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ORIGINAL INVESTIGATION

Haploinsufficiency of the *HIRA* gene located in the 22q11 deletion syndrome region is associated with abnormal neurodevelopment and impaired dendritic outgrowth

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Abstract

The 22q11.2 deletion syndrome (22q11DS) is associated with a wide spectrum of cognitive and psychiatric symptoms. Despite the considerable work performed over the past 20 years, the genetic etiology of the neurodevelopmental phenotype remains speculative. Here, we report de novo heterozygous truncating variants in the *HIRA* (Histone cell cycle regulation defective, S. Cerevisiae, homolog of, A) gene associated with a neurodevelopmental disorder in two unrelated patients. *HIRA* is located within the commonly deleted region of the 22q11DS and encodes a histone chaperone that regulates neural progenitor proliferation and neurogenesis, and that belongs to the WD40 Repeat (WDR) protein family involved in brain development and neuronal connectivity. To address the specific impact of *HIRA* haploinsufficiency in the neurodevelopmental phenotype of 22q11DS, we combined *Hira* knock-down strategies in developing mouse primary hippocampal neurons, and the direct study of brains from heterozygous $Hira^{+/-}$ mice. Our in vitro analyses revealed that *Hira* gene is mostly expressed during neuritogenesis and early dendritogenesis stages in mouse total brain and in developing primary hippocampal neurons. Moreover, shRNA knock-down experiments showed that a twofold decrease of endogenous *Hira* expression level resulted in an impaired dendritic growth and branching in primary developing hippocampal neuronal cultures. In parallel, in vivo analyses demonstrated that $Hira^{+/-}$ mice displayed subtle neuroanatomical defects including a reduced size of the hippocampus, the fornix and the corpus callosum. Our results suggest that *HIRA* haploinsufficiency would likely contribute to the complex pathophysiology of the neurodevelopmental phenotype of 22q11DS by impairing key processes in neurogenesis and by causing neuroanatomical defects during cerebral development.

Introduction

The 22q11.2 deletion syndrome (22q11DS), also known as DiGeorge syndrome or velocardiofacial syndrome, is the most frequent chromosomal microdeletion syndrome. The phenotypic expression of the 22q11DS is highly

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heterogeneous and includes congenital heart defects, thymus and parathyroid hypoplasia or agenesis leading, respectively, to immune deficiency and hypocalcemia. The phenotypic spectrum also includes a large variety of neurodevelopmental disorders (NDD). Indeed, intellectual disability (ID) is present in about 50% of the patients with a mean total IQ of approximately 70 (Swillen et al. 1997; De Smedt et al. 2007). The frequency of Autism Spectrum Disorders (ASD) is estimated to range between 15 and 50% (Fine et al. 2005; Vorstman et al. 2006; Bruining et al. 2010). Psychiatric disorders are also very frequent as Attention Deficit/Hyperactivity Disorder is present in 37% of affected children and psychotic disorders in 41% of the adult patients (Schneider et al. 2014). This makes the 22q11DS the highest known genetic risk factor for developing schizophrenia and the second most common genetic cause of developmental delay (Rauch et al. 2006). Considerable work has been done over the past 20 years to address the complex pathophysiology of the neurodevelopmental phenotype of the 22q11DS. Nevertheless, until now, the etiology of the cognitive and psychiatric phenotype remains speculative.

Among the 32 protein-coding genes located in the 1.5 Mb proximal 22q11.2 deletion, *HIRA* (Histone cell cycle regulator [MIM: 600237]) encodes a histone chaperone and plays an important role in the epigenetic regulation of gene expression since its tissue-specific knock-out affects many basic cellular processes including DNA damage, limited de novo methylation, and global aberrant transcription (Nashun et al. 2015; Dilg et al. 2016; Valenzuela et al. 2017). The HIRA protein contains a WD40 repeat (WDR) domain and belongs to the WDR family proteins, recently pointed out for their putative role in brain development and neuronal connectivity (Kannan et al. 2017). Finally, it has been recently shown that HIRA regulates neural progenitor cell proliferation and neurogenesis particularly by controlling the Wnt signaling pathway (Li and Jiao 2017).

Here, we report the identification of de novo truncating variants in the *HIRA* gene in two unrelated patients referred to the genetic consultation for diagnostic purpose of a neurodevelopmental disorder. To provide evidence for the pathogenicity of these variants, we performed functional analyses combining in vitro mouse neuronal models in which *Hira* gene expression was decreased, with the neuroanatomical analysis of *Hira* heterozygous knock-out mice, and the neurodevelopmental expression profile of *Hira* mRNA. Our findings revealed that *HIRA* haploinsufficiency leads to abnormal defects in both in vitro and in vivo models, suggesting that this gene would be involved in the regulation of neuronal differentiation and maturation.

Materials and methods

Patients

The study was approved by the local institutional review boards, and written informed consent was obtained from the patients' parents, including explicit permission to share clinical and identifying information. Genomic DNA and RNA were obtained from the University Hospital of Tours (France) and from the Radboud University Medical Center in Nijmegen (The Netherlands).

Hira mRNA neurodevelopmental expression analyses

Total RNAs were extracted from neuronal cultures or from mouse brains using Trizol (Invitrogen, USA) and purified by the DirectZol kit (Ozyme, France). Complementary DNAs were obtained from 2 µg of total RNAs using the Prime Script RT Reagent commercial kit (Takara Bio, Nojihigashi, Japan). Primers were designed with Primer3 software: forward primer (5′CGTACTGCTGCTGTGCTGTT3′) was located in exon 9 of *Hira* and the reverse primer (5′CTG GGAGAAGTCGAGGAATG3′) was located in exon 11. qPCR experiments were run in duplicates on the LightCycler 480 (Roche Diagnostics, Meylan, France) using the SYBR Green Master Mix commercial kit (Applied Biosystem, Foster City, CA, USA). Relative expression was assessed using the advanced E-method from LightCycler software (Roche Diagnostics, Meylan, France). Hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) for human samples, Cyclin C1 (*Cyc1*) and Beta-2-microglobulin (*B2m*) for mouse samples were used as reference genes for normalization.

Hira knock-down expression analysis

Plasmids containing a small hairpin RNA (shRNA) targeting *HIRA* human and murine transcripts, as well as plasmids containing a scramble shRNA were used (Origene, TF500962; Rockville, MD, USA). The target sequence of the effective shRNA (5′GACAGTATGAATGCTACCTCTACT CCTGC3′) overlapped exons 3 and 4 of *Hira* mRNA. The target sequence of the scramble shRNA is as follows, 5′GCA CTACCAGAGCTAACTCAGATAGTACT3′. The expression of the shRNA was driven by a U6 promoter. These constructions contained the chloramphenicol resistance gene and a CMV driven *RFP* gene, which expresses RFP protein constitutively in mammalian cells. The plasmidic DNAs were extracted using the Endo Free Plasmid Maxi Kit (Qiagen, Venlo, Netherlands). Both effective shRNA and non-effective shRNA were transfected in HEK293T

cells to evaluate their efficiency to repress the expression of endogenous *HIRA*. HEK293T cultures were maintained in Dulbecco's modified Eagle's medium (DMEM)-Glutamax (Invitrogen, USA) supplemented with 10% Fetal Bovine Serum (Eurobio, France) and were transfected with the appropriate plasmid using Lipofectamine 2000 (Invitrogen, USA). In brief, 2.5 µg of DNA was combined with 6 µL of Lipofectamine 2000 in 300 µL of DMEM. The DNA/Lipofectamine 2000 mixture was incubated for 20 min at room temperature. Complexes were added dropwise onto cells growing after half of the medium was removed. Next, cells were incubated for 4 h at 37 °C and 5% CO2. The transfection medium was then replaced by DMEM/10% Fetal Bovine Serum, and total RNA lysates were obtained 72 h post-transfection. To measure the expression levels of *HIRA* in each condition, RT-qPCR analyses were performed on 5 independent transfections.

Primary hippocampal cultures

All mouse experiments were performed according to the protocols approved by the University Francois-Rabelais of Tours and INSERM (Project authorization number 01456.03, Ministry of Research). Hippocampi or cortices were dissected from embryonic day 17.5 C57BL/6 J mouse embryos (Janvier Labs, Le Genest Saint-Isle, France), manually dispersed in cold PBS (Fisher Scientific, Waltham, MA, USA), and triturated with papain (10 U/mL; Worthington Biochemical, Lakewood, NJ, USA) for 22 min at 37 °C. Cells were resuspended in DMEM/F12 (Fisher Scientific) with 10% fetal bovine serum (Eurobio, Courtaboeuf, France) and centrifuged at 250 g for 3 min, and the final pellet was resuspended in primary neuron growth medium (PNGM; Lonza, Basel, Switzerland). Dissociated cells were then plated onto glass coverslips coated in poly-D-lysine (Sigma Aldrich, Saint-Louis, MO, USA) and laminin (Fisher Scientific) at a density of 400 cells per mm^2. The cultures were kept in Primary Neuron Growth Medium, and half of the medium was changed twice a week.

Transfections and cellular analyses

Hippocampal neurons were transfected 11 days after plating (Day in vitro 11, DIV11) with the appropriate plasmids (non-effective shRNA and effective shRNA) with the DNAin Neuro transfection Kit (Amsbio, Abingdon, UK). In brief, 500 ng of plasmid DNA was combined with 1 µL of DNAin Neuro in 100 µL of PNBM medium. The DNA and DNAin Neuro mixture was incubated for 15 min at room temperature. Complexes were added dropwise onto cells growing after half of the medium was removed and stored. Next, cells were incubated for 4 h at 37 °C and 5% CO2. After incubation, the medium was changed. Cells were fixed at DIV14 using 4% paraformaldehyde and coverslips were mounted on a glass slide using ProLong Diamond Antifade Mountant with DAPI (Invitrogen). Individual neurons were directly imaged under fluorescence and confocal microscopy (Leica SP8, Leica, Wetzlar, Germany) using RFP (562 nm) labeling as a tracer of morphology. Image analysis was done using Imaris software (Bitplane Scientific Software, Zurich, Switzerland) allowing the evaluation of the number of neurites per neurons, the number of neurites by branching level and the mean neurite length. The neuronal branching was evaluated using Sholl analysis on ImageJ software (Wayne Rasband, NIH, Bethesda, MD, USA). Statistical analysis was performed using the GraphPad Prism 6.0 software (La Jolla, CA, USA). For the neuronal morphological study, the data were first analyzed using the D'Agostino-Pearson normality test. When the result was positive, the data were then analyzed using an Ordinary one-way ANOVA followed by Tukey's multiple comparisons. $P < 0.05$ was assumed significant.

Generation of *Hira*$^{+/-}$ knock-out mice

The construction of the *Hira* allele was based on the "Knockout-first allele" method (Skarnes et al. 2011). A "critical" exon (exon 4) present on all transcripts was first identified. A promotor-less targeting cassette was inserted in intron 3 in a C57BL/6 N blastocyst, which created a frameshift mutation, thus generating the KO-first allele (also known as the tm1a allele). A heterozygous-by-heterozygous ("het-by-het") breeding scheme was used to propagate the line. After weaning, animals were housed three–four mice per cage with WT controls housed separately, in specific-pathogen-free environment in individually ventilated cages under 12/12 light/dark cycle with temperature-controlled conditions and free access to food and water with hardwood bedding. All animals were regularly monitored for health and welfare concerns.

Mouse *Hira*$^{+/-}$ neuroanatomical study

The neuroanatomical study was carried out using a cohort of 10 male mice aged 16 weeks ($n = 5$ per genotype). Mouse brain samples were fixed in 4% buffered formalin for exactly 48 h. A total of 40 brain parameters, consisting of surface area and length measurements, were taken blind to the genotype across one sagittal section at Lateral + 0.60 mm. These parameters were distributed across 22 developmentally distinct brain regions related to the cerebrum, the cortex, the pons, the cerebellum, the ventricle, the corpus callosum, the thalamus, the caudate putamen, the hippocampus, the fimbria, the anterior commissure, the stria medullaris, the fornix, the optic chiasm, the hypothalamus, the pontine nuclei, the substantia nigra, the transverse fibers of the pons,

the cingulate gyrus, the dorsal subiculum, the inferior colliculus and the superior colliculus. These brain regions were further delineated into six main brain categories (brain size, commissures, ventricles, cortex, subcortex and cerebellum). Data were analyzed using a two-tailed Student t-test assuming equal variance to determine whether a brain region is associated with neuroanatomical defects or not.

Ethical considerations in animal use

The care and use of *Hira*$^{+/-}$ mice in the Wellcome Sanger Institute study was carried out in accordance with UK Home Office regulations, UK Animals (Scientific Procedures) Act of 1986 under two UK Home Office licences (80/2485 and P77453634) that approved this work, which were reviewed regularly by the Wellcome Sanger Institute Animal Welfare and Ethical Review Body.

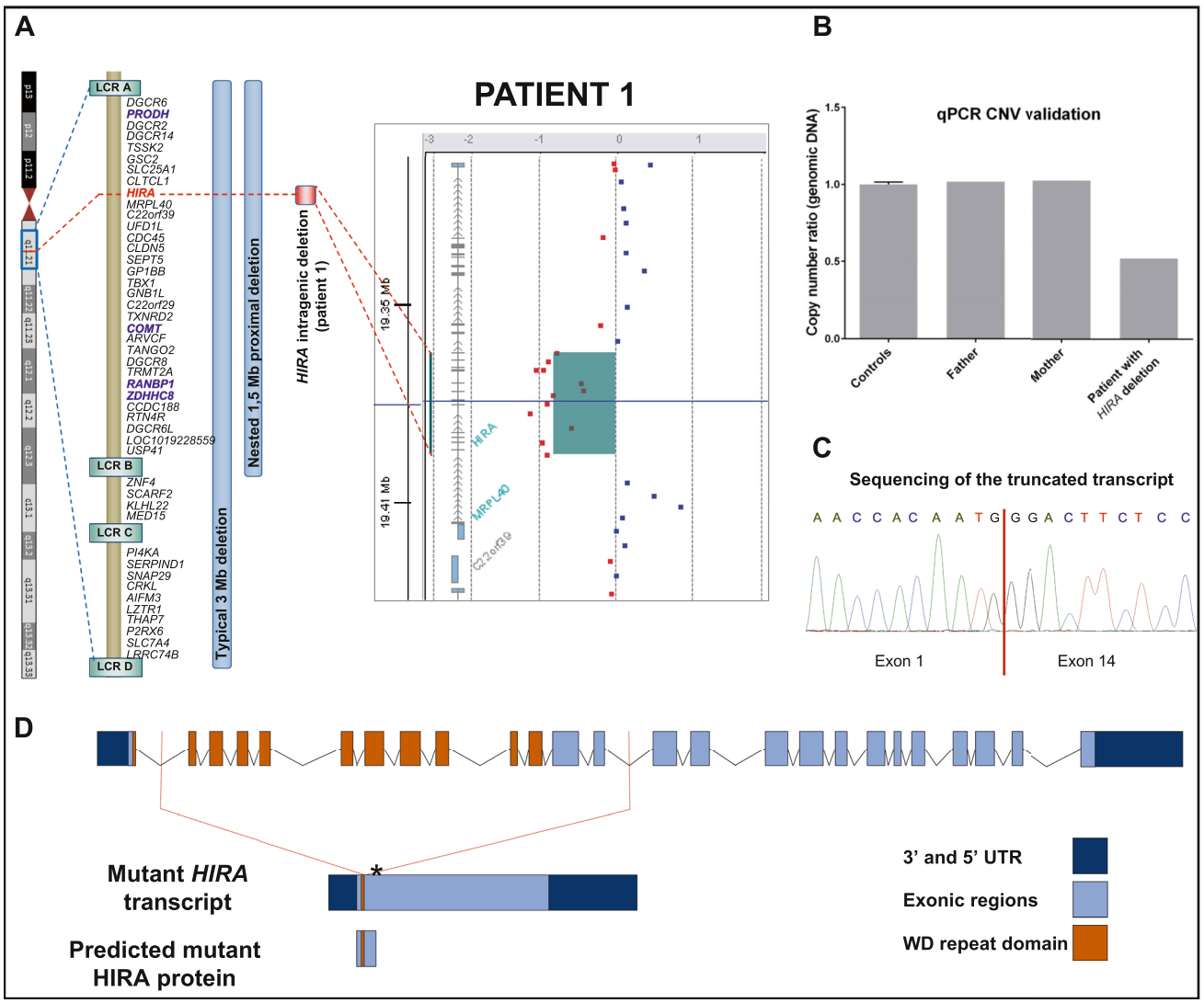


Fig. 1 Molecular genetic investigations in the two patients with *HIRA* genetic alteration. **a** Schematic representation of the 22q11.2 region and array CGH profile showing a 28 kb intragenic deletion of the *HIRA* gene in the DNA from Patient 1. Major candidate genes for the neurodevelopmental phenotype in the literature are written in blue characters. Green boxes represent the low copy repeats (LCR) regions. **b** Representative graph of qPCR analyses performed on genomic DNA from patient 1 and her respective parents confirming the de novo occurrence of the deletion; **c** DNA sequencing electrophoregram of the deletion breakpoint; **d** Schematic representation of the predicted truncated *HIRA* transcript and protein resulting from patient 1 variant; **e** Agarose gel electrophoresis of the amplified *HIRA* transcript from patient 2 compared to control showing the presence of a truncated transcript; **f** DNA sequencing electrophoregram of the truncated transcript showing the skipping of the exon 4 resulting from the splice site variant carried by patient 2; **g** Schematic representation of the predicted *HIRA* transcript and protein resulting from patient 2 variant

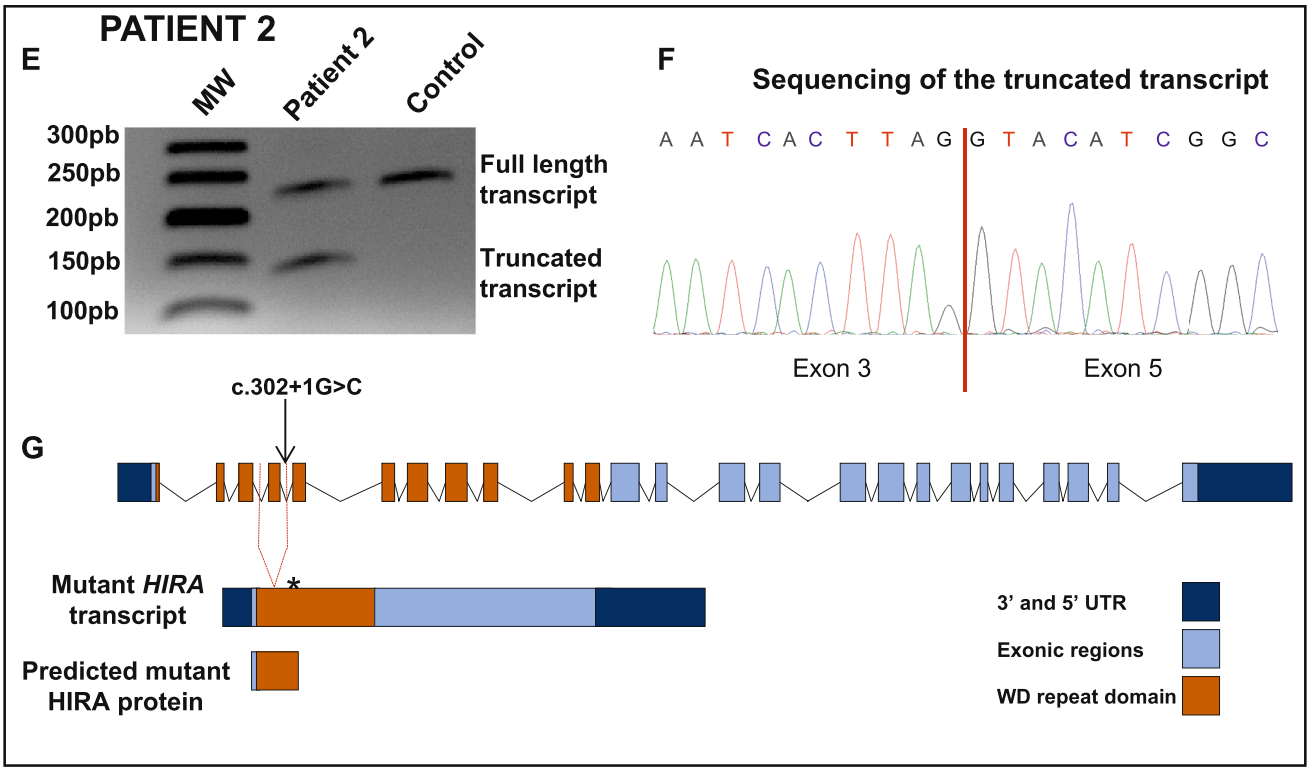


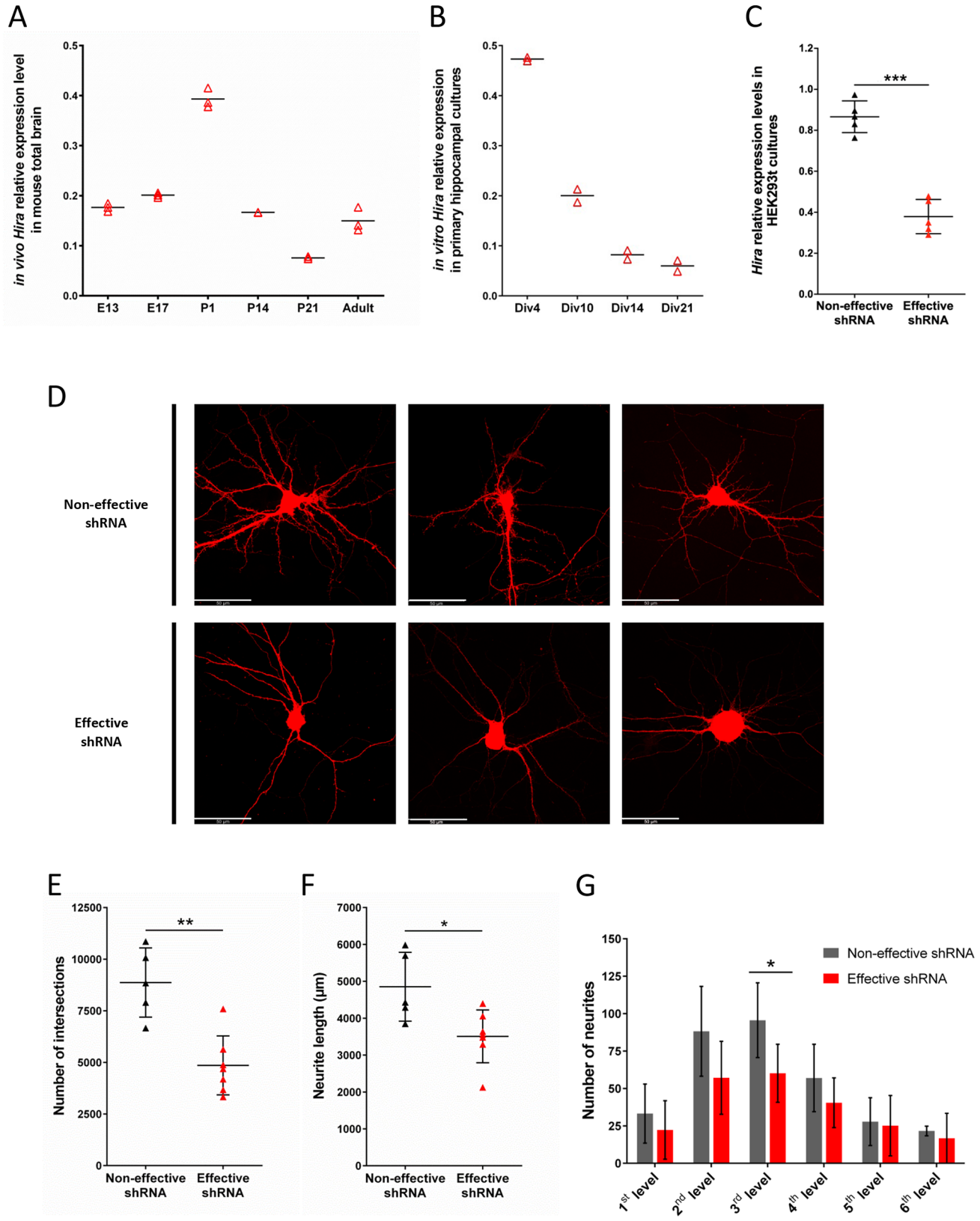
Fig. 1 (continued)

Results

We identified two cases with neurodevelopmental disorder and de novo genetic variants of *HIRA*. The patient 1 was a 4-year-old female born to non-consanguineous Algerian parents. She was born at 38 weeks. Birth parameters were unknown except for the weight which was at the 25th percentile. Neonatal period was marked by hypotonia and feeding difficulties with velopharyngeal insufficiency. She had psychomotor retardation, moderate ID and motor stereotypies. She also had growth retardation and mild microcephaly (OFC: − 2.8 SD). At physical examination, facial features were suggestive of 22q11DS as she had narrow and slightly upslanted palpebral fissures, bilateral ptosis, tubular nose with hypoplastic alae nasi. The mouth was small, with a high palate and a short uvula. Ears were posteriorly rotated with small lobes. Brain MRI showed diffuse atrophy of the white matter. Metabolic investigation, blood count and calcemia were normal. Array-CGH (cytoshure ISCA 60 k, Agilent, Santa-Clara, CA, USA) identified a de novo 28 kb intragenic deletion encompassing exons 2–13 of *HIRA* (Fig. 1). This deletion resulted in a disruption of the open reading frame of HIRA leading to a premature stop codon in exon 14 (Fig. 1).

The Patient 2 was a 6-year-old female who presented behavioural problems mainly confined to symptoms of ASD. She had average head circumference for age. She also had diabetes mellitus type 1 and recurrent infections since 4 years of age. Array-CGH did not show any pathogenic CNV but whole-exome sequencing revealed a de novo heterozygous splice site variant in the *HIRA* gene, (hg19, chr22:19394706C > G; NM_003325.4: c.302 + 1G > C). The cDNA analysis confirmed the predicted deletion of exon 4 leading to a frameshift with a premature stop codon in exon 6, p.(Thr101Thrfs*20) (Fig. 1).

We also looked in public databases (DECIPHER, ClinVar, denovoDB) for additional de novo heterozygous *HIRA* variants potentially associated with pathogenicity. Interestingly, the denovo-db database (release v.1.6.1, May 2020 accessed) included two additional cases. This includes a case from the Deciphering Developmental Disorders (DDD) study (individual DDD4K.03620) (Deciphering Developmental Disorders Study 2017), with NDD associated with a de novo truncating variant in *HIRA* consisting in a stop-gained mutation, hg19 chr22:19341607G > A, NM_003325.4:c.2596C > T and leading to a truncated HIRA protein, p.(Gln866*). We also found one variant of unknown significance, chr22:19341564A > G; NM_003325.4:c.269 T > C, p.(Met880Thr), carried by a patient for which the phenotype was not clearly described but included in a large autism spectrum disorder cohort (de Rubeis et al. 2014).


A
in vivo Hira relative expression level in mouse total brain
0.5
0.4
0.3
0.2
0.1
0.0
E13
E17
P1
P14
P21
Adult
B
in vitro Hira relative expression in primary hippocampal cultures
0.5
0.4
0.3
0.2
0.1
0.0
Div4
Div10
Div14
Div21
C
Hira relative expression levels in HEK293t cultures
***
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Non-effective shRNA
Effective shRNA
D
Non-effective shRNA
Effective shRNA
E
Number of intersections
**
12500
10000
7500
5000
2500
0
Non-effective shRNA
Effective shRNA
F
Neurite length (μm)
*
7000
6000
5000
4000
3000
2000
1000
0
Non-effective shRNA
Effective shRNA
G
Number of neurites
*
150
125
100
75
50
25
0
Non-effective shRNA
Effective shRNA
1st level
2nd level
3rd level
4th level
5th level
6th level

◂**Fig. 2** Neuronal expression study and functional analyses of *Hira* in mouse brain tissue and primary hippocampal cultures. **a** Relative *Hira* mRNA expression levels at different stages of embryonic and postnatal development. *E* embryonic day, *P* postnatal day. RT-qPCR analysis of *Hira* mRNA expression level is shown relative to the *Gapdh* reference gene. Data are presented as dot plots with mean. For each time point, the experiments were performed using total RNAs extracted from a pool of total brain extracts from 5 different mice (25 mouse brains in total); each dot plot represents a technical replicate ($n=3$); **b** Relative *Hira* mRNA expression level in developing mouse primary hippocampal neuronal cultures. Expression of *Hira* mRNA is shown relative to the *Gapdh* reference gene at the culture days 4, 10, 14 and 21. $n=2$ independent cultures, *Div* Day in vitro, Data are presented as dot plots with mean; **c** Relative *HIRA* mRNA expression in HEK293T cultures transfected with the non-effective shRNA and with the effective shRNA ($n=5$ independent transfections). Statistical significance was assessed using a non-parametric Mann–Whitney test; **d** Altered morphology of primary embryonic hippocampal neurons transfected with the effective shRNA or with the non-effective shRNA, observed in confocal microscopy (representative images of 3 transfected neurons that are also Red fluorescent protein (RFP)-labeled); scale bar: 50 µm. $n=2$ independent transfections leading to the analysis of 5 neurons with non-effective shRNA and 7 neurons with *Hira* shRNA). **e** Graphical representation of the quantification of the number of neurite intersections (i.e., branching numbers) from the transfected hippocampal neurons (each dot indicates data for one transfected neuron); **f** Graphical representation of the quantification of the total neuritic length (each dot indicates data for one transfected neuron); **g** Graphical representation of the quantification of the number of neurites per branching level (each dot indicates data for one transfected neuron). Statistical significance was evaluated using Mann–Whitney test for the number of neurite intersections on Scholl analysis, the mean neurite length. The data on the number of neurites per branching level were analyzed using an Ordinary one-way ANOVA followed by Tukey's multiple comparisons. Data are represented as the mean ± SD. $*P<0.05$, $**P<0.01$, $***P<0.001$

Following these observations, we hypothesized that haploinsufficiency of *HIRA* might cause the phenotype observed in our patients and play a role in the developmental phenotype of the 22q11DS. We, therefore, studied the cerebral pattern of *Hira* expression and assessed the impact of haploinsufficiency of *Hira* in the neuronal plasticity and brain neuroanatomy in mice.

Expression analysis of *Hira* mRNA by RT-qPCR in mouse total brain extracts revealed a maximal level at birth with an approximately twofold increased expression compared to embryonic and postnatal expression (Fig. 2). Furthermore, RT-qPCR analyses performed in developing primary hippocampal cultures from mouse embryonic brains showed that *Hira* mRNA expression was maximal at 4 days of culture (Day in Vitro, DIV4) and significantly decreased when neurons become fully mature with functional synapses (DIV10 to DIV21). The pattern of *Hira* mRNA expression observed in total brain and in primary hippocampal neurons would, thus, correlate with the neuritogenesis and early dendritogenesis stages suggesting that *Hira* might modulate neuronal differentiation and early maturation processes.

To address the impact of *Hira* haploinsufficiency in developing primary hippocampal neuronal cultures, we used a shRNA approach to knock-down endogenous *Hira* gene expression. We first sought to validate the efficiency of our approach by transfecting of a shRNA expression plasmid targeting *HIRA* in HEK293T cell lines. Our experiments resulted in a 56% ($P=0.0079$) reduction of *HIRA* mRNA expression level, in accordance with a haploinsufficiency model. As the shRNA targets a sequence of *HIRA* gene that is identical between human and mouse orthologs, we then assessed the impact of *Hira* haploinsufficiency in developing mouse neuronal cultures. Primary hippocampal neurons transfected at DIV11 with the effective shRNA displayed morphological impairments compared to controls. Particularly, they displayed a 46% ($P=0.0051$) decrease in the number of neuritic intersections per neuron, a 28% ($P=0.0177$) decrease in the mean total neurite length per neuron and a 23% decrease in the number of neurites per neuron compared to neurons transfected with the non-effective shRNA (i.e., scramble shRNA). The decrease in the number of neurites per neurons was not statistically significant ($P=0.1490$) but the assessment of the number of neurites by branching level showed a 33% ($P=0.0241$) decrease in the number of neurites in the third branching level compared to neurons transfected with the non-effective shRNA. These results suggested that a twofold reduction of *Hira* gene expression dysregulates dendritic arborization in primary neuronal cultures, suggesting that the HIRA protein is essential for dendritic development growth and stability.

Since no previous mouse studies reported the implication of *Hira* in the context of brain development, and in order to study the role of *Hira* haploinsufficiency in vivo, we developed a heterozygous mouse model using the knock-out first allele method (Skarnes et al. 2011). The strategy relies on the identification of an exon common to all transcript variants (exon 4), upstream of which a LacZ cassette was inserted. Exon 4 of the *Hira* allele was flanked by loxP sequences bilaterally. The resulting $Hira^{tm1a(EUCOMM)Wtsi}$ mice were then phenotyped. At weaning age, mouse survival was assessed from 187 successfully genotyped mice originating from several litters and derived from a het-by-het breeding scheme. We obtained no homozygous mice suggesting that *Hira* is essential for viability. To determine the window of death, we carried out a recessive lethality screen at mouse embryonic day 14.5 (E14.5). We looked at 41 embryos, none of which were homozygous, suggesting that homozygous mice die before E14.5. Using a recently developed and highly robust approach for the assessment of 40 brain parameters distributed across 22 developmentally distinct brain regions (Collins et al. 2018), we analyzed neuroanatomical defects in $Hira^{+/-}$ transgenic mice (data available upon request). These parameters encompass six main brain categories: brain size,

commissures (callosal, anterior and stria medullaris), ventricles (lateral and fourth), cortex (motor and cingulate), subcortex (hippocampus, caudate putamen, fimbria, thalamus, hypothalamus, substantia nigra, subiculum and colliculus), and cerebellum (granule layer, cerebellar nuclei, number of folia, pons, nerves, and pontine nuclei). This consisted of a systematic quantification on a single sagittal brain section at Lateral + 0.60 mm. To minimize environmental and genetic variations, mice of each genotype were analyzed at 16 weeks of age and bred on the same genetic background (Bl/6 N). Minor anomalies were identified in *Hira*$^{+/-}$ mice when compared to wild type, with suggestive evidence of possible convergence with microcephaly in some brain regions. For example, the hippocampus showed reduced height in the molecular layer (− 20%, $P = 0.04$). The corpus callosum and the fornix showed a marginally reduced area (− 10%, $P = 0.04$ and − 9%, $P = 0.016$, respectively) (Fig. 3).

Discussion

We report here de novo truncating variants in the *HIRA* gene, which is located in the commonly deleted region of the 22q11DS, identified in patients presenting with ID or ASD symptoms. These two variants were not found in control databases (GnomAD) and were predicted to be pathogenic and to lead to a loss-of-function, knowing that *HIRA* is predicted intolerant to loss-of-function (pLI = 1). This observation and the further identification of two additional cases with NDD and a de novo *HIRA* truncating mutation led us to consider *HIRA* as a candidate gene for aspects of the neurodevelopmental phenotype of 22q11DS.

Several genes have already been proposed as candidate genes for the neuropsychiatric phenotype of the 22q11DS, such as *COMT, PRODH, RANBP1* or even *ZDHHC8* (Goodman et al. 2000; Jacquet et al. 2002; Bassett et al. 2007; Raux et al. 2007; Mukai et al. 2008, 2015; Beemer et al. 2009; Vorstman et al. 2009; Philip and Bassett 2011; Gothelf et al. 2013; Meechan et al. 2015; Paronett et al. 2015; Moutin et al. 2017). Nevertheless, the pathophysiology of the neurodevelopmental phenotype remains unexplained. The role of *HIRA* in development has already been suggested as the homozygous KO is lethal in mice, which we also show in this study, and the heterozygous KO results in gastrulation defects (Roberts et al. 2002). Moreover, as a chromatin organization regulator HIRA is supposed to be necessary during brain development. Indeed, the chromatin remodeling process is frequently impaired in neurodevelopmental disorders and genes involved in this process are known to be responsible for multiple conditions associated with ID or ASD. A recent study described that genes located in the 22q11.2 region are significantly associated with networks involved in brain development and associated with pathogenesis of schizophrenia and ASD (Forsyth et al. 2020). In particular, *HIRA* was highlighted as a candidate driver of neurodevelopmental disorders such as schizophrenia and ASD.

Here, we bring new evidence of the implication of *HIRA* in brain development and disorder. We observed that mouse *Hira* was significantly expressed in both total brain and primary hippocampal cultures during times corresponding to neuritogenesis and early dendritogenesis stages. Furthermore, we demonstrated that a twofold reduction in *Hira* gene expression induced alteration of neuronal morphology in primary hippocampal cultures by impairing both dendritic growth and branching. Interestingly, primary hippocampal neurons from mouse models of 22q11.2 deletion also have impaired dendritic growth (Mukai et al. 2008; Fénelon et al. 2013; Moutin et al. 2017). Although it has been demonstrated that *ZDHHC8* is involved in this neuronal phenotype, our results suggest that *HIRA* is also involved in the impaired dendritic growth observed in the 22q11DS mouse model. Additionally, it has been demonstrated that HIRA regulates neurogenesis by controlling the Wnt signaling pathway (Li and Jiao 2017). Indeed, HIRA regulates β-catenin levels by recruiting the H3K4 trimethyltransferase SETD1A to the β-catenin promoter. In humans, loss-of-function variants in *SETD1A* have been associated with schizophrenia and developmental disorders (Singh et al. 2016; Kummeling et al. 2020). Moreover, HIRA defects inhibit β-catenin expression and result in altered neurogenesis. Years of studies have revealed that the Wnt signaling pathway is necessary for the guidance and branching of the axon and dendrites, as well as synapses formation and their structural remodeling. Therefore, we could hypothesize that the impaired dendritic growth and branching observed in our neuronal model results from the alteration of the Wnt pathway caused by the haploinsufficiency of *HIRA*.

As shown in our study, it has been previously demonstrated that homozygous KO of *Hira* is lethal in mice (Roberts et al. 2002). Although conditional KO models in heart or muscle tissues have been described, there is no mouse model studying the impact of *Hira* haploinsufficiency on neurological phenotype (Dilg et al. 2016; Valenzuela et al. 2017). Therefore, we developed and analyzed a heterozygous *Hira*$^{+/-}$ mouse model to assess the impact of *Hira* haploinsufficiency on brain anatomy. We showed that heterozygous *Hira* knock-out mice resulted in subtle neuroanatomical defects with some evidence of possible microcephaly in some brain regions. In particular, the corpus callosum and the molecular layer of the hippocampus displayed a statistically significant reduction in surface area and height, respectively. It is likely that neuroanatomical anomalies exhibited in mice at the tissue level might be partially caused by abnormal neuritogenesis seen in primary neurons. It is noteworthy

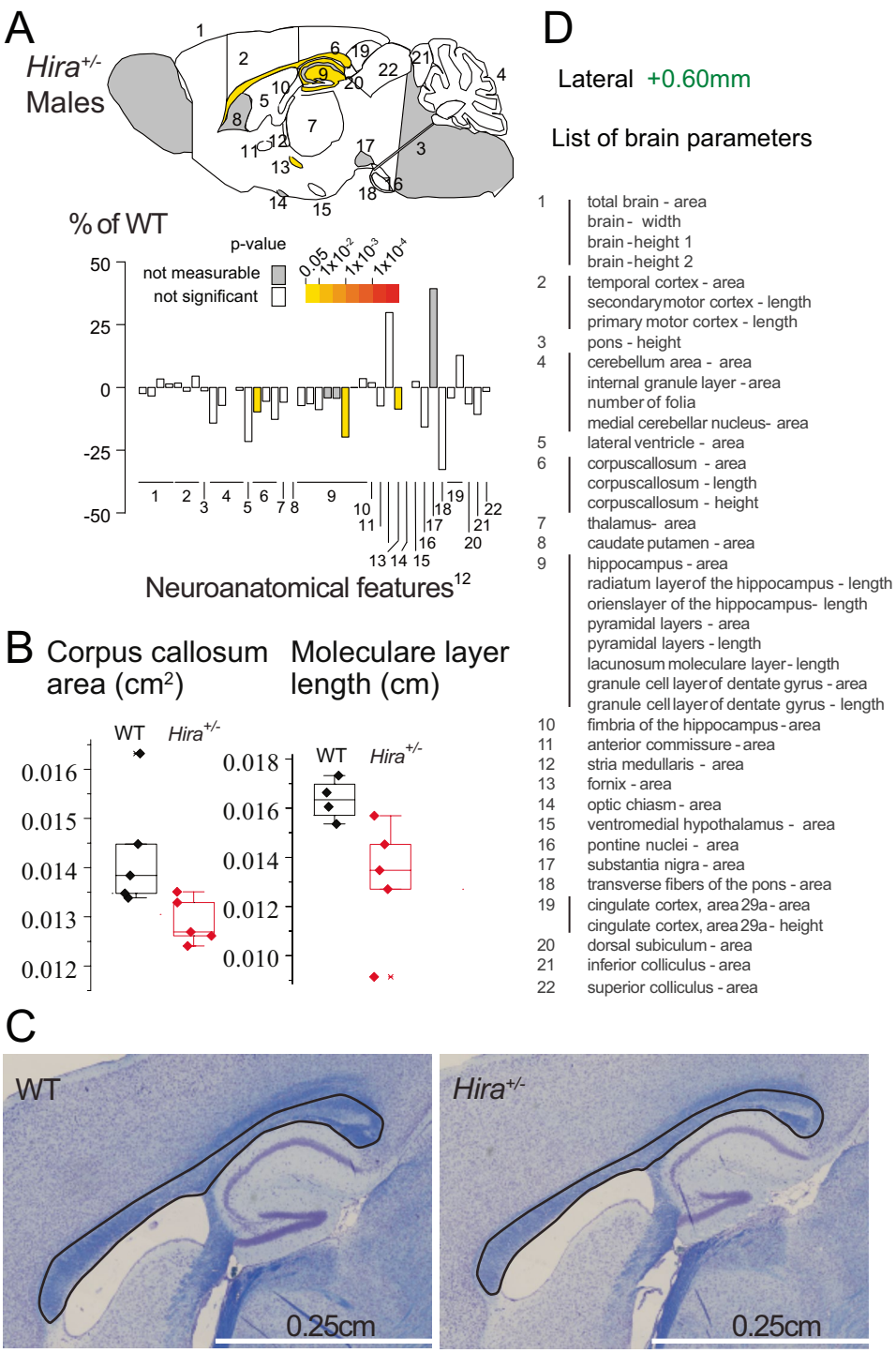


Fig. 3 Mouse *Hira*$^{+/-}$ displays minor reductions in neuroanatomical phenotypes. **a** Histogram comparing male *Hira*$^{+/-}$ to WT and showing variation (decreased-minus scale or increased-positive scale) in areas and lengths expressed as percentage of WT together with a color map indicating the significance level. A schematic representation of a section at Lateral + 0.60 mm. White color indicates a *P*-value higher than 0.05 and gray means that data were not enough to calculate a *P*-value. Three parameters were failed due to poor quality (folia, caudate putamen and optic chiasm). **b** Box plot and raw data points plots of the corpus callosum area and length of the lacunosum moleculare layer. **c** Sagittal sections of the hippocampus and the corpus callosum from WT and *Hira*$^{+/-}$ adult male mice stained with cresyl violet/luxol blue. Scale bar 0.25 cm. **d** List of brain parameters measured

that nearly 50% of 22q11DS patients have microcephaly. Furthermore, MRI studies in 22q11DS patients have noted a decrease in total brain volume by 9–11% (Kates et al. 2004; Simon et al. 2005). Hypoplastic corpus callosum, abnormalities of the septum pellucidi and hippocampal abnormalities have also been reported (Ryan et al. 1997; Andrade et al. 2013; Bohm et al. 2017). Although mild, the neuroanatomical findings of the *Hira*$^{+/-}$ mouse model should be put in the context of the neuroradiographic abnormalities reported in 22q11DS patients, potentially sharing common features such as corpus callosum hypoplasia and hippocampal malformations.

In conclusion, neurobiological and genetic analyses have provided important insights into the genetic basis of psychiatric and cognitive symptoms observed in patients carrying 22q11.2 microdeletions. Nevertheless,

the neurodevelopmental pathogenesis of this syndrome seems to be extremely complex and may probably require the effects of reduced dosage for multiple genes within the 22q11.2 deletion, possibly interacting with permissive variants in modifier genes elsewhere in the genome. Our results demonstrate that the haploinsufficiency of *HIRA* impairs dendritic growth, neuronal branching and neuroanatomical development suggesting that *HIRA* should be considered as a major gene in the landscape of the complex pathophysiology of the neurodevelopmental phenotype of 22q11DS.

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Author contributions MJ, FL, BY, and AT contributed to the study conception and design. Clinical and genetic data collection and analysis were performed by MJ, M-LV, SW, DH, NC, RP, JK, M-PM, TK and AT. Material preparation, in vitro and in vivo functional experiments and analyses were performed by MJ, DCU, VEV, CW, SC, SM, BY and FL. The first draft of the manuscript was written by MJ, M-LV, BY, FL and AT, and all authors commented on previous versions of the manuscript. All authors have reviewed and approved the finalized manuscript.

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Data availability The data and materials used and analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval The study was approved by the local institutional review boards, and written informed consent was obtained from the patients' parents, including explicit permission to share clinical and identifying information. All mouse experiments performed at the University of Tours/INSERM were approved by the French Ministry of Research (Project authorization number 01456.03). The care and use of *Hira*$^{\pm}$ mice in the Wellcome Sanger Institute study was carried out in accordance with UK Home Office regulations, UK Animals (Scientific Procedures) Act of 1986 under two UK Home Office licences (80/2485 and P77453634) that approved this work, which were reviewed regularly by the Wellcome Sanger Institute Animal Welfare and Ethical Review Body.

Consent to participate The study was approved by the local institutional review boards, and written informed consent was obtained from the patients' parents, including explicit permission to share clinical and identifying information.

Consent for publication The study was approved by the local institutional review boards, and written informed consent was obtained from the patients' parents, including explicit permission to share clinical and identifying information.

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