015]. These genes are primarily expected to account for the recent globularization of the human skull/brain and ultimately, for the cognitive changes resulting in our species-specific ability for acquiring and using languages. These genes fulfil several of the following criteria: (1) they have changed (and/or interact with genes that have changed) after our separation from extinct hominins, particularly from Neanderthals, these changes including either changes in their coding regions, in their regulation, or in both; (2) they play a role in brain development, wiring, and/or function; and/or (3) they are candidates for language deficits in broader, highly prevalent cognitive disorders, particularly ASD and SZ [for further details, see Benítez-Burraco and Murphy, 2016; Murphy and Benítez-Burraco, 2016, 2017]. A potential limitation of this approach is that not all the genes within these 2 sets result from GWAs based on large samples. Both sets of genes are listed in online supplementary File 2 (for all online suppl. material, see [www.karger.com/doi/10.1159/000524710](http://www.karger.com/doi/10.1159/000524710)). String 11.0 predicts physical and functional associations between proteins relying on different sources (genomic context, high-throughput experiments, conserved coexpression, and text mining) [for details, see Szklarczyk et al., 2015]. Because we were interested in finding robust functional links, we limited our search to protein-protein interaction databases and curated databases of protein interactions (BioCarta, BioCyc, GO, KEGG, and Reactome).

## **Results**

### *Clinical History*

The proband was born by caesarean section after an uncomplicated pregnancy. She weighed 2.6 kg at birth and presented with congenital torticollis that required a year of rehabilitation. She was fed with artificial lactation, and the transition to solid feeding went smoothly. At 9 months of age, she was referred to the Paediatric Neurology Unit, because her parents reported that the girl's de-

velopment was delayed compared to her older sister. She was diagnosed with global psychomotor developmental delay. At 13 months of age, a cranial MRI was performed. Results were suggestive of an increase in extra-axial spaces, supratentorial ventriculomegaly, and mega cisterna magna. At that age, the girl also began to attend day care. At 15 months of age, she seemed to evolve favourably despite her maturational delay. At 17 months of age, urine and metabolic studies were required, as the girl suffered from anemia. At 21 months of age, her verbal comprehension abilities seemed to have slightly improved, but her expressive impairment was still dramatic, as she was only able to produce a few single words. Regarding her family history, it is of interest that her paternal uncle suffered from SZ and ID, her father presented with motor tics, and her paternal great-grandmother suffered from cognitive and motor impairment.

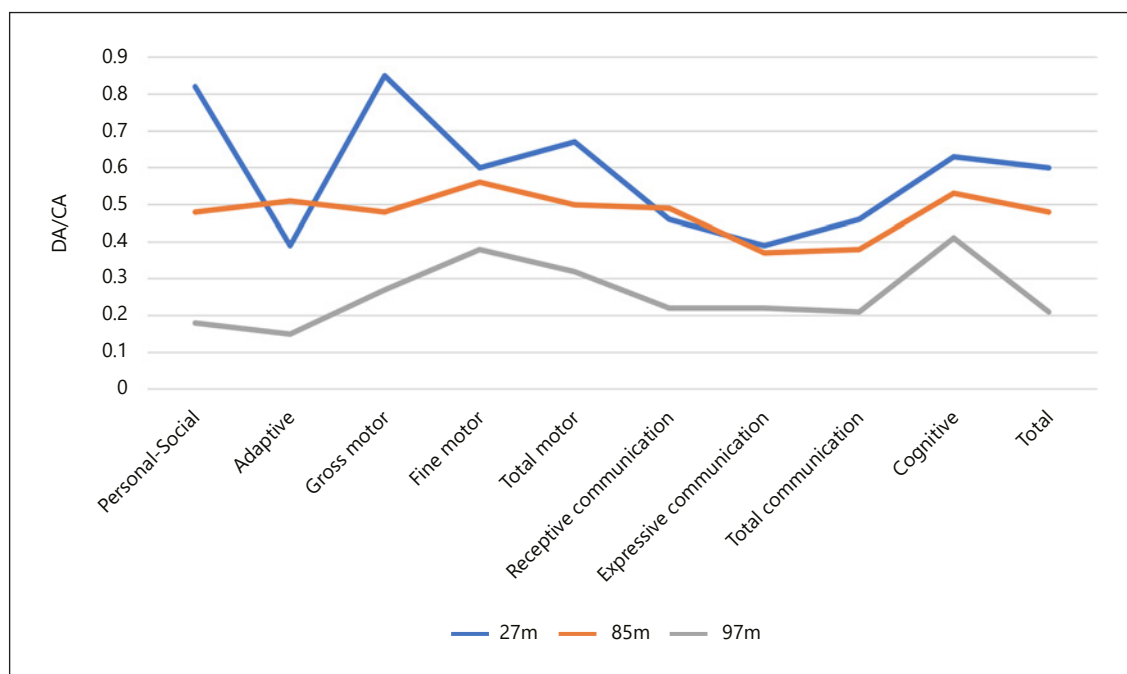
### *Language and Cognitive Development*

At 27 months of age, the proband was assessed with the Battelle Developmental Inventory (see Fig. 1). At this moment, the girl exhibited a 16-month delay in her adaptive abilities and her expressive communication skills, and a 14-month delay in her receptive communication abilities. She also presented with a 10-month cognitive delay. By contrast, her personal social skills and her gross motor abilities were less impaired.

At 42 months of age, when the proband began to attend school, she was diagnosed with a severe developmental delay. Regarding her cognitive (dis)abilities, the girl showed scattered attention, problems with making associations, poor imitation capacity, absence of symbolic play, and difficulties with normal social interaction. She also experienced difficulties expressing and identifying emotions, although she did not present with behavioral problems. Concerning her language development, the girl exhibited a severe mixed language delay. She was unable to construct simple sentences and her speech was barely intelligible.

At 60 months of age, the proband's language abilities had improved only slightly. On the expressive domain, she occasionally uttered 2-word phrases, and her speech difficulties persisted. At that age, the girl started to receive speech therapy.

At the age of 72 months, when the girl entered the primary school, her cognitive and behavioral abilities were examined again. The girl was reported to exhibit moderate ID, emotional and behavioral deficits, as well as learning difficulties: for successfully achieving and performing specific activities, she needed a very structured learning



**Fig. 1.** Results of the assessment with the Battelle Developmental Inventory at 27, 85, and 97 months of age. DA/CA, developmental age/chronological age.

environment with constant monitoring and aid from adults. Overall, she showed a notable developmental delay compared to her peers.

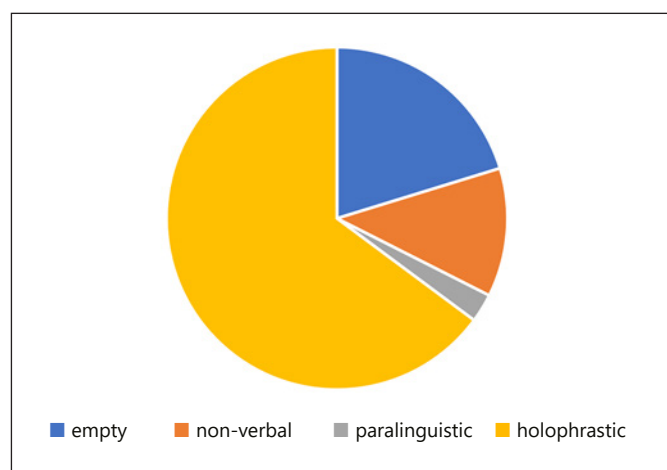
At 85 months of age, the proband was assessed again with the Battelle Developmental Inventory. As shown in Figure 1, the gap between the girl's chronological age and her developmental age had increased in all the assessed areas, being expressive language the most impaired domain. At that moment, the proband presented with ID and significant problems in the areas of social interaction and verbal communication. She also exhibited ritualized and disordered behaviors, such as smelling objects, as well as high levels of impulsivity and attention deficit. Her broad independence and adaptive skills were also below expectations for her chronological age.

At 97 months of age, a third evaluation with the Battelle Developmental Inventory was carried out, which showed a significant setback with respect to the 2 previous assessments, the proband's personal/social and adaptive skills being the most affected domains, along with her expressive language (Fig. 1).

In addition, at that age, we also examined in detail the structural and functional features of the child's language, as well as her communication skills through the analysis of a naturally occurring interaction with her mother and

sister. The full record can be found in online supplementary File 1. The proband's speech featured frequent omissions of initial consonants (e.g., siguiente > iguiente\* "following"; quita > ita\* "remove"; ventana > entana\* "window"), as well as consonant clusters simplification (e.g., verde > vele\* "green"; espejo > epejo\* "mirror"; pastel > fafé\* "cake"; armario > ababio\* "closet"), and sound substitutions (e.g., ducha > lucha\* "shower/fight"; roja > doja\* "red"; armario > armadio\* "closet"; aquí > adí\* "here"; música > muzita\* "music"; pastel > fafé\* "cake"). Consonant frontalization was also frequently observed (e.g., aquí > adí\* "here"; armario > ababio\* "closet"; pastel > fafé\* "cake"), particularly, of alveolar /s/ into interdental /θ/ (e.g., silla > zilla\* "chair"; esto > ezto\* "this"; eso > ezo\* "that"; sí > zí\* "yes"; música > muzita\* "music"). Additionally, the mean length of utterance in words of the girl's discourse was very low, of 0.981. This roughly corresponds to a restricted holophrastic (i.e., one-word) language, which is typically found in children from 1 to 2 years of age. Although the proband did not produce truly syntactic structures, a hallmark of her discourse was the avoidance or the phonetic alteration of definite and indefinite articles (e.g., la ropa > e dopa\* "the clothes"; las manos > a mano\* "the hands").





**Fig. 2.** Nature of the conversational turns provided by the child during a spontaneous conversation with a peer. Empty turns: the child provided no answer at all, ignoring her interlocutor and focusing on the objects she was playing with. Non-verbal turns: the child produced a gestural action in response to her interlocutor's request. Paralinguistic: the child tried to take the turn but was only able to produce fillers, such as non-verbal vocalizations. Holophrastic: the child generated single-word responses.

With regards to the pragmatic component, the child did not seem able to properly engage in conversation. Most of her turns at talk, even being verbal by nature (Fig. 2), were reactive, in response to her interlocutor's requests. Usually, the interlocutor needed to repeat the question twice or 3 times in order to obtain a minimal answer (verbal or nonverbal). In the infrequent cases when the child took the floor, she could generate echolalic utterances only.

Furthermore, the pragmatic skills needed for understanding the logic of conversation as described by Grice [1975] were seriously impaired. Specifically, transgressions of the maxim of relation (i.e., say things relevant to the topic under discussion) were pervasive (e.g., when asked to name a music player, she might say "dance"; when asked about a picture, she might reply "paper"; or when asked about some windows in the room, she might reply by counting them: "one, two, etc."), as well as transgressions of the maxim of quantity (i.e., provide the right amount of information), so that the child might refer to the majority of the things around by means of demonstrative pronouns like "this" and "that", which are not informative enough. Finally, her communicative use of gaze was also impaired. Usually, she did not establish visual contact with her conversational partner, and she experienced difficulties with joint attention when asked to name

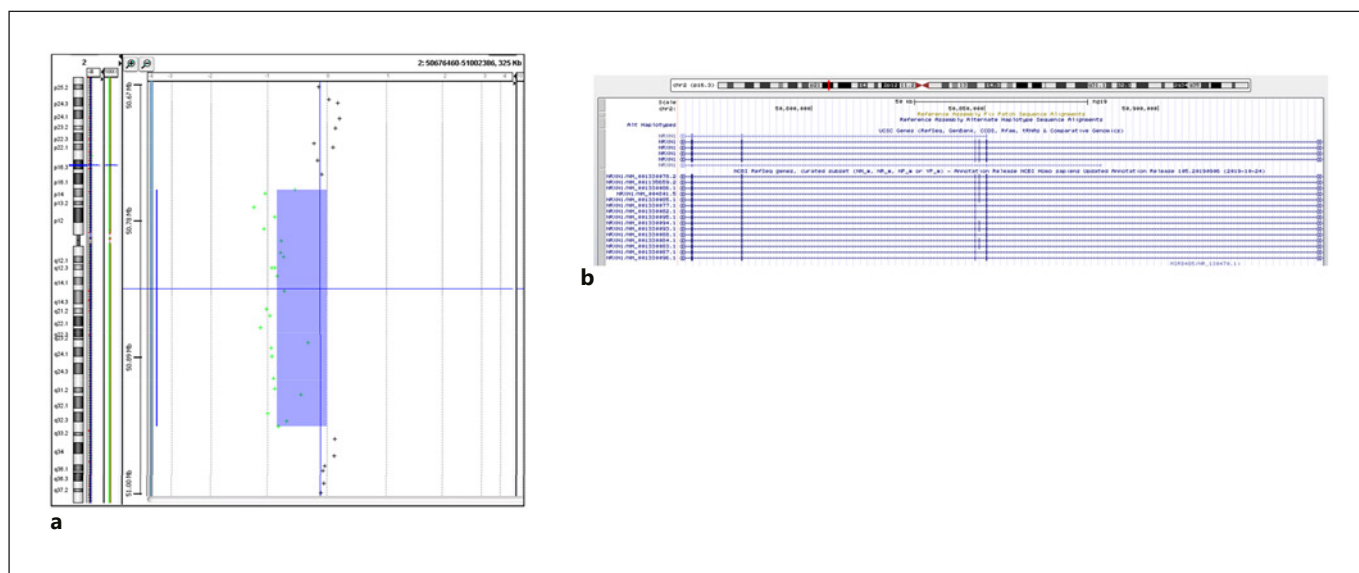
an object. No compensatory strategies aimed to improve her communication deficits were observed (e.g., asking for help when she was unable to find a word).

To sum up, our proband exhibits severe speech and language deficits, with her expressive skills being dramatically affected, to the point that she is unable to pronounce words correctly, to build up simple sentences, and to name familiar things around her. These deficits hinder the intelligibility of her discourse and preclude from successfully communicating. Language comprehension is also seriously impaired, as well as pragmatic abilities: she shows no metapragmatic awareness (i.e., she is unable to realize which of her utterances are problematic or failed, and she is unable to generate compensatory strategies aimed to improve communicative success); she frequently produces non-related, tangential and/or insufficiently informative answers; she is not able to properly engage in conversation according to the turn-taking rules [Sacks et al., 1974], and she makes no communicative use of gaze.

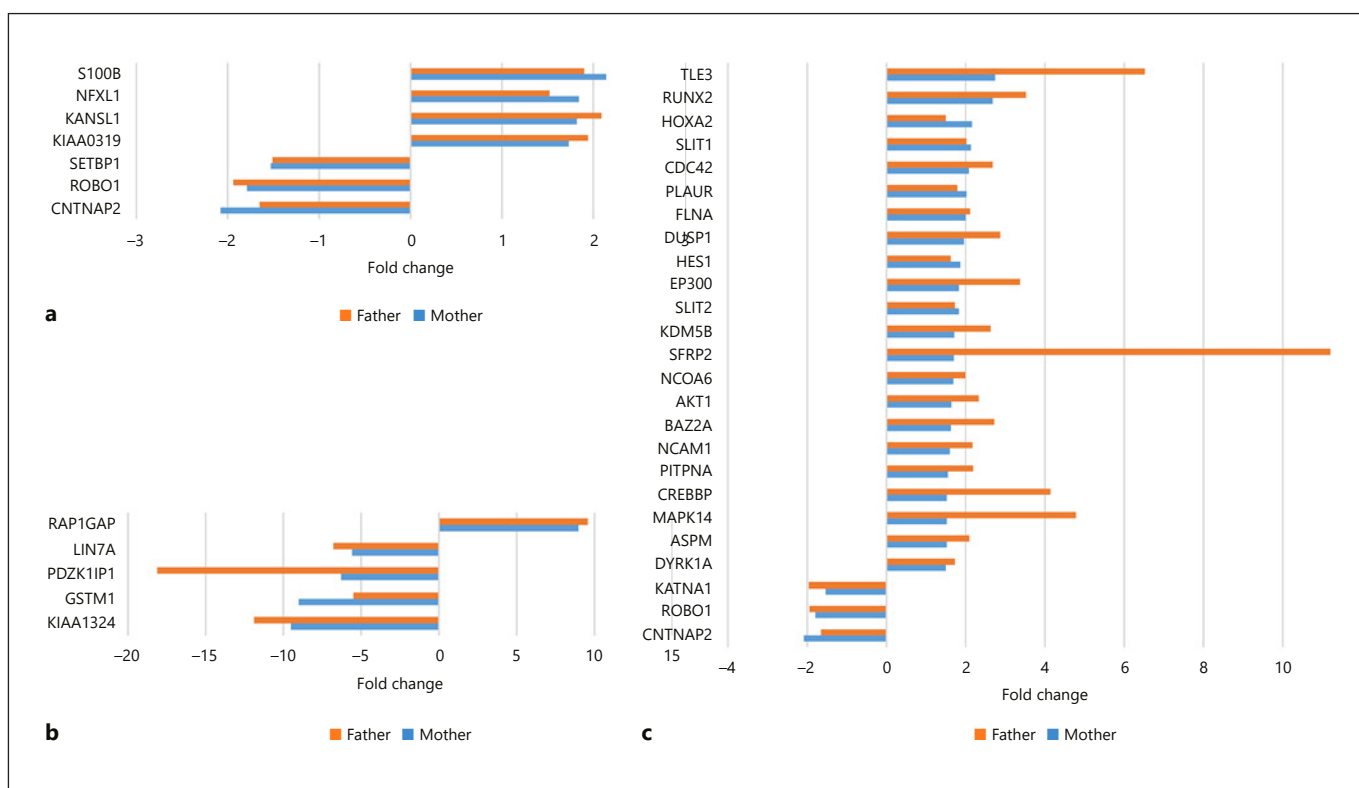
### *Cytogenetic and Molecular Analyses*

An array comparative genomic hybridization (array-CGH) was performed when the proband was 5 years and 1 month old. The array confirmed the presence of a deletion of 0.186 Mb in the 2p16.3 region (arr[hg19] 2p16.3(50761778\_50947729)×1), predicted to be pathological, and affecting exons 3–7 of the *NRXN1* gene (Fig. 3a, b). The deletion was inherited from the proband's healthy father.

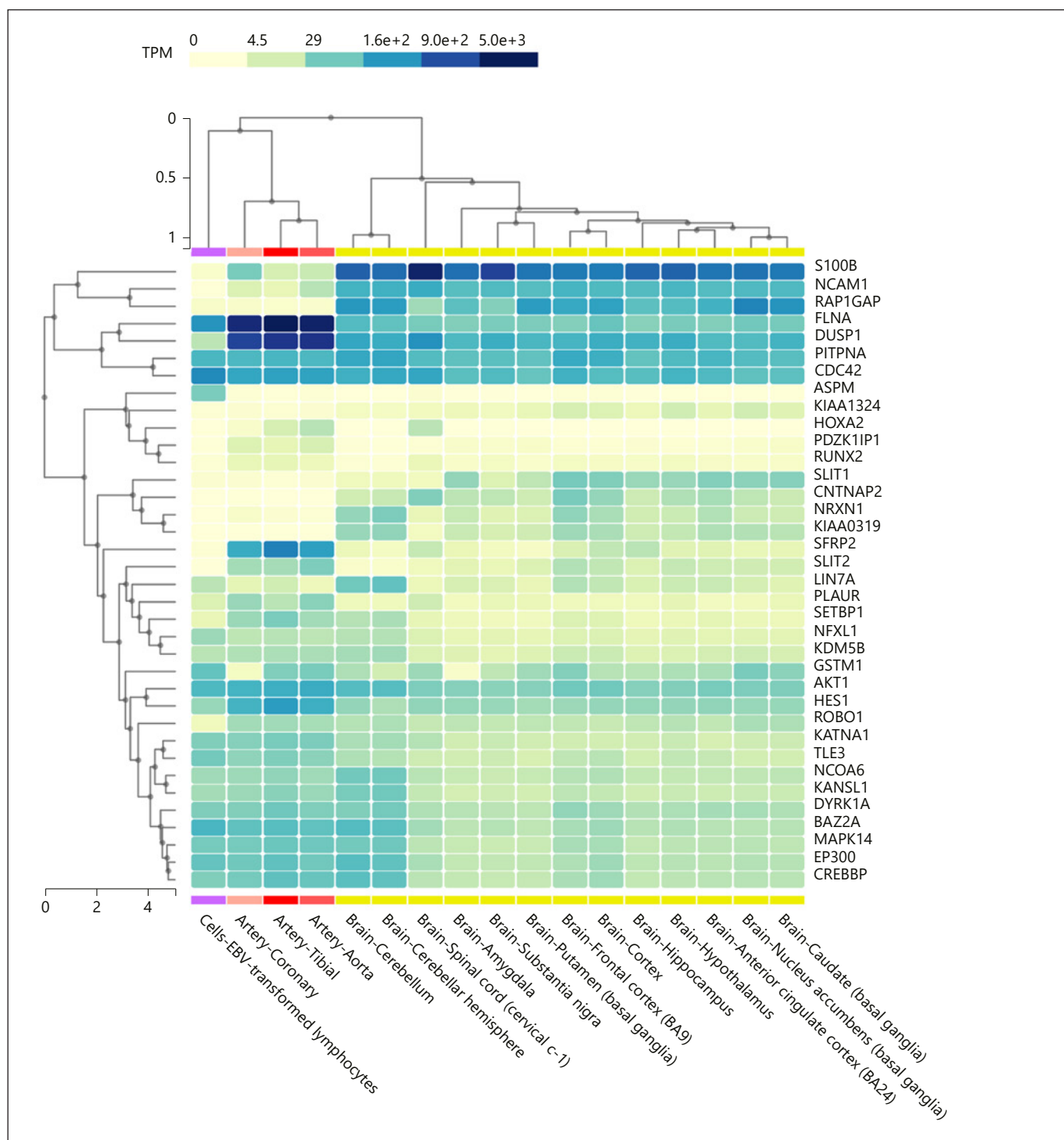
We then used String 11.0 (<https://www.string-db.org>) for finding functional links between *NRXN1* and the products of genes important for language, specifically, candidates for DD and SLI, but also candidates for language evolution. Because String did not predict any functional link of interest between *NRXN1* and the products of these genes (data not shown), we conducted additional in vitro analyses. Specifically, we performed microarray analyses of blood samples from the proband to determine whether she exhibited altered patterns of gene expression that may account for the observed deficits. We used her healthy carrier father and her healthy non-carrier mother as controls. The results of the microarrays are shown in online supplementary File 3. We found that *NRXN1* was only slightly upregulated in the proband (1.27-fold change [FC] compared to her healthy non-carrier mother; 1.1 FC compared to her healthy carrier father). Additionally, we checked whether the candidate genes for language disorders and/or evolution used in our in silico analyses were differentially expressed in the girl compared to her parents. For most of the candidates for language disorders,



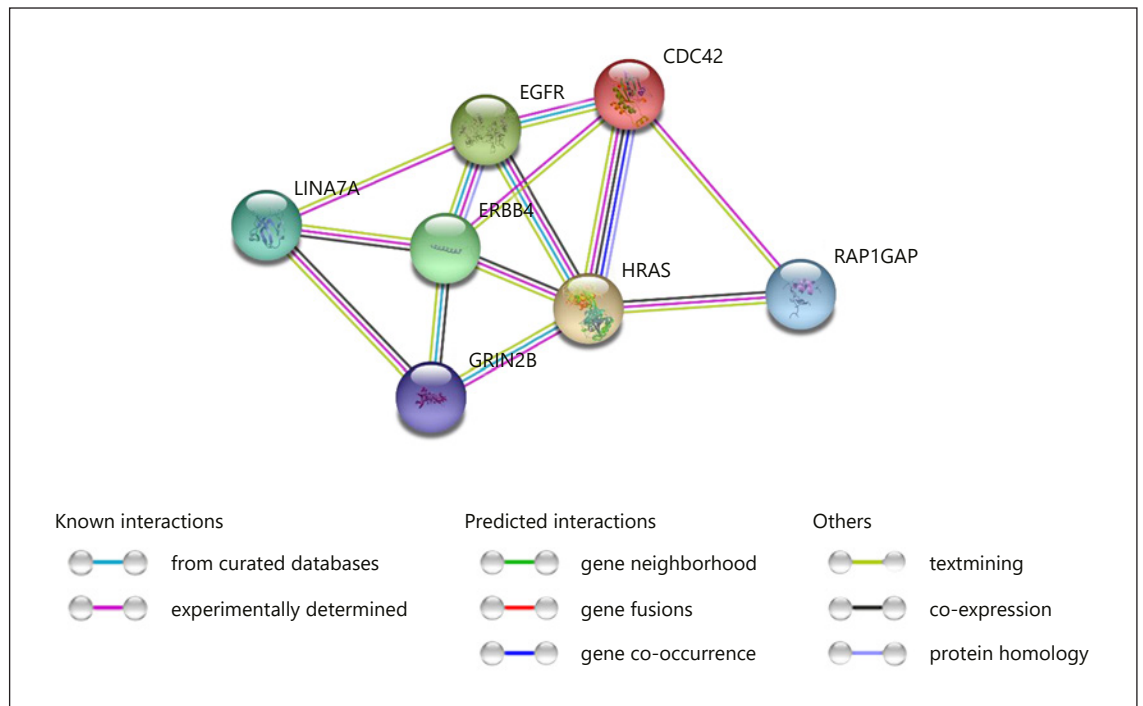
**Fig. 3.** Chromosomal alterations found in the proband. **a** Screen capture of the array-CGH of the proband's chromosome 2 showing the microdeletion at 2p16.3. **b** Screen capture of the UCSC Genome Browser (<https://genome.ucsc.edu/>) showing the extension of the deletion in *NRXN1*.



**Fig. 4.** Variation in the expression levels of genes of interest in the proband's blood compared to her healthy parents. **a** Fold changes in the expression levels of candidates for language disorders. **b** Genes exhibiting fold changes >5 in the proband compared to both healthy parents. **c** Fold changes in the expression levels of candidates for language evolution.



**Fig. 5.** Expression pattern in the blood and the brain of genes of interest. Expression levels of the genes were retrieved from the Genotype-Tissue Expression (GTEx) project (<https://www.gtexportal.org/home/>). Statistical analysis and data interpretation were performed by the GTEx Consortium Analysis Working Group using the GTEx Multi Gene Query tool. TPM, transcripts per million.



**Fig. 6.** Interaction network of genes found strongly dysregulated in the proband and the proteins encoded by candidate genes for language disorders and/or evolution. The network was drawn with String, version 11.0 [Szklarczyk et al., 2015] license-free software (<http://string-db.org/>), using the molecular action visualization. Colored nodes symbolize genes/proteins included in the query. The color of the lines represents different kind of evidence, as

shown in the legend. Stronger associations between proteins are represented by thicker lines. The medium confidence value was 0.0400 (a 40% probability that a predicted link exists in a particular database). The diagram only represents the potential connectivity between the involved proteins, which has to be mapped onto particular biochemical networks, signaling pathways, cellular properties, aspects of neuronal function, or cell types of interest.

we found no significant differences (i.e.,  $FC > 1.5$  compared to both healthy parents). However, some of them (*CNTNAP2*, *ROBO1*, and *SETBP1*) were significantly downregulated in the proband, whereas some others (*KIAA0319*, *KANSL1*, *NFXL1*, and *S100B*) were significantly upregulated (Fig. 4a). Regarding candidates for language evolution, we found that most genes that were differentially expressed genes ( $FC > 1.5$  compared to both healthy parents) were upregulated in the proband (including *DYRK1A*, *ASPM*, *NCAM1*, *BAZ2A*, *SLIT2*, *DUSP1*, *FLNA*, *PLAUR*, *CDC42*, *SLIT1*, *RUNX2*, and *TLE3*) (Fig. 4c). Finally, we searched for additional candidates for the proband's language deficits, looking for genes exhibiting the strongest FC in our subject (i.e.,  $FC > 5$  compared to both unaffected parents). We found that several genes of interest were strongly downregulated (*KIAA1324*, *GSTM1*, *PDZK1IP1*, and *LIN7A*), whereas only 1 gene (*RAP1GAP*) was strongly upregulated (Fig. 4b).

We then checked the expression patterns in the brain of genes of interest using the Genotype-Tissue Expression (GTEx) project interface (<https://www.gtexportal.org/home/>). Our results are summarized in Figure 5. With regards to the candidates for language disorders and/or evolution that are significantly down- or upregulated in the blood of our proband, we found that around 50% are more expressed in the brain than in the blood. These include *S100B* (strongly expressed in the spinal cord, the substantia nigra, the hypothalamus, and the hippocampus), *NCAM1* (highly expressed across the whole brain), *SLIT1* (expressed throughout the brain), *CNTNAP2* (more expressed in the cortex), and *KIAA0319* (highly expressed in the cortex and parts of the basal ganglia). Finally, with regards to the genes that are strongly downregulated in the proband, we found that *LIN7A* is expressed at similar levels in the nervous system and in the blood, although it is upregulated in the cerebellum. *PDZK1IP1* is expressed at lower levels in the brain than in the blood. Transcripts of this gene are preferentially



**Table 1.** Summary table with the most relevant clinical features of our proband compared to patients with deletions of *NRXN1* according to Brignell et al., 2018 and DECIPHER

Cognitive/behavioural features	
Intellectual disability	+
Autism spectrum disorder	+
Attention deficit hyperactivity disorder	+
Schizophrenia	
Anxiety	
Speech impairment	+
Expressive language impairment	+
Receptive language impairment	+
Impaired pragmatics (social communication difficulties)	+
Brain/neurological findings	
Childhood apraxia of speech	+
Dysarthria	
Hypotonia	+
Epilepsy	
Complex neurodevelopmental disorder	+

+, features present in our proband.

found in the spinal cord. *GSTM1* is expressed at similar levels in the blood and in the brain, where it is mostly expressed in the frontal cortex and the basal ganglia. *KIAA1324* is expressed at higher levels in the brain, particularly in the cortex, the hypothalamus and parts of the basal ganglia. Lastly, *RAP1GAP* is expressed at higher levels in the brain than in the blood, particularly in the cortex and the basal ganglia.

Finally, we used String 11.0 for knowing more about functional interactions of interest between the proteins encoded by the genes found strongly dysregulated in the proband and the proteins encoded by candidate genes for language disorders and/or evolution. We found predicted functional links between *LIN7A* and *GRIN2B*, *ERBB4*, and *EGFR*, as well as between *RAP1GAP* and *CDC42* and *HRAS* (Fig. 6).

## Discussion

The widespread application of NGS facilities, particularly array-CGH, to people with conditions featuring cognitive and language deficits has resulted in an increasing list of candidate genes for these problems. Nonetheless, it is usually difficult to obtain robust genotype-to-phenotype links, particularly in the case of complex, multifactorial traits like language. In this paper, we provide a de-

tailed characterization of the language problems exhibited by a girl with a microdeletion in 2p16.3 affecting part of the *NRXN1* gene. Mutations or genomic losses in this gene have been associated with diverse cognitive and behavioral problems, including language and communication deficits [Béna et al., 2013; Curran et al., 2013; Brignell et al., 2018; Al Shehhi et al., 2019], although we still lack a mechanistic account of these problems.

Our proband exhibits most of the cognitive, behavioral, and neurological anomalies observed in patients with losses of *NRXN1*. This is specifically true for her speech, language, and communication (dis)abilities (Table 1).

From our detailed analysis of the proband's speech problems, we conclude that they are indicative of childhood apraxia of speech (CAS), a condition characterized by inconsistent errors on consonants and vowels in repeated productions of words and inappropriate prosody. Nonetheless, we have also found evidence of impaired phonological awareness. Our results are thus in line with findings by Brignell et al. [2018], who suggest that articulation and phonological problems co-occur in children with *NRXN1* deletions.

We also found that besides her severe expressive language delay, the receptive skills of our proband are also notably affected. This finding is also in line with previous research [e.g., Ching et al., 2010; Gregor et al., 2011; Béna et al., 2013; Curran et al., 2013; Dabell et al., 2013].

Finally, we report for the first time on the pragmatic impairment associated with deletions of *NRXN1*. This pragmatic deficit consists of an inability to properly engage in turn-taking during conversation together with a reduced metapragmatic awareness, which impedes perceiving the violation of the logic and the rules of conversation. All this results in different abnormal behaviors during conversation, like producing tangential or perseverating answers to new questions, or interpreting utterances in a literal way. These pragmatic problems seemingly become exacerbated by other co-occurring problems, like her reduced vocabulary, her altered expressive language/grammatical skills, her attention deficit, and her ASD-like features.

Overall, this language and communication profile partially overlaps with the deficits found in other neurodevelopmental disorders, such as ASD, ADHD, and moderate to severe ID, which are commonly comorbid conditions in children with *NRXN1* deletions (ASD: 43–65% [Schaaf et al., 2012; Béna et al., 2013; Dabell et al., 2013; González-Mantilla et al., 2016]; ADHD: 9–41% [Schaaf et al., 2012; Lowther et al., 2017]; ID: 77–92% [Schaaf et al.,

2012; Béna et al., 2013; Dabell et al., 2013]). In fact, as pointed out by Lowther et al. [2017], around 46% of individuals with *NRXN1* deletions present with dual neurodevelopmental disorder diagnoses, as it is the case of our proband, who was diagnosed with a moderate ID, but who also exhibits ADHD features, as well as ASD-like traits, including problems with social interactions, some ritualized behaviors, and altered sensory experience. Brignell et al. [2018] have suggested that the speech and language problems of individuals with *NRXN1* deletions could result from their ID and ASD features, a possibility that is supported by several studies pointing to a correlation between ID, ASD, and language difficulties [Levy et al., 2010; Liao et al., 2015].

For this reason, besides providing a more detailed profile of the problems with language exhibited by our proband, as well as some hints about their cognitive causes, we also aimed to advance an alternative hypothesis about the causes of the language problems resulting from losses of the *NRXN1* gene. The known roles of *NRXN1* in synapse development and maintenance, and neuron network assembly during cortical development [Missler and Südhof, 1998; Südhof, 2008; Gjølund et al., 2012; Jenkins et al., 2016], are compatible with a putative role in the development and maintenance, specifically, of brain circuits underlying language function. This possibility is reinforced by the fact that the gene is expressed in areas involved in language processing, particularly, the cerebellum, which contributes significantly to motor planning, but also to many aspects of language processing [Vias and Dick, 2017; Mariën and Borgatti, 2018], along with the prefrontal cortex region, which is a core region for speech and language [Jenkins et al., 2016]. Deficits in the frontal cortex are known to impact on executive function, and problems with executive function have been hypothesized to contribute to diverse neurodevelopmental disorders, including ASD, ADHD, and SZ [Ozonoff and Jensen, 1999; Martos-Pérez and Paula-Pérez, 2011]. As we have repeatedly pointed out, our proband exhibits ASD- and ADHD-like features. Additionally, her paternal uncle, who was also a carrier of the *NRXN1* deletion inherited by our proband, presented with SZ. Furthermore, executive dysfunction has been claimed to account for the generativity deficit observed in ASD (i.e., the lack of new ideas, behaviors, and utterances tailored to new contexts) and ultimately, for the stereotypies, echolalic utterances and restrained interests typical of this condition [Turner, 2000; Gilotty et al., 2002]. Additionally, some studies [e.g., Perner and Lang, 2000; Russell, 2000; Ibáñez-Barasasi, 2005] have associated executive dysfunctions with an

impaired theory of mind. Overall, one could hypothesize that the *NRXN1* deletion found in our proband may impact on her executive function, thus affecting a wide range of cognitive abilities, such as attention (which plays a central role in learning and communication), theory of mind (necessary for the development of adequate communication skills) and, of course, speech and language.

Brignell et al. [2018] support the view that there is no specific linguistic profile for deletions of *NRXN1*. We agree that the speech, language, and communication deficits found in our proband can be expected to result to a great extent from the impairment of more basic cognitive functions, which are also affected in other high-prevalent, co-morbid neurodevelopmental conditions in children with *NRXN1* deletions. Nonetheless, we think that it is possible to identify and characterize a specific molecular pathway accounting for the speech and language problems observed in deletions of *NRXN1*. Accordingly, our hypothesis is that changes in *NRXN1* dosage might impact on strong candidates for language development and evolution. In our proband, *NRXN1* is not significantly dysregulated compared to her healthy parents. We did not find either in silico evidence of a functional connection between the protein NRXN1 and the products of candidates for language development, impairment, and/or evolution. Nonetheless, we have found that several candidates for language disorders and/or language evolution are indeed dysregulated in the proband's blood. While as noted we have no evidence of a direct effect of the loss of *NRXN1* on the dysregulation of these genes, these changes can be expected to contribute to the language deficits observed in the proband. Among the genes found downregulated, we wish to highlight the DD candidates *ROBO1* and *SETBP1*. The former encodes a protein involved in thalamocortical axon development and plays a critical role in the establishment of vocal learning pathways in many species [Pfenning et al., 2014]. Mutations in the latter give rise to Schinzel-Giedion syndrome, a clinical condition entailing occasional epilepsy and severe developmental delay [Ko et al., 2013; Miyake et al., 2015], as well as behavioral and social deficits [Coe et al., 2014]. Also the DD candidate *CNTNAP2* is found strongly downregulated in our proband. This gene encodes a protein associated with K<sup>+</sup> voltage-gated channels in the pyramidal cells of the temporal cortex [Inda et al., 2006], which contributes to regulate brain connectivity and morphology [Scott-Van Zeeland et al., 2010; Tan et al., 2010; Dennis et al., 2011], dendritic arborization and spine development [Anderson et al., 2012], and axonogenesis in conjunction with ROBO factors [Banerjee et

al., 2010]. *CNTNAP2* has been also associated with language and speech regression [Strauss et al., 2006; Marchese et al., 2016; Smogavec et al., 2016], SLI [Newbury et al., 2011], ASD [Alarcón et al., 2008; Bakkaloglu et al., 2008], CAS [Worthey et al., 2013], and language impairment in SZ [Poot, 2015]. As noted, CAS is a core symptom in our proband, whereas one of her relatives has been diagnosed with SZ. Moreover, several candidates for DD are upregulated in the proband, including *KIAA0319*, *KANSL1*, and *S100B*. *KIAA0319* encodes a membrane protein contributing to the interactions between neurons and radial glial cells during neuronal migration [Paracchini et al., 2006; Velayos-Baeza et al., 2008]. Mutations in *KANSL1*, which encodes a component of the NSL1 complex, important for chromatin organization and gene transcription regulation, result in Koolen-de Vries syndrome (OMIM #610443). This clinical condition features epilepsy, developmental delay, and moderate ID, which impacts mostly on expressive language development [Koolen et al., 2016]. Finally, *S100B* encodes a calcium-binding protein involved in neurite extension, axonal proliferation, and synaptic plasticity and learning [Sorci et al., 2013].

Among the candidates for language evolution (many of them resulting in cognitive disorders like ASD or SZ when mutated) that are found dysregulated in the proband, we wish to highlight *SLIT1*, *SLIT2*, and *HES1*. The SLIT/ROBO pathway is thought to play a crucial role in the externalization of language (i.e., speech) [for details, see Boeckx and Benítez-Burraco, 2014b]. Several other genes related to (the evolution of) speech are upregulated in the girl, including *DUSP1*, involved in vocal learning in songbirds [Horita et al., 2010, 2012]; *PLAUR*, part of FOXP2's interactome [Royer-Zemmour et al., 2008; Roll et al., 2010] and a candidate for speech dyspraxia [Roll et al., 2006]; *TLE3*, a target of FOXP2 in the inferior prefrontal cortex [Spiteri et al., 2007]; and *FLNA*, which encodes an actin-binding protein needed for cytoskeleton remodeling and neuronal migration [Fox et al., 1998], and that interacts with *CDC42*, which is also found upregulated in our proband. *CDC42* regulates cortical interneuron migration [Katayama et al., 2013] and dendritic spine growth and function [Datta et al., 2015]. In mice, *SLIT2* reduces *CDC42* activity in living growth cones [Myers et al., 2012], whereas depletion of *ROBO1* prevents *SLIT2* inhibition of *CDC42* activity [Yiin et al., 2009]. The abnormal expression pattern of these candidates for DD and speech-related genes might account for the speech and phonological problems exhibited by our proband.

Also of interest for explaining the language deficits observed in the proband is the upregulation of *NCAM1*, a gene related to working memory performance [Bisaz et al., 2013], but also to neuropsychiatric conditions like SZ, bipolar disorder, and Alzheimer disease [Atz et al., 2007]. *NCAM1* is a target of FOXP2 [Konopka et al., 2009], but also of *RUNX2* [Kuhlwilm et al., 2013]. *RUNX2* is strongly upregulated in the proband. This is a key gene hypothesized to account for some of the changes in the human brain morphology and configuration resulting in our cognitive uniqueness, particularly, our cross-modal thinking [for a detailed discussion, see Boeckx and Benítez-Burraco, 2014a]. Also *ASPM* is found upregulated in our proband. This is a strong candidate for microcephaly, but also for the increase in brain size in the human lineage [Bond et al., 2002; Zhang, 2003]. Finally, the finding that *DYRK1A* is upregulated in the girl is of interest too. Mutations in this gene result in microcephaly, as well as in ID and absence of speech [Van Bon et al., 2011; Courcet et al., 2012]. The gene seems to play a role in learning and memory [Hämmerle et al., 2003] via its effects on synaptic plasticity and on the balance between excitation and inhibition [Souchet et al., 2014]. Overall, the abnormal expression patterns of these genes can also contribute to the speech problems of the proband through their effects on the FOXP2 interactome, but also to her problems with grammar through their effect on the *RUNX2* network.

Lastly, it is worth considering the biological roles played by the genes that are strongly dysregulated in the blood of the girl compared to her healthy parents. The most promising genes for explaining her language deficits are *LIN7A* (found strongly downregulated) and *RAP1GAP* (found strongly upregulated). Both genes are expressed in the brain, particularly in the cerebellum (*LIN7A*) and the striatum (*RAP1GAP*), which are 2 regions that are crucially involved in language processing [Silveri, 2020], particularly, in speech production [Konopka and Roberts, 2016]. Deletions in *LIN7A*, a gene encoding a scaffold protein important for synaptic function, impact on the development of the cerebral cortex and result in ID [Matsumoto et al., 2014]. Regarding *RAP1GAP*, it encodes a GTPase-activating-protein acting as a negative regulator of *RAP1*, which is involved in cell proliferation, adhesion, differentiation, and embryogenesis and, specifically, in neuronal migration [Yang et al., 2019] and the regulation of dendritic spine morphology in the striatum [McAvoy et al., 2009] and the cortex [Chen et al., 2005]. *RAP1GAP* mediates aversive behaviors in the nucleus accumbens [Lin et al., 2020]. Interestingly, we have



found that the products of these 2 genes interact with the products of several candidates for language impairment and/or evolution, particularly, *GRIN2A*, *GRIN2B*, *HRAS*, and *ERBB4*. *GRIN2A* and *GRIN2B* are two of the components of the NR2 subunit of a receptor for N-methyl-D-aspartate (NMDA), involved in long-term potentiation and ultimately in memory and learning. Mutations in *GRIN2A* give rise to speech impairment and language regression, as part of epileptic syndromes like Landau-Kleffner syndrome, continuous spike and waves during slow-wave sleep (CSWSS) syndrome, or rolandic epilepsies [Carvill et al., 2013; Lesca et al., 2013; Dimassi et al., 2014]. The speech problems observed in patients include syllable repetitions, imprecise articulation, and problems with pitch and prosody, which are usually labelled as dyspraxia or dysarthria [Turner et al., 2015], and which parallel the speech deficits observed in our proband. By contrast, mutations in *GRIN2B* are mostly related to behavioral problems, but also to motor and cognitive problems [Freunsch et al., 2013; Hu et al., 2016; Smigiel et al., 2016]. Concerning *HRAS*, it is a candidate for DD and ASD, and encodes a GTPase that contributes to regulate neural growth and differentiation, long-term potentiation, and synaptic plasticity [Comings et al., 1996]. Lastly, *ERBB4* is involved in the regulation of interneuron migration and in synchronizing neural oscillations in the cortex [Li et al., 2012; Hou et al., 2014]. Mutations in *ERBB4* result in ID and speech delay [Kasnauskiene et al., 2013]. In our view, the strong dysregulation of these genes could contribute as well to explain the speech, language, cognitive, and behavioral problems exhibited by our proband.

## Conclusions

Although the exact molecular causes of the speech and language problems observed in our proband remain to be fully elucidated, we think that they might result from the dysregulation of several known candidates for language impairment and/or evolution with a known function on brain development and with a role in cognitive diseases impacting on speech and language, particularly, ASD, ID, and DD. This hypothesis needs to be properly tested. Particularly, the link between the loss of *NRXN1* and the dysregulation of these genes needs to be probed in vitro and in vivo. Still, we hope that our findings contribute to a better understanding of the speech, language, cognitive, and behavioral phenotype resulting from mutations and CNVs of this key neurexin. More detailed accounts of the neurobiological basis of the deficits (and strengths) ex-

hibited by patients are necessary for improving (psycho) pedagogical strategies aimed to ameliorate their problems.

## Acknowledgments

We would like to thank the proband and her family for their participation in this research. We wish to thank also Dr. Montserrat Barcos Martínez and Dr. Isabel Espejo Portero, from the “Reina Sofía” hospital in Córdoba, for granting us access to the patient and her clinical history.

## Statement of Ethics

Ethics approval for this research was granted by the Comité Ético del Hospital “Reina Sofía”. Written informed consent was obtained from the proband’s parents for conducting the psycholinguistic evaluations and the molecular analyses, and for publicizing this case report and all the accompanying tables and images in scientific journals and meetings.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.

## Funding Sources

This research was conducted in absence of external financial aid.

## Author Contributions

Antonio Benítez-Burraco conceived the paper, planned the molecular experiments, analyzed the genomic/genetic data, and wrote the paper. Maite Fernández-Urquiza and M. Salud Jiménez-Romero conducted the clinical evaluation of the proband, analyzed the psycholinguistic data, and wrote the paper. All authors approved the final version of the manuscript.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article. A preprint version of this article is available on PsyArXiv [https://doi.org/10.31234/osf.io/aw25n].



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