Language Impairment with a Partial Duplication of *DOCK8*

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Keywords

9p24.3 duplication · *DOCK8* · Cognitive delay · Language deficits · Working memory · SLIT/ROBO/FOXP2

Abstract

Duplications of the distal region of the short arm of chromosome 9 are rare, but are associated with learning disabilities and behavioral disturbances. We report in detail the cognitive and language features of a child with a duplication in the 9p24.3 region, arr[hg19] 9p24.3(266,045–459,076)×3. The proband exhibits marked expressive and receptive problems, which affect both structural and functional aspects of language. These problems might result from a severe underlying deficit in working memory. Regarding the molecular causes of the observed symptoms, they might result from the altered expression of selected genes involved in procedural learning, particularly some of components of the SLIT/ROBO/FOXP2 network, strongly related to the development and evolution of language. Dysregulation of specific components of this network can result in turn from an altered interaction between *DOCK8*, affected by the microduplication, and *CDC42*, acting as the hub component of the network encompassing language-related genes.

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Introduction

Copy number variants (CNVs) identified in subjects with language problems provide important evidence of how changes in gene dosage affect the wiring and functioning of brain areas involved in language processing, particularly if they result in syndromic features and encompass few genes. The terminal region of the short arm of chromosome 9 is prone to deletion, giving rise to a complex syndromic condition entailing mental retardation, delayed psychomotor development, and speech delay (OMIM #158170). Specifically, and according to the Unique database (<https://www.rarechromo.org/>), deletions of the 9p24.3 region are associated with mild learning disabilities, that commonly improve with appropriate early/supporting intervention, and with language and speech delay, with a greater impact on the expressive domain. Although deletions of 9p are more frequent than duplications, an interstitial duplication at 9p24.3, encompassing the genes *DOCK8* and *KANK1*, has been recently associated with multiple neurodevelopmental disorders, including schizophrenia, bipolar disease, autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), and depression – all of them conditions entailing problems with language [Glessner et al., 2017].

In this study, we report on a boy with a partial duplication of *DOCK8* resulting from a de novo microduplication in 9p24.3 and provide a detailed characterization of his language (dis)abilities. A growing body of evidence points to an association of *DOCK8* deletions, duplications, and translocations interrupting its functionality, with intellectual disability and developmental delay [Griggs et al., 2008; Krgovic et al., 2018]. According to DECIPHER (<https://decipher.sanger.ac.uk/>), nearly 60% of the 143 CNVs involving *DOCK8* are losses and about 40% are gains. *DOCK8* losses are associated with intellectual disability, global developmental delay, abnormal facial features, and microcephaly, whereas *DOCK8* gains are mostly associated with intellectual disability, global developmental delay, ASD, clinodactyly, and delayed speech and language development. However, these CNVs usually involve other genes besides *DOCK8* and/or are found in patients with other chromosomal abnormalities. Accordingly, we expect that smaller CNVs affecting *DOCK8* only, like the one found in our patient, help to achieve more robust genotype-phenotype correlations.

DOCK8 plays an important role in modulating the immune response, and mutations in the gene are known to cause immunodeficiency disease [Biggs et al., 2017; Broides et al., 2017; Kearney et al., 2017]. Because the gene is also expressed in the fetal and adult brains [Griggs et al., 2008], it is expected to play a key role as well in brain development and function. Accordingly, in mice the *DOCK8* protein contributes to Schwann cell precursor migration during embryonic development of the peripheral nervous system [Miyamoto et al., 2016]. *DOCK8* is upregulated in neuronally differentiated cells after the overexpression of *MECP2_e1*, which is the etiologically relevant variant of the *MECP2* protein for Rett syndrome [Orlic-Milacic et al., 2014]. *DOCK8* is a candidate for ASD too. Two de novo loss-of-function variants of *DOCK8* have been found in probands from the Autism Sequencing Consortium and the Autism Clinical and Genetic Resources in China (ACGC) cohort [De Rubeis et al., 2014; Wang et al., 2016]. The 9p24.3 region overlaps with linkage regions found in large ASD extended pedigrees [Allen-Brady et al., 2009; Coon et al., 2010]. Nonetheless, little is known about the causes of the neuropsychiatric features and neurodevelopmental anomalies associated with mutations of *DOCK8* or CNVs encompassing the gene. This is particularly true of the language deficits that are commonly observed in patients, among other reasons, because we still lack a detailed account of the language problems caused by *DOCK8* mutations or dosage alterations. In this study, we build on our

current knowledge of the genetic aspects of language development and language evolution, as well as on the expression pattern of selected genes in the blood of our proband, for advancing a hypothesis of the molecular causes of language dysfunction associated with *DOCK8* alterations.

Case Report

Clinical History

The proband is a boy born after 9 months of gestation by cesarean section. At birth, his weight was 3,450 g (65th percentile), his height was 50 cm (54th percentile), and his occipitofrontal circumference was 37 cm (98th percentile). The Apgar scores were 9 (at 1') and 9 (at 5'). At delivery, no signs of disease were observed, and the newborn was fed with both maternal and artificial milk. Brain MRI, performed at 4 years of age in view of the developmental and cognitive delay exhibited by the child, yielded no pathological results. The child has suffered from recurrent sinusitis, resulting in frequent nose infections and headaches. At the time of our evaluation, he was 11 years and 6 months old. He exhibited mild dysmorphic facial features, particularly hypertelorism and mild retrognathia.

Language and Cognitive Development

Early developmental milestones were achieved normally by the child, including sphincter control at the age of 2 years. Cognitive anomalies were first reported by the teachers of the nursery school when the proband was around 2 years old. At the age of 3 years, language delay was evident. According to his parents, the child's language was gibberish, with poor articulation, and he usually helped himself with body and hand gestures to communicate better. The child received early childhood intervention from the age of 4 years to the age of 6 years, and speech therapy from the age of 3 years to present. At the age of 6 years and 1 month, the child was evaluated by the hospital's neuropsychologist because of his language delay and learning difficulties. His language skills were poor. He uttered pseudosentences consisting of 3–4 content words with no functional words, such as articles. Expressive language was more impaired than comprehension. The child scored low in the Leiter International Performance Scale, 3rd Edition (Leiter-3), a test aimed to evaluate nonverbal cognitive, attentional, and neuropsychological abilities in typical and atypical populations [Roid et al., 2013]. The resulting IQ was 66, with an estimated mental age of 4 years (more than 2 years below his chronological age). He was diagnosed with global developmental delay primarily affecting language. Motor delay was attested too.

At the age of 9 years, our proband was evaluated by a child psychologist. The boy scored abnormally high in the Spanish version of the Conners Comprehensive Behavior Rating Scales (Conners CBRS) [Conners, 2008] as well as abnormally high (1st percentile) in the Escalas para la Evaluación del Trastorno por Déficit de Atención con Hiperactividad [Scales for the Evaluation of ADHD (EDAH)] [Farré and Narbona, 2013], this being suggestive of the presence of ADHD. Likewise, he scored very low in the Spanish version of the Differences Perception Test (CARAS-R) [Thurstone and Yela, 2012], which suggested that he was unable to judge prop-

Table 1. Summary table of the scores obtained by our proband in the WISC-V test

Scale	Subtest name	Total Raw score	Scaled score	Per-centile rank	Age equivalent	SEM	Total Scaled score	Composite score	Percentile rank	Confidence interval (95% level)	Classification	SEM
Verbal comprehension	Similarities	15	3	1	<6y 2m	1.04	4	55	0.1	51–69	Very low	4.50
	Vocabulary	12	1	0.1	<6y 2m	1.27						
	(Information)	8	2	0.4	<6y 2m	1.08						
	(Comprehension)	12	3	1	6y 2m	1.37						
Visual spatial	Block design	14	4	2	<6y 2m	1.41	11	75	5	69–86	Low	4.74
	Visual puzzles	12	7	16	7y 10m	0.99						
Fluid reasoning	Matrix reasoning	5	1	0.1	<6y 2m	1.04	8	67	1	62–77	Very low	3.67
	Figure Weights	17	7	16	8y 6m	0.73						
	(Arithmetic)	10	3	1	6y 6m	0.85						
Working memory	Digit Span	18	5	5	6y 10m	0.90	8	67	1	62–78	Very low	4.74
	Picture Span	11	3	1	<6y 2m	1.34						
	(Letter-number Seq.)	5	1	0.1	<6y 2m	1.20						
Processing speed	Coding	15	1	0.1	<8y 2m	1.31	5	56	0.2	52–70	Very low	5.41
	Symbol search	13	4	2	<8y 2m	1.37						
	(Cancellation)	65	9	37	10y 6m	1.20						
Total Scale							22	54	0.1	50–63	Very low	3.00

SEM, standard error of measurement; y, years; m, months. Subtests used to derive the IQ are in bold. Secondary subtests are in parentheses.

erly about similarities and differences and paid reduced attention to details because of his reduced visuo-perceptive abilities. He also scored very low in the impulsivity control index, which pointed to a high impulsivity and lack of inhibitory control. In the Spanish version of the Behavior Assessment System for Children (BASC-2) [Reynolds and Kamphaus, 2004], which evaluates different aspects of the child's behavior, our proband scored abnormally high in the subtests assessing the presence of aggressiveness, hyperactivity, attention deficit, and depression. Finally, regarding his intellectual development, the boy obtained 77 points (6th percentile) in the Spanish version of the Reynolds Intellectual Assessment Scales (RIAS) [Reynolds and Kamphaus, 2013]. He obtained 82 points (11th percentile) in the Verbal Index of the test, 77 points (6th percentile) in the Nonverbal Index, and 80 points (9th percentile) in the Memory Index. Overall, the obtained scores pointed to a mild to moderate impact of cognitive impairment on functional abilities. Similarly, the IQ of our proband according to the Spanish version of the Cattell intelligence test [Cattell and Cattell, 1994] was 65 (4th percentile), which was indicative of low to moderate intellectual disability.

At the age of 11 years, the proband was again evaluated by the school's psychologist, who reported cognitive delay, difficulties with recalling information, problems with understanding, poor reasoning, and problems with spatio-temporal perception. He also

exhibited low working memory abilities. In the language domain, articulatory problems were observed, in part resulting from an orofacial hypotonia, as well as expressive and comprehensive deficits in the oral and written domains. Although our subject had learnt to read and write, he made frequent mistakes. He also exhibited behavioral disturbances, including low tolerance to frustration, variable mood, frequent distractions, and difficulties with interacting with some of his peers (being instead attentive and kind with adults). Minor coordination problems in the motor domain were confirmed. However, they did not preclude him from mastering the needed skills for skiing, biking, or skating. At that time, he was attending a normal school, although he was aided by an assistant teacher several hours a week.

When the child was 11 years and 6 months old, we assessed in detail his global development with the Spanish versions of the Wechsler Intelligence Scale for Children, 5th Edition (WISC-V) and the Inventory for Client and Agency Planning (ICAP). In the WISC-V the child scored low in most of the subtests (Table 1), resulting in a IQ of only 54. As also shown in Table 1, verbal comprehension, fluid reasoning, working memory, and processing speed were significantly impaired, with the visual spatial abilities being the most preserved domain. Regarding the ICAP, which was administered to assess the child's adaptive behavior, the obtained scores were indicative of a delay of 5 years and 3 months in his

Fig. 1. Distinctive clinical features of the proband. **a** Developmental profile of the proband at age 11 years and 6 months according to the Inventory for Client and Agency Planning (ICAP). DA, developmental age; CA, chronological age. **b** Samples of the child's writing and drawing abilities. His handwritten name is displayed above. Below, 2 different drawings are shown: the proband's sister leaving for a plane trip (left) and a motorcycle (right). The size of the drawings correlate with their emotional contents.

global development, with social and communication skills being the most impaired domain; community living skills were relatively spared (Fig. 1a; see online suppl. file 1 for details; for all suppl. material, see www.karger.com/doi/10.1159/511972).

Because of the significant impairment reported by the WISC-V, we assessed in detail the linguistic abilities of the proband at the age of 11 years and 6 months, using different tests aimed to evaluate his expressive and comprehensive abilities as well as how they were put into use for communicating.

Table 2 summarizes the scores obtained by our proband in the subtests comprising the Clinical Evaluation of Language Fundamentals (CELF-4), which is aimed to provide a detailed overview of the language deficits and strengths of the subject. The Core Language Score of the CELF-4 was indicative of a moderate language disorder. The most affected domains were Working Memory and Language Memory, which were severely impaired. Language Content, Receptive Language and Expressive Language were moderately affected. Interestingly, as also shown in Table 2, in some of the subtests our proband scored like neurotypical children, significantly in tasks evaluating semantic knowledge. Conversely, he obtained the lowest scores in tasks assessing his ability to understand and compose sentences, and to retrieve and process verbal information. Regarding the subtests aimed to establish the nature of the underlying cognitive processes plausibly accounting for these problems (Level 3 of the CELF-4), he scored atypically (in

terms of time of execution and performed errors) in the tasks assessing his ability to produce automatic speech (Rapid Automatic Naming) and scored very low in the Working Memory Index (Table 2). On the contrary, he scored only slightly below his age-matched peers in the tasks evaluating his phonological competence (Phonological Awareness) and his semantic category knowledge (Word Associations). Last, regarding his pragmatic abilities (Level 4 of the CELF-4), our proband exhibited problems with properly putting his language knowledge into use. The Pragmatic Profile suggested that he was performing below his chronological age. The checklist in the Observational Rating Scale component corroborated the findings at the other levels of the test, pointing to widespread problems in all the assessed domains: language comprehension, language production, and literacy. The proband was reported to have difficulties with finding the correct or needed words, and with describing things, expressing what he meant, and describing events in a coherent fashion. Likewise, he was reported to have problems with sustained attention and with following and recalling oral instructions. On the contrary, he was able to follow the other's gaze and paid attention to nonverbal, gestural, and corporal language during conversational exchanges. Moreover, he seemingly had a good command of the principles regulating conversation (for instance, he knows how to ask for clarifications and for help when he does not understand something). Last, this part of the test also corroborated his generalized problems with reading

Table 2. Summary table of the scores obtained by our proband in the CELF-4 test

	Core language CLS	Receptive language RLI	Expressive language ELI	Language content LCI	Language memory LMI	Working memory WMI	SD	Developmental age	Developmental age/Chronological age	Classification
Concepts & following directions	4	4			4		−2 SD	6y 11m	0.5	Very low
Recalling sentences	6		6		6		−1.5 SD	6y 9m	0.6	Low
Formulated sentences	3		3		3		−2 SD	<9y	0.7	Very low
Word classes-receptive		8					−1 SD	8y 3m	0.7	Average
Word classes-expressive			9				−1 SD	8y 3m	0.7	Average
Word classes-Total	9			9			−1 SD	9y 3m	0.8	Average
Word definitions				5			−2 SD	<10y	0.8	Low
Understanding spoken paragraphs				1			−3 SD	No age equivalents available		Very low
Number repetition 2-Total						5	−1.5 SD	8y 6m	0.7	Low
Familiar sequences 2						2	−3 SD	No age equivalents available		Very low
Sum of subtest Scaled scores	22	12	18	15	13	7				
Standard score	73	77	77	70	68	60				
Standard score points +/−	6	9	6	5	7	10				
Confidence interval (95%Level)	67–79	68–86	71–83	65–75	61–75	50–70				
Percentil rank	4	6	6	2	2	0.4				
Severity of the disorder										
Distance from the mean	−2 SD	−2 SD	−2 SD	−2 SD	−3 SD	−3 SD				
Classification	Low/moderate	Low/moderate	Low/moderate	Low/moderate	Very low/severe	Very low/severe				

SD, standard deviation; y, years; m, months.

and writing. He experiences difficulties with understanding written texts and extracting the main theme. Likewise, he exhibited difficulties with writing, particularly with the shape of the letters, and with drawing, although his drawing abilities are in line with his cognitive deficits and language problems (Fig. 1b).

We assessed in more detail aspects of the phonetic, semantic, and grammatical profile of our proband, in order to refine our view of his deficits and strengths in the language domain. Articulatory problems were evaluated with the Registro Fonológico Inducido, which pointed out that he has problems mostly with liquid consonants (i.e., [l], [r], [r]). Accordingly, he regularly assimilates [r] to [l], like in ['flesa], instead of ['fresa] “strawberry”, or ['bluja], instead of ['bruja] “witch”. Polysyllabic words were also problematic for him, as in [pre'ðrjoðiko] instead of [pe'rjoðiko] “newspaper” (for details, see online suppl. file 1). Semantic problems were assessed in more depth with the Peabody Picture Vocabulary Test (PPVT-3). The resulting scores pointed to a delay of 2 years in his

receptive vocabulary (online suppl. file 1). Finally, we used the Test de Comprensión de Estructuras Gramaticales (CEG) for refining our characterization of the proband’s grammatical knowledge. The global score obtained by the child in this test was 62 (10th percentile). This low performance was mostly caused by generalized difficulties with computing marked word order (as in topicalized or cleft sentences), anaphora (particularly in complex sentences with relative pronouns), and clitics. On the contrary, sentence length did not have any significant impact on his performance (online suppl. file 1).

Finally, we examined in even more depth the child’s language in use and his communication skills through the analysis of several naturally occurring interactions with his mother and his speech therapist (online suppl. file 2). We confirmed that the proband’s speech exhibits frequent (but nonsystematic) substitutions of specific consonants and vowels (e.g., [r] > [d], [r] > [d], [d] > [r], [s] > [θ], [r] – [r] > [l], [e] > [a], [e] > [i], [o] > [a]) as well as recur-

Fig. 2. Chromosomal alterations found in our proband. **a** Screen capture of the array-CGH of the proband's chromosome 9 showing the microduplication at 9p24.3. **b** Screen capture of the UCSC Genome Browser (<https://genome.ucsc.edu/>) showing the gene partially duplicated in the proband. **c** Screen capture of the array-CGH of the proband's chromosome 8 showing the microdeletion at 8p23.1. **d** Screen capture of the UCSC Genome Browser (<https://genome.ucsc.edu/>) showing the genes partially deleted in the proband.

rent simplification of consonant clusters (e.g., [tar'xeta] > [ta'xeta] "card", [pri'mero] > [pi'mero] "first", [traβa'xar] > [taβa'xar] "work", [gusta] > [guta] "I like", [grita] > [rita] "he shouts", [en'tran] > [in'tan] "they go into"). We also found occasional diphthong simplifications (e.g., [we] > [e]), sound assimilations ([deθi'males] > [diθi'males] "decimals"; ['poster] > ['potter] "poster", [ernja] > [ennja] "hernia") and metatheses or sound inversions (e.g., [aðxe'tiβos] > [axeθ'tiβos] "adjectives"). Speech prosody and fluency were significantly impaired due to the articulatory difficulties experienced by the child, which hinders his speech intelligibility.

Concerning language structure and complexity, the mean length of utterance in words was 4.2, which is within the range of children aged between 4 years and 6 months, and 5 years [Rice et al., 2010], and thus remarkably below his chronological age. The child produced morphosyntactic errors such as substitutions of inflectional morphemes (dicen > dice "they say", as in *y todos dice "and all of them say"), substitution of conjunctions (que > a "that", as in *tenemos a poner "we have to put"), and omissions

of prepositions (de > ø "of" as in *qué color soy "(of) which color I am").

Finally, we observed that the child usually avoided the indirect or reported speech, which is more complex syntactically. Instead, when requested to retell a story with several characters, he represented the different perspectives by dramatizing each character by means of direct reported speech, as is commonly observed in people with Williams Syndrome [Losh et al., 2000; Reilly et al., 2004; Gonçalves et al., 2010; Shiro et al., 2016].

Despite these problems with some structural components of language, the boy's interactive pragmatic skills seemed to be quite preserved. Accordingly, he had mastered most of the conversational abilities, including turn-taking and repair strategies [Sacks et al., 1974]. For instance, he made an adequate communicative use of gaze when taking the floor, but also when ceding it to the other participants. Likewise, in order to compensate his expressive difficulties, he might explicitly request for help and make visual contact with his interlocutor. Additionally, he used fillers, word repetitions, and reformulations to keep the turn and extend the time

of elocution, in order to gain some extra seconds to build up his utterances. Likewise, his echolalic replies reflected his willingness to collaborate with the interlocutor during verbal interactions despite his comprehension problems. Interestingly, the child made extensive use of interjections along with facial expressions as a sort of “inference-launching mechanism” that seemingly allowed him to communicate implicit propositional contents avoiding at the very same time his expressive difficulties. That is to say, the intentional transgression of the conversational maxims of manner (“be as clear, as brief, and as orderly as you can in what you say, and avoid obscurity and ambiguity”) and quantity (“be as informative as possible and give as much information as needed, but no more”) resulted in successful implicit communication. However, pathological transgressions of the maxims of quality (“be truthful and do not give information that is false or that is not supported by evidence”) and relation (“be relevant and say things that are pertinent to the discussion”) were also observed. For example, when asked simple questions about the characteristics of a pineapple, he would say that it is not yellow, but triangular and with seeds. This might result from an aberrant reasoning and/or lack of awareness of the world of reference shared by his interlocutors, which might explain as well his difficulties to understand even the dynamics of simple games.

Cytogenetic and Molecular Analyses

Routine cytogenetic and molecular analyses of the proband were performed when he was 6 years and 6 months old. The karyotype was normal, although the analysis did not discard the presence of minor chromosomal aberrations. PCR analysis of the *FMR1* fragile site was normal (28 repetitions). MLPA of subtelomeric regions detected an increase of gene dosage in 9p24.3 involving *DOCK8* (P070-B2 Human Telomere-5 probemix). A comparative genomic hybridization array (array-CGH) was performed a month later, when the proband was 6 years and 7 months old. The arrays confirmed the presence of a duplication of nearly 0.2 Mb in the 9p24.3 region, arr[hg19] 9p24.3(266,045–459,076)×3, predicted to be probably benign, and affecting the gene *DOCK8* (Fig. 2a, b). However, it also uncovered a microdeletion of 0.5 Mb in the 8p23.1 region, arr[hg19] 8p23.1(7,169,490–7,752,586)×1, predicted to be benign. This microdeletion in chromosome 8 encompasses 19 protein-coding genes: 12 genes coding for defensins, 2 genes coding for proline-rich domain proteins, 2 genes coding for sperm-associated antigen proteins, 2 genes coding for ubiquitin peptidase-like proteins, and 1 gene coding for a zinc finger protein (Fig. 2c, d). Both alterations occurred de novo in the proband.

In order to delve into the molecular causes of the speech and language deficits exhibited by the child, we conducted several silico analyses. First, we surveyed the literature looking for patients with language problems in which any of the genes deleted or duplicated in our proband were affected. None of the genes located within the deleted region in chromosome 8 have been associated with language deficits. Defensins are peptides with a broad-spectrum antimicrobial activity and with no known role in brain development or function, beyond host defense against central nervous system pathogenesis [Hao et al., 2001]. Defensin CNVs are well-known, but they have been related mostly to differential susceptibility to infection and disease [Linzmeier and Ganz, 2005]. Likewise, the 2 sperm-associated antigen proteins (SPAG11A and SPAG11B) encode β -defensins involved in sperm maturation [Chan and Zhang, 2005; Hu et al., 2014], with no known role in

Table 3. Summary table with the most relevant clinical features of our proband compared to patients with *DOCK8* deficiency^a and with duplications affecting or encompassing *DOCK8* (up to 1 Mb)^b

<i>Clinical features of DOCK8 deficiency</i>	
Cognitive/behavioral features	
Mental retardation	x
Absence of speech/language impairment	x
Physical features	
Dysmorphic facial features	x
Increased nose width	
Retained primary teeth	
High palate	
Other medical problems	
Neurological complications	
Skin disease (eczema, abscesses)	
Lung disease/abnormalities (pneumonia, bronchiectasis)	
Atopy (asthma, allergies)	
Autoimmune disease	
Malignancies	
Hyperflexibility	
High susceptibility to superficial, localized, and invasive infections	
<i>Clinical features of DOCK8 duplications (up to 1 Mb)</i>	
Cognitive/behavioral features	
Intellectual disability	x
Global developmental delay	x
Autism	
Behavioral abnormality	x
Delayed speech and language development	x
Neurological findings	
Motor delay	x
Epileptic spasms	
Febrile seizures	
Physical features	
Macrocephaly	
Dolichocephaly	
Dysmorphic facial features (long philtrum)	x

^a According to Griggs et al., 2008, Biggs et al., 2017, and Engelhardt et al., 2017. ^b According to DECIPHER. The observed features are marked with x.

brain development either. The 2 proline-rich domain proteins (PRR23D1 and PRR23D2), the 2 ubiquitin peptidase-like proteins (USP17L1P and USP17L4), and the zinc finger protein (ZNF705G) play unknown roles. On the contrary, our proband exhibits several of the neurological, cognitive, and behavioral features of *DOCK8*-deficient patients, as well as individuals with duplications of the 9p24.3 region encompassing *DOCK8* (Table 3).

Additionally, we surveyed DECIPHER (<https://decipher.sanger.ac.uk/>) to find other patients with deletions and duplications that are similar or smaller than the fragments deleted or duplicated in our proband that might help associate his language problems to specific genes. We found that microdeletions in 8p23.1 are usually reported as benign (patients 298702, 285986, 298151), of uncertain pathogenicity (patient 323189), or of un-

Fig. 3. DECIPHER patients of interest bearing chromosomal duplications at 9p24.3 with similar sizes as the one found in the pro-

band. Patients with cognitive deficits are highlighted in red, cases with no reported cognitive problems are highlighted in blue.

known pathogenicity (patients 317632 and 317616). DECIPHER patients exhibiting developmental delay (298702, 285986, and 298151) bear other CNVs in other chromosomes that are reported as pathogenic and that seemingly account for their problems in the cognitive/behavioral domains. Three DECIPHER patients (323189, 317632, and 317616) bear unique deletions in chromosome 8 that are similar to the one found in our patient, but neither of them is reported to present with language delay, speech problems, cognitive disabilities, mental retardation, or learning impairment. By contrast, DECIPHER patients with duplications in 9p24.3 encompassing *DOCK8* frequently present with intellectual disability, developmental delay, behavioral abnormality, and/or delayed speech and language development, even in the absence of additional CNVs (e.g., patients 328340 and 251154) (Fig. 3).

Finally, we used String 10.5 (www.string-db.org) for uncovering potential functional links between the genes deleted and duplicated in our proband and genes important for language. We focused on candidate genes for (i) prevalent language disorders (developmental dyslexia and specific language impairment (SLI), as listed by Paracchini et al. [2016], Pettigrew et al. [2016], and Chen et al. [2017], and (ii) language evolution, as discussed by Boeckx and Benítez-Burraco [2014a,b] and Benítez-Burraco and Boeckx [2015] (many of the genes belonging to this second group are also candidates for language dysfunction in broader cognitive disorders, particularly ASD and schizophrenia, as discussed in detail in Benítez-Burraco and Murphy [2016] and Murphy and Benítez-Burraco [2016, 2017]. The whole set of genes considered in this analysis is listed in online supplementary file 3. String 10.5 predicts physical and functional associations between proteins relying on different sources (genomic context, high-throughput experiments, conserved coexpression, and text mining) [Szklarczyk et al., 2015]. Because we were interested in finding robust functional links, we restricted our search to significant protein interactions gathered from protein-protein interaction databases and curated databases (BioCarta, BioCyc, GO, KEGG, and Reactome). String did not pre-

dict any functional link between any of the proteins coded by the genes within the 8p23.1 deleted region and the proteins coded by candidates for language disorders and/or evolution (data not shown). By contrast, it predicted a robust direct link between *DOCK8* and *CDC42* as well as robust indirect links with nearly 30 other candidates for language disorders and/or evolution, including *SLIT1*, *SLIT2*, and *ROBO1*, which play a key role in the externalization of language (speech) [for review, see Boeckx and Benítez-Burraco, 2014b] (Fig. 4).

Besides in silico analyses, we also conducted in vitro analyses. Specifically, we performed microarray analyses of blood samples from the proband to determine whether he exhibited altered patterns of gene expression that may account for the observed symptoms. We used his healthy parents as controls. The results of the microarrays are shown in online supplementary file 4. First, we checked whether the genes within the deleted or duplicated chromosomal fragments were differentially expressed in the proband compared to his unaffected parents (Fig. 5a). We found that *DOCK8* was slightly upregulated in the proband. Regarding the genes within the deleted fragment in 8p23.1, we found that several genes encoding defensins were downregulated, particularly *DEFB105A*, *DEFB105B*, *DEFB106B*, *DEFB107A*, and *DEFB107B*. By contrast, *SPAG11B* and *ZNF705G* were slightly upregulated.

Second, we checked whether the genes encoding proteins predicted by String 10.5 to interact with *DOCK8* were differentially expressed in the boy compared to his healthy parents (Fig. 5b). For most genes, we found no significant differences. However, some of them were downregulated (*CDC42BPB*, *DOCK4*, *EGFR*), whereas *SRGAP3* was upregulated in the proband. Minor differences were found in *ROBO1* (slightly upregulated) and *SLIT* factors (slightly downregulated) as well as in *FOXP2* (slightly upregulated; see online suppl. file 4).

Finally, we searched for additional candidates for the proband's language deficits, looking for genes exhibiting strong fold changes in our subject (i.e., with fold changes >4 compared to both unaffected parents). We found several strongly downregulated genes

Fig. 4. Interaction network of the DOCK8 protein with proteins encoded by core candidates for language development and evolution. The network was drawn with String [version 10.5; Szklarczyk et al., 2015] license-free software (<http://string-db.org/>), using the molecular action visualization. It includes DOCK8 and the products of nearly 30 strong candidates for language development/evolution. Colored nodes symbolize gene/proteins included in the query (small nodes are for proteins with unknown 3D structure, while large nodes are for those with known structures). The color of the edges represents different kind of known protein-protein associations. Green: activation, red: inhibition, dark blue: binding, light blue: phenotype, dark purple: catalysis, light purple: post-translational modification, black: reaction, yellow: transcriptional

regulation. Edges ending in an arrow symbolize positive effects, edges ending in a bar symbolize negative effects, whereas edges ending in a circle symbolize unspecified effects. Gray edges symbolize predicted links based on literature search (co-mentioned in PubMed abstracts). Stronger associations between proteins are represented by thicker lines. The medium confidence value was 0.0400 (a 40% probability that a predicted link exists between 2 enzymes in the same metabolic map in the KEGG database: <http://www.genome.jp/kegg/pathway.html>). 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.

(*HERC5*, *SERPING1*, *IFIT2*, *IFI44L*, *IFIT3*, *CA1*, *IFIT1*, and *RSAD2*) as well as several strongly upregulated genes (*FCER2*, *BACH2*, *NRCAM*, *PLEKHG1*, *VPREB3*, *TCL1B*, *TCL6*, *AFF3*, and *TCL1A*) (Fig. 5c).

Because changes in gene expression levels with an impact on the proband's language abilities are expected to occur in the brain, we

determined whether the genes highlighted above (Fig. 5) are expressed in the brain at similar levels to the blood. Blood and brain expression profiles of genes were retrieved from the Genotype-Tissue Expression (GTEx) database (<https://www.gtexportal.org/home/>) and compared using the GTEx Multi Gene Query tool (Fig. 6). We further searched for the brain expression profiles

Fig. 5. Variation in the expression levels of genes of interest in the proband's blood compared to his healthy parents. **a** Fold changes in the expression levels of *DOCK8*, the only gene within the region duplicated at 9p24.3 in the proband (left), and of the genes within the region deleted at 8p23.1 (right). **b** Fold changes in the expres-

sion levels of predicted functional partners of *DOCK8* with a known role in language development, language impairment, and/or language evolution. **c** Genes exhibiting fold changes >4 in the proband compared to both healthy parents.

Fig. 6. Expression pattern in the blood and the brain of genes of interest. **a** Expression profiles of *DOCK8* and of the genes within the region deleted at 8p23.1 in the proband. **b** Expression profiles of predicted functional partners of *DOCK8* with a known role in language development, language impairment, and/or language evolution. **c** Expression profiles of genes exhibiting fold changes >4

in the proband compared to both healthy parents. Expression levels of the genes were retrieved from the Genotype-Tissue Expression (GTEx) project (GTEx Consortium, 2013) (<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. (Figure continued on next pages.)

across development of individual genes via the Human Brain Transcriptome Database (<http://hbatlas.org/>) (online suppl. file 5), focusing on Stage 11 (middle and late childhood, 6–12 years). We found that *DOCK8* is expressed at lower levels in the nervous system than in the blood, mostly in the spinal cord, but also in the brain, with the exception of the cerebellum. Regarding the genes located within the deleted fragment in 8p23.1, neither of them are expressed in the brain at any significant level (Fig. 6a). By contrast, most of the potential interactors of *DOCK8* with a known play in language development and/or evolution are highly expressed in the brain (with the exception of *CDH1*, *DCC*, and *MET*). In fact, they are expressed in the brain at higher levels than in the blood (with the exception of *MAPK14*). Specifically, most of them are preferentially expressed in the cerebellum and to some extent in the cortex and the basal ganglia (Fig. 6b). Finally, with regards to the genes that are strongly downregulated in our proband, we found that

many of them were highly expressed in most brain areas, particularly *IFT1*, *IFT2*, *IFT3*, and *SERPINC1*. Regarding the genes found strongly upregulated in the boy, *NRCAM* is highly expressed in all brain areas, whereas *AFF3* and *PLEKHG1* are preferentially expressed in the cerebellum and the spinal cord, respectively.

Materials and Methods

Linguistic, Cognitive, and Behavioral Assessment

The global developmental profile of the proband was defined with the Spanish versions of the Wechsler Intelligence Scale for Children, 5th Edition (WISC-V) [Hernández et al., 2015] and the ICAP [Montero, 1996].

Wechsler Intelligence Scale for Children

The WISC-V was used for testing multiple cognitive abilities of the child. It consists of 10 primary subtests and 5 secondary subtests, which are combined to generate different composite score indices. The 5 primary composite score indices are: Verbal Comprehension Index (VCI), Visual Spatial Index (VSI), Fluid Reasoning Index (FRI), Working Memory Index (WMI), and Processing Speed Index (PSI). The VCI measures verbal reasoning, understanding, concept formation, and crystallized intelligence. The VSI measures nonverbal reasoning and concept formation, visual perception and organization, visual-motor coordination, and the child's ability to analyze and synthesize abstract information, and to distinguish figure-ground in visual stimuli. The FRI evaluates quantitative reasoning, classification and spatial abilities, and knowledge of part to whole relationships, as well as fluid reasoning abilities. The WMI assesses the child's ability to sustain auditory attention, concentrate, and exert mental control. Last, the PSI de-

termines the speed and accuracy of the child when processing information.

The Inventory for Client and Agency Planning

The ICAP was used for evaluating the subject's functional abilities and maladaptive behaviors in the following general areas: motor skills, social and communication skills, personal living skills, and community living skills. The ICAP measures the frequency and severity of 8 types of behavioral disturbances, which are organized in 3 subscales: asocial maladaptive behavior (i.e., uncooperative behavior and socially offensive behavior), internalized maladaptive behavior (i.e., withdrawn or inattentive behavior, unusual or repetitive habits, and self-harm), and externalized maladaptive behavior (i.e., disruptive behavior, destructive to property, and hurtful to others). Behavior is rated as normal or abnormal, whereas behavioral problems are subsequently rated as marginally serious, moderately serious, serious, or very serious.

Regarding the language profile of our proband, his abilities were evaluated in depth with the Spanish versions of the Clinical Evaluation of Language Fundamentals, 4th Edition (CELF-4) [Semel et al., 2006], the Peabody Picture Vocabulary Test, 3rd Edition (PPVT-3) [Dunn et al., 2006], the Test de Comprensión de Estructuras Gramaticales (Test for evaluating the comprehension of grammatical structures; CEG) [Mendoza et al., 2005], and the Registro Fonológico Inducido (Triggered Phonological Register) [Juárez and Monfort, 1998].

Clinical Evaluation of Language Fundamentals

This test was used to evaluate the language (dis)abilities of the child and to gain a general view of his strengths and deficits during communication, and of the language domains that were more severely impaired. For subjects above 9 years old, like our proband, the CELF-4 encompasses 16 subtests, which are organized in 4 levels. Level 1 is aimed to identify whether or not the child suffers from a language disorder (expressed by the Core Language Score; CLS). Level 1 comprises the following 5 subtests: (1) Concepts & Following Directions (C&FD), in which the subject is expected to point to images of objects in response to oral commands, and which is aimed to evaluate the ability to interpret spoken directions, remember names and features of objects, and identify them among other pictured objects; (2) Recalling Sentences (RS), in

which the subject is expected to imitate different target sentences and which is aimed to assess the child's ability to listen and repeat sentences in an accurate way; (3) Formulated Sentences (FS), in which the subject is expected to formulate full sentences about different visual stimuli that are presented using target words or phrases, and which is aimed to evaluate the ability to create well-formed and congruent spoken sentences relying on given words and the contextual information provided by illustrations; (4) Word Classes 2-Receptive (WC 2-R), in which the subject is expected to point to the 2 pictured objects that are most related among a set of different objects, and which is aimed to evaluate the child's ability to understand logical relationships in the meanings of associated words, and (5) Word Classes 2-Expressive (WC 2-E), in which the subject is expected to describe the relationship that holds between the 2 objects chosen in subtest WC 2-R, and which is aimed to assess the ability to explain logical relationships in the meanings of associated words. At this Level 1, the WC 2-R and the WC 2-E scores are usually merged in a Word Classes Total score (WC-T).

Level 2 is aimed to identify the nature of the disorder through 4 index scores, resulting from different subtests. The Receptive Language Index (RLI) measures listening and auditory comprehension. It is calculated from the scores obtained in the C&FD and the WC 2-R subtests. The Expressive Language Index (ELI) measures the expressive language skills and is obtained from the scores

obtained in the RS, FS, and WC 2-E subtests. The Language Content Index (LCI) measures the subject's ability to understand and produce linguistic meaning by means of a set of subtests assessing the proband's lexical and morphosyntactic knowledge along with his pragmatic skills. It results from the WC-T score, plus the scores obtained in 2 other specific subtests: Words Definitions (WD) and Understanding Spoken Paragraphs (USP). In the WD, the subject is expected to provide a definition of several target words, that are orally presented, followed by an introductory sentence that includes the word. This subtest is aimed to evaluate the subject's expressive vocabulary, and particularly his ability to analyze words for their meaning features, define words by referring to class relationships and shared meanings, and describe meanings that are unique to the reference or instance. In the USP, the subject is expected to answer to different questions about a paragraph presented orally by the examiner. The questions target the paragraph's main idea, some of its details, and its sequencing, but also different kind of inferential and predictive information. This subtest is aimed to assess the subject's ability for sustained attention and focusing, but also to understand oral texts, and respond to questions about the contents of the given information. Finally, the Language Memory Index (LMI) measures the subject's ability to apply working memory to linguistic content and structure. It is calculated from the scores obtained in the C&FD, RS, and FS subtests.

Level 3 is aimed to assess underlying behaviors of clinical significance that may account for the attested language problems. It makes use of 3 specific subtests: (1) Phonological Awareness (PA), in which the subject is expected to identify, segment, blend, or delete sounds and syllables, and which evaluates his knowledge of the sound structure and rules of his language, and his ability to manipulate the sound units making up words and sentences; (2) Word Associations (WA), in which the subject is asked to recall words of specific semantic categories while being timed, and which is aimed to evaluate his semantic category learning and knowledge, in turn a measure of the development and adequacy of his vocabulary knowledge and use in receptive and expressive tasks, and (3) Rapid Automatic Naming (RAN), in which the subject is asked to name familiar colors, shapes, and shape-color combinations while being timed, and which is aimed to evaluate his ability to produce automatic speech. Level 3 also includes a Working Memory Index (WMI), which assesses working memory (important for attention, concentration, and recall) through 2 specific subtests: Number Repetition 2 Total (NR 2-T) and Familiar Sequences 2 (FS 2). In the NR2-T the subject is asked to repeat series of numbers forward and then backwards, with the aim of evaluating his abilities to repeat random number sequences. In the FS the subject is asked to name the days of the week, count backward, or recall other kind of information while being timed, with the aim of evaluating his ability to quickly sequence auditory and verbal information, but also his attention, concentration, processing speed, and auditory/verbal working memory.

Finally, Level 4 is aimed to evaluate language and communication in use by eliciting information from parents, teachers, or the subject himself about the subject's social and communication skills. This level encompasses 2 tasks: Pragmatics Profile (PP) and Observational Rating Scale (ORS). In the PP, the examiner elicits information from a parent or teacher about the subject's social language skills. This task is aimed to determine his overall pragmatic development. In the ORS, a parent, teacher, and the subject each rate the subject's interaction and communication skills.

The Peabody Picture Vocabulary Test

This test was used to evaluate in more detail the receptive abilities of the proband. The PPVT-3 is a performance test that assesses vocabulary acquisition via several tasks in which the child is asked to point to 1 of 4 color pictures on a page after hearing a word. The test is designed for subjects from 2.5 to 90 years, and it can also be used for a rapid screening of difficulties with language or abnormal verbal attitudes.

Test de Comprensión de Estructuras Gramaticales

This test, which is based on the Test for Reception of Grammar (TROG-2) [Bishop, 2003], was also used to gain a more accurate view of the receptive abilities of the proband. Similarly to the PPVT-3, the CEG does not demand any verbal response from the child, which is asked to point to 1 of 4 pictures (1 correct picture and 3 distractors) after listening to the target sentences. Each grammatical structure is tested via 4 different items. Twenty different grammatical constructions are assessed (see online suppl. file 1). The resulting scores enable the quantification of the developmental delay of the proband as well as the identification of the grammatical aspects that are more of a problem for him.

Registro Fonológico Inducido

This test assesses phonological and articulatory abilities of Spanish-speaking children between 3 and 7 years of age by means of elicited word production and repetition. Word production is induced by showing the child different pictures that it has to name. If the child is unable to produce the name, the speech-language therapist will provide the correct name and will ask the child to repeat it. The test comprises every possible sound of the Spanish language, so that it can be used to detect dyslalia as well as phonological impairment.

Natural Speech Sample Analysis

Additionally, we assessed the proband's language in use and overall communication skills through the systematic analysis of a 15-min sample of the child's talk while he was playing and chatting about familiar topics (family and school) with his mother and his speech language therapist (M.S.J.-R., one of the authors of this study). The conversation was video-recorded and then transcribed and coded using CHAT (Codes for the Human Analysis of Transcripts) software, a tool of the CHILDES Project [MacWhinney, 2000]. CHAT codes the speech production phenomena on the main lines of the transcript, whereas the phonological, morphological, syntactic, and pragmatic phenomena are coded on the dependent lines. Information about prosody and communicative gestures and gaze is included within square brackets in the main lines. Pauses are also coded, with an estimation of duration (for details, see <https://talkbank.org/manuals/CHAT.pdf>). We then proceeded to evaluate the most relevant aspects of language in use, which entails assessing every linguistic phenomenon from a pragmatic perspective [Verschuere, 1999]. Hence, we first tagged the child's errors, with an indication of their nature (phonological, morphological, syntactic, lexical). Afterward, we determined their impact on communication and labeled them according to PREP-CORP (Pragmatic Evaluation Protocol for the analysis of oral CORPpora), which has been previously used for obtaining the pragmatic profile of other developmental disorders [Fernández-Urquiza et al., 2015, 2016; Shiro et al., 2016; Díez-Itza et al., 2017]. Last, a global description of the pragmatic linguistic profile of the

proband was built up by automatically computing the number of occurrences of each label by means of CLAN (Computerized Language Analysis) [Conti-Ramsden, 1996].

Cytogenetic and Molecular Analyses

Karyotype Analysis

Peripheral venous blood lymphocytes were grown following standard protocols and collected after 72 h. A moderate resolution G-banding (550 bands) karyotyping by trypsin (Gibco 1x trypsin® and Leishmann stain) was subsequently performed. Microscopic analysis was conducted with a Nikon® eclipse 50i optical microscope and the IKAROS Karyotyping System (MetaSystem® software).

DNA from the patient and his parents was extracted from 100 µL of EDTA-anticoagulated whole blood using MagNA Pure (Roche Diagnostics, West Sussex, UK) and used for subsequent analyses.

Fragile X Syndrome Analysis

CGG expansions affecting the gene *FMRI* (the main determinant factor for Fragile X syndrome) were analyzed in the proband according to standard protocols. Polymerase chain reaction (PCR) of the fragile site was performed with specific primers for the fragile region of the *FMRI* locus and the trinucleotide repeat size of the resulting fragments was evaluated by electrophoresis in agarose gel.

Multiplex Ligation-Dependent Probe Amplification

MLPA was conducted to detect abnormal copy-number variations (CNVs) in subtelomeric regions of the chromosomes as well as frequent interstitial CNVs. MLPA consists on the amplification of different probes using a single PCR primer pair. Each probe detects a specific subtelomeric DNA sequence. Two different kits from MRC-Holland were used: SALSA® MLPA® probemix P036-E1 and SALSA MLPA probemix P070-B2 Human Telomere-5. SALSA MLPA probemix P036-E1 contains 46 MLPA probes with amplification products between 130 and 483 nt: 2 probes for each chromosome. Forty-one probes are located in subtelomeric regions. No probes are present for the subtelomeric regions of the 5 acrocentric chromosomes (13, 14, 15, 21, and 22). For these, an extra probe is included detecting the q arm, close to the centromere. The subtelomeric probes for the X and Y chromosome are identical as they detect sequences in the pseudoautosomal regions (PAR1 and PAR2) which are identical in chromosome X and Y. The following genes are detected by the probes included in this probemix: *TNFRSF4*, *SH3BP5L*, *ACPI*, *CAPN10*, *CHL1*, *BDH1*, *PIGG*, *TRIML2*, *PDCCD6*, *GNB2L1*, *IRF4*, *PSMB1*, *ADAP1*, *VIPR2*, *FBXO25*, *ZC3H3*, *DMRT1*, *EHMT1*, *DIP2C*, *PAOX*, *RIC8A*, *NCAPD3*, *SLC6A12*, *ZNF10*, *PSPC1*, *F7*, *CCNB1IP1*, *MTA1*, *MKRN3*, *ALDH1A3*, *POLR3K*, *GAS8*, *RPH3AL*, *TBCD*, *USP14*, *RBFA*, *CDC34*, *CHMP2A*, *SOX12*, *OPRL1*, *RBM11*, *PRMT2*, *BID*, *RABL2B*, *SHOX*, and *VAMP7*, respectively.

Regarding SALSA MLPA probemix P070-B2 Human Telomere-5, it also contains 46 MLPA probes with amplification products between 132 and 484 nt: 2 probes for each chromosome. As with the former probemix, no probes are present for the subtelomeric regions of the 5 acrocentric chromosomes (13, 14, 15, 21, and 22). For these, an extra probe is included detecting the q arm, close to the centromere. Likewise, the subtelomeric probes for the X and Y chromosome are also identical as they detect sequences in

the pseudoautosomal regions (PAR1 and PAR2) which are identical in chromosome X and Y. The following genes are detected by the probes included in this probemix: *TNFRSF18*, *SH3BP5L*, *ACPI*, *ATG4B*, *CHL1*, *KIAA0226*, *PIGG*, *FRG1*, *CCDC127*, *GNB2L1*, *IRF4*, *TBP*, *SUN1*, *VIPR2*, *FBXO25*, *RECQL4*, *DOCK8*, *EHMT1*, *ZMYND11*, *ECHS1*, *BET1L*, *IGSF9B*, *JARID1A*, *ZNF10*, *PSPC1*, *CDC16*, *PARP2*, *MTA1*, *NDN*, *TM2D3*, *DECR2*, *GAS8*, *RPH3AL*, *SECTM1*, *THOC1*, *CTDP1*, *PPAP2C*, *CHMP2A*, *ZCCHC3*, *UCKL1*, *HSPA13*, *S100B*, *IL17RA*, *ARSA*, *SHOX*, and *VAMP7*, respectively.

The PCR products were analyzed by capillary electrophoresis in an automatic sequencer Hitachi 3500 and further analyzed with the Coffalyser V 1.0 software from MRC-Holland.

Microarrays for Whole-Genome CNVs Search and Chromosome Aberrations Analysis

The DNA from the patient and his parents was hybridized on a CGH platform (Agilent Technologies). The derivative log ratio spread value was 0.11. 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).

In order to determine the genes that could be differentially expressed in the proband compared to his 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 above 9. An Affymetrix® Scanner 3000 7 G 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 his parents. Statistical analyses were conducted with the LIMMA (Linear Models for Microarray Analysis) package of BioConductor, using the TAC 4.0 software.

Discussion

The generalized application of next-generation sequencing facilities, particularly array-CGH, to the genetic examination of subjects with cognitive and language disorders has resulted in a growing list of genes and chromosomal regions associated with these conditions. Nonetheless, in most cases, no robust genotype-phenotype links have been established, and no molecular causative factors have been identified to date. In this study, we have provided a detailed description of the cognitive and linguistic features of a boy with a microdeletion in 8p23.1 and a microduplication in 9p24.3. It

is our contention, however, that his abnormal phenotype, and particularly his problems with language, result from the partial duplication of the *DOCK8* gene in 9p24.3. On the one hand, the boy exhibits many of the physical, neurological, cognitive, behavioral, and language features of people with CNVs encompassing this gene (Table 3; Fig. 3). Moreover, we have found no evidence in the literature or in patient databases of the involvement of any of the genes within the deleted region in 8p23.1 in language development, or in cognitive and behavioral aspects relevant to language function. Finally, as we discuss below in more detail, *DOCK8* is functionally connected with many genes that are robust candidates for language development, impairment, and/or evolution. Although alterations of *DOCK8* have a known impact on language abilities and although the gene is known to play a role in brain development and function, we still lack a detailed characterization of the language phenotype resulting from *DOCK8* alterations. One important reason is that the linguistic evaluation of patients usually involves psychological tests only. In our work, we have not only conducted a fine-grained analysis of the language strengths and deficits of our subject using a battery of specific diagnostic tools, but also examined samples of naturalistic speech, looking for structural and functional anomalies.

In the expressive domain, our proband shows signals of a slightly delayed phonological development, characterized by sound substitutions, simplification of consonant clusters, assimilations, omissions, weak syllable deletions, and problems with syllable segmentation. His score in the Phonological Awareness subtest of the CELF-4 reinforces this view. Additionally, his ability to assemble morphemes into words and words into sentences is also impaired. His mean length of utterance in words is shorter than expected according to his chronological age. Some functional components of language, such as inflectional affixes or prepositions, are particularly problematic for him. He scored very low in tasks assessing his ability to compose sentences and to retrieve and process verbal information. These difficulties with utterance production, as well as the reduced intelligibility resulting from his articulatory and phonological problems, explain his frequent repetitions of words and phrases, and ultimately, his perseverative discourse. Receptive language is also affected, as evidenced by the child's low scores in tests evaluating his receptive vocabulary and his comprehension of the grammar as well as by his echolalic responses during conversation. Complex sentences such as those with non-canonical word order (object-

verb-subject), passives, and with relative clauses are particularly problematic for him. Finally, he exhibits a good semantic knowledge at word level, but he has problems with aspects of sentence semantics involving structural aspects.

In our opinion, the observed deficits mostly result from the impairment of more basic cognitive abilities, specifically working memory. On the one hand, the child's nonverbal IQ is suggestive of a moderate mental retardation. On the other hand, he scored very low in tasks evaluating working memory (e.g., the WMI of the CELF-4) and exhibited difficulties in sustaining focus. Deficits in working memory have been reported to result in learning disabilities, poor phonological awareness, and impaired sequential processing in subjects with other chromosomopathies such as Fragile X syndrome [Johnson-Glenberg, 2008]. In particular, problems with sequential processing can contribute to explain the syntactic perseveration observed in our proband. At the same time, we cannot rule out the possibility that his problems with language are exacerbated because of his reduced executive function and/or attention, as the child presents with lack of inhibition and behaves impulsively and inappropriately to age. The co-occurrence of deficits in attention, working memory, and inhibition together with language problems has been reported in other clinical conditions, like Fragile X syndrome [Artigas-Pallarés et al., 2001]. That said, in spite of these pervasive problems with structural aspects of language, the child has mastered some key aspects of language use, such as the dynamics of turn-taking and compensatory behaviors aimed to circumvent expressive difficulties. His willingness to communicate is remarkable. Still, he frequently violates conversational principles (like the maxims of quality and relationship) and seems unable to understand the logics of conversation. These circumstances make his discourse incomplete, awkward, and incoherent at times. Because his semantic knowledge is spared, we contend that these problems with language use might be caused by a deviant reasoning, lack of attention, and reduced encyclopedic knowledge (i.e., awareness of the common ground shared with his interlocutors). Limited memory abilities, ultimately boiling down to his working memory deficit, seemingly contribute to these problems.

To sum up, our proband exhibits selected deficits in several structural components of language (notably, inflectional morphology, complex syntax, and sentence semantics), both in the expressive and receptive domains, together with a pragmatic impairment, which seemingly

result from his intellectual disability, and particularly from his severe deficit in working memory.

Regarding the molecular causes of the observed symptoms, we contend that they might arise from an abnormal interaction in the brain between *DOCK8* and *CDC42* resulting in the dysregulation of several key genes involved in language development and/or evolution. We cannot rule out the possibility that changes in the expression levels of selected genes outside the duplicated region and not interacting with *DOCK8* also contribute to the problems exhibited by the proband.

In the blood, *DOCK8* activates *CDC42* at the leading edge membrane of migrating cells, with this activation promoting changes in cell morphology that are needed for proper 3D cell migration [Harada et al., 2012]. *CDC42* regulates neural crest cell proliferation [Fuchs et al., 2009] and cortical interneuron migration [Katayama et al., 2013], and *CDC42* signaling is involved as well in dendritic spine growth and function [Datta et al., 2015]. *CDC42* polymorphisms have been associated with increased schizophrenia risk [Gilks et al., 2012]. As shown in Figure 4, *CDC42* interacts with the products of nearly 30 candidates for language development, evolution, and/or impairment. Most of these genes do not show fold changes in our proband compared to his healthy parents, but some of them appear slightly down- or upregulated. In view of the speech and language problems exhibited by the child, *SLIT2* (slightly downregulated) and *ROBO1* (slightly upregulated) are of particular interest. *Slit2* reduces *Cdc42* activity in living growth cones [Myers et al., 2012]. In turn, depletion of *Robo1* prevents *Slit2* inhibition of *Cdc42* activity [Yiin et al., 2009]. The *SLIT/ROBO* pathway has been claimed to play an important role in the externalization of language (i.e., speech) [for details, see Boeckx and Benítez-Burraco 2014b]. Specifically, the *SLIT*-mediated interaction between *FOXP2/foxp2* (also found slightly upregulated in our proband and reviewed above) and *ROBO1/robo1* is critical to the establishment of vocal learning pathways across species [Pfenning et al., 2014].

Another gene of interest is *CDC42BPB*, which is found downregulated in our proband. This gene encodes an effector of *CDC42* and is regulated by *FOXP2* [Spiteri et al., 2007]. *FOXP2* is a strong candidate for speech and language impairment [Vargha-Khadem et al., 2005; Kurt et al., 2012; Graham et al., 2015], playing a key role in neurogenesis, neuron differentiation, and neuron migration in brain areas involved in language processing [Tsui et al., 2013; Chiu et al., 2014; Garcia-Calero et al., 2016]. Pathogenic mutations in humans have been proven to affect

auditory-motor association learning when mimicked in mice [Kurt et al., 2012]. Specifically, the gene seems to facilitate the transition from declarative (i.e., less automatized) to procedural (i.e., more automatized) Murphy performance [Schreiweis et al., 2014; Chandrasekaran et al., 2015].

Interesting too is that *SRGAP3* is found upregulated in our proband. This gene has been associated with absence of speech co-occurring with severe mental retardation [Endris et al., 2002; Bacon et al., 2013] as well as schizophrenia [Wilson et al., 2011; Waltereit et al., 2012]. *SRGAP3* is involved in cytoskeletal reorganization and contributes to different neurodevelopmental processes, including neuronal differentiation and neurite outgrowth, which may be required for normal cognitive function [Endris et al., 2011; Bacon et al., 2013; Ma et al., 2013]. *SRGAP3* interacts with *ROBO1*, thus contributing to modulate the *SLIT/ROBO* pathway [Wong et al., 2001].

To finish, it is also of interest finding that *DOCK4* and *EGFR* are downregulated in our proband. A microdeletion of *DOCK4* has been suggested to contribute to dyslexia as well as to ASD [Pagnamenta et al., 2010]. Concerning *EGFR*, this gene interacts with *VCAN*, a gene involved in neuronal attachment, neurite outgrowth, and synaptic transmission [Xiang et al., 2006].

Last, it is worth considering as well the biological roles played by the genes that are more strongly dysregulated in the blood of our proband compared to his healthy parents. Among the genes found strongly downregulated, most of them are involved in antiviral response (*RSAD2*, *IFIT1*, *IFIT2*, *IFIT3*, and *IFI44L*). *CA1* plays diverse biological roles, but although it is highly expressed in the blood, it is not expressed in the brain (Fig. 6). Accordingly, the only potential candidate for the cognitive and language deficits exhibited by our proband is *SERPING1*. This gene encodes a plasma protein involved in the regulation of the complement cascade. This cascade has been related to synaptic plasticity, as the knockdown of *Serpining1* in mice impairs synapse formation [Ren et al., 2020]. The gene has been also linked to cortical development, particularly to neuronal migration [Gorelik et al., 2017].

The genes found strongly upregulated in the proband are of more interest in view of his language and cognitive phenotype. Several of them (*FCER2*, *TCL1A*, *TCL1B*, and *TCL6*) are not expressed in the brain or are expressed at very low levels and have been associated with conditions not involving cognitive deficits, like cancer (*TCL1A*, *TCL1B*, *TCL6*) or asthma (*FCER2*). Interestingly though, *FCER2* and *TCL1B* have been found to be dysregulated in the blood of patients with Parkinson dis-

ease [Infante et al., 2015]. Regarding the genes that are expressed in the brain, *NRCAM* is the most interesting candidate. This gene is highly expressed in most brain areas (particularly the cortex and thalamus) and encodes a neuronal cell adhesion protein with attested roles in neuron-neuron adhesion, as well as axonal cone growth and axonal guidance, being ultimately involved in the organization of neural circuits during brain development [Sakurai, 2012]. Variants of this gene have been associated with ASD, particularly with the subtype co-occurring with obsessive-compulsive behavior [Sakurai et al., 2006] as well as with addictive behavior because of its regulatory role in glutamatergic and GABAergic innervation [Ishiguro et al., 2014, 2019]. All this evidence suggests that *NRCAM* might play some important role in the reward circuitry, in part through its involvement in the fasciculation of axon fibers in the amygdala [Mohan et al., 2019]. In view of the attention problems exhibited by our proband, it is interesting that *Nrcam*-null mice show decreased sociability and learning deficits [Moy et al., 2009] as well as altered impulsivity [Matzel et al., 2008]. Two other genes of interest, that are also strongly upregulated in our proband and also expressed in the brain, are *AFF3* and *BACH2*. They are mostly expressed in the cerebellum, particularly after birth (online suppl. file 5). *AFF3* encodes a nuclear transcription factor required for normal cellular migration in the developing cortex [Moore et al., 2014]. Silencing of this gene results in intellectual disability [Metsu et al., 2014]. Regarding *BACH2*, it encodes a transcription factor which is part of a regulatory network involved in the development of cortico-striatal projections within the telencephalon, also encompassing *FOXO1* and *ISL1* [Waclaw et al., 2017]. *FOXO1* is a target of *FOXP2* [Vernes et al., 2011], whereas the *FOXO1* protein is phosphorylated by *DYRK1A* [Huang and Tindall, 2007], associated to mental retardation and absence of speech [Van Bon et al., 2011; Courcet et al., 2012], and involved in learning and memory [Hämmerle et al., 2003]. In turn, *FOXO1* upregulates *RELN* [Daly et al., 2004], a candidate for ASD and for lissencephaly with language loss [Hong et al., 2000; Wang et al., 2014]. Finally, *PLEKHG1* encodes an activator of *CDC42* [Reinhard et al., 2019] and has been associated to panic disorder [Otowa et al., 2009] and to white matter hyperintensities [Traylor et al., 2019].

In conclusion, although the exact molecular causes of the language problems (and other cognitive, behavioral and even motor deficits) observed in our proband remain to be fully elucidated, we hypothesize that his dis-

tinctive features might result from the altered expression of selected genes involved in procedural learning, particularly some components of the *SLIT/ROBO/FOXP2* network, which are strongly related to the development and evolution of language. Dysregulation of specific components of this network can be hypothesized to result in turn from an altered interaction between *DOCK8*, affected by the microduplication in 9p24.3 carried by our proband, and *CDC42*, acting as the hub component of the network encompassing language-related genes. Still, some genes found strongly upregulated in the subject, and not related to these genes, can contribute to the observed problems in the language domain as well as to specific features of the proband, particularly impulsivity.

Although this hypothesis needs to be properly tested, we hope that our findings contribute to a better understanding of the language, cognitive, and behavioral phenotype resulting from CNVs of the distal region of the short arm of chromosome 9. A more detailed knowledge of the neurobiological basis of the deficits (and strengths) exhibited by patients is necessary for improving (psycho) pedagogical strategies aimed to ameliorate their problems. This is particularly true of low-prevalent clinical conditions, such as these CNVs, which are mostly understudied and for which optimized intervention tools are not usually available.

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Statement of Ethics

Ethics approval for this research was granted by the Comité Ético del Hospital “Reina Sofía”, Córdoba, Spain. Written informed consent was obtained from the proband’s parents for conducting the psycholinguistic evaluations and the molecular analyses, and for publishing 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.

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Author Contributions

A.B.-B. conceived the paper, performed the molecular experiments, analyzed the genomic/genetic data, and wrote the paper. M.F.-U. and M.S.J.-R. conducted the clinical evaluation of the proband, analyzed the psycholinguistic data, and wrote the paper. All authors approved the final version of the manuscript.

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