Received: 15 June 2020 | Revised: 10 July 2020 | Accepted: 11 July 2020

DOI: 10.1111/ejn.14914

RESEARCH REPORT

EJN European Journal of Neuroscience FENS WILEY

Asymmetric distribution of pallial-expressed genes in zebrafish (*Danio rerio*)

Andrea Messina | **Alessandra Boiti** | **Giorgio Vallortigara**

Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy

Correspondence
Andrea Messina, Center for Mind/Brain of Trento, Piazza della Manifattura 1, 38068 Rovereto, Trento, Italy.
Email: andrea.messina@unitn.it

Funding information
H2020 European Research Council, Grant/Award Number: 833504-SPANUMBRA; PRIN 2017, Grant/Award Number: 2017PSRHPZ

Abstract

The left and right distribution of a set of twenty-six genes in the zebrafish pallium was examined by RT-qPCR experiments. The analysis comprised four general pallial markers (*eomesa*, *emx2*, *emx3* and *prox1*); eight genes, *dapper1*, *htr3a*, *htr3b*, *htr4*, *id2*, *ndr2, pkcβ* and *lmo4*, that have been described as asymmetric distributed in the brain of mammals (human and mouse); six genes, *arrb2*, *auts2*, *baiap2*, *fez1*, *gap43* and *robo1*, asymmetrically distributed in the mammalian cortex, that have been associated with autism in humans; and, eight genes, *baz1b*, *fzd9*, *limk1*, *tubgcp5*, *cyfip1*, *grik1a*, *nipa1* and *nipa2*, which have been associated with developmental dyscalculia, a brain disability linked to brain laterality in humans. We found a leftward bias in the expression of 10 genes (*dapper1, htr3a, htr3b, htr4, id2, ndr2, pkcß, auts2, baiap2 and grik1a*) and a rightward bias for 5 genes (*lmo4, arrb2, fez1, gap43, robo1)* in agreement with the data reported in mammals. We also found a rightward lateralization for *nipa1 and nipa2*, whereas the remaining genes (*eomesa, emx2, emx3, prox1, baz1b, cyfip1, fzd9, limk1 and tubgpc5*) were bilaterally distributed. These findings suggest a basic homology in the asymmetric expression of several pallial vertebrate genes.

KEYWORDS

autism-related genes, brain asymmetry, *Danio rerio*, developmental dyscalculia-related genes, telencephalic lateralization

Abbreviations: *18S*, ribosomal RNA 18S; *arrb2*, arrestin beta 2; *auts2*, activator of transcription and developmental regulator AUTS2; *baiap2*, BAR/IMD domain containing adaptor protein 2; *baz1b*, bromodomain adjacent to zinc finger domain 1 B; *cyfip1*, cytoplasmic FMR1 interacting protein 1; *dapper1*, dishevelled binding antagonist of beta catenin 1; *emx2*, empty spiracle 2; *emx3*, empty spiracle 3; *eomesa*, eomesodermin homolog a; *fez1*, fasciculation and elongation protein zeta 1; *fzd9*, frizzled class receptor 9; *gap43*, growth-associated protein 43; *grik1a*, glutamate receptor, ionotropic, kainate 1a; *htr3a*, 5-hydroxytryptamine receptor 3a; *htr3b*, 5-hydroxytryptamine receptor 3b; *htr4*, 5-hydroxytryptamine receptor 4; *id2*, inhibitor of DNA binding 2; *kctd12.1/lev*, potassium channel tetramerization domain containing 12.1/leftover; *ktcd12.2/ron*, potassium channel tetramerization domain containing 12.2/right-on; *ktcd8/dex*, potassium channel tetramerization domain containing 8/dexter; LI, Index of Laterality; *limk1*, LIM domain kinase 1; *lmo4*, LIM domain only 4; *ndr2*, nodal-related 2; *nipa1*, nonimprinted in Prader-Willi/Angelman syndrome 1 homolog; *nipa2*, nonimprinted in Prader-Willi/Angelman syndrome 2 homolog; *pkcβ*, protein kinase C beta; *prox1*, prospero homeobox 1; RNA, ribonucleic acid; *robo1*, roundabout 1; RT-qPCR, reverse transcription–quantitative polymerase chain reaction; *tubgcp5*, tubulin gamma complex-associated protein 5.

Edited by Yoland Smith

1 | INTRODUCTION

The two sides of the vertebrate brain appear as near-mirror images of each other, being grossly characterized by similar cell composition, subdivisions and connections. However, a large number of studies have revealed fundamental differences in the ability of the left and right side to perform different functions and to process specific information (Güntürkün & Ocklenbrug, 2017; Rogers & Vallortigara, 2015). Functional and anatomical differences (lateralization) are well-documented in all vertebrate taxonomic groups, from fish to mammals (see for reviews Güntürkün & Ocklenbrug, 2017; MacNeilage, Rogers, & Vallortigara, 2009; Rogers, Rigosi, Frasnelli, & Vallortigara, 2013; Rogers, Vallortigara, & Andrew, 2013; Vallortigara & Rogers, 2020; Vallortigara & Versace, 2017). It has been argued that possession of a slight asymmetry in the brain would confer biological advantages (Ghirlanda, Frasnelli, & Vallortigara, 2009; Ghirlanda & Vallortigara, 2004; Rogers, Zucca, & Vallortigara, 2004; Vallortigara, 2006; Vallortigara & Rogers, 2020). Impairment in the development of brain laterality has been linked to neurodevelopmental disorders such as autism (Paquet, Golse, Girard, Olliac, & Vaivre-Douret, 2017), developmental dyscalculia (Shalev, Manor, Amir, Wertman-Eiad, & Gross-Tsur, 1995) and developmental dyslexia (Milne, Syngeniotis, Jackson, & Corballis, 2002).

In the last twenty years, zebrafish (*Danio rerio*) has become a prominent model to study the molecular mechanisms of brain lateralization (Carl et al., 2007; Concha, Burdine, Russell, Schier, & Wilson, 2000; Duboc, Dufourcq, Blader, & Roussigné, 2015; Liang et al., 2000; Miletto Petrazzini, Sovrano, Vallortigara, & Messina, 2020). Research with zebrafish established that vertebrates laterality seems to be guided by four major pathways (nodal, fibroblast growth factors, notch and Wnt/beta catenin), which are involved in the positioning of some genes (*ndr2*, *lefty 1* and *pitx2*) on the left side of the forming neural tube (Carl et al., 2007; Concha et al., 2000; Essner, Amack, Nyholm, Harris, & Yost, 2005; Hüsken & Carl, 2013; Liang et al., 2000; Raya et al., 2003; Regan, Concha, Roussigne, Russell, & Wilson, 2009) and contributing to the generation of zebrafish thalamus asymmetries with the positioning of *kctd12.1/leftover* in the left and of *ktcd8/dexter* and *ktcd12.2/right-on* in the right primordium of habenular nuclei (Gamse et al., 2005; Gamse, Thisse, Thisse, & Halpern, 2003).

Disrupting of the signalling cascades from these pathways—and the consequent disorganization of the correct left-right laterality of zebrafish thalamus—leads to a loss of habenular responsiveness to either odour or light stimuli (Dreosti, Vendrell Llopis, Carl, Yaksi, & Wilson, 2014). In zebrafish transgenic line *frequent-situs-inversus (fsi)*, the reversal of nodal signalling appears to reverse visceral and diencephalic asymmetries and at least some behavioural asymmetries (Barth et al., 2005).

During the years, it has become apparent that very conserved gene expression patterns are present in forebrain, midbrain and hindbrain of fish and other vertebrates (Wullimann et al., 2011; Wurst & Bally-Cuif, 2001), and similarities have been observed in the neurochemistry (Mueller, 2012; Panula et al., 2010; Schweitzer & Driever, 2009) and regional connectivity of thalamus (Mueller, 2012), optic tectum (Wullimann, 1994), hypothalamus (Herget, Wolf, Wullimann, & Ryu, 2014), cerebellum (Hashimoto & Hibi, 2012) and medulla oblongata (Kinkhabwala et al., 2011). Relatively little is known, however, as to telencephalic structures, in particular with respect to brain asymmetry.

In the earlier years of 2000s, the study on the molecular bases of brain lateralization converged on the hypothesis that an essential step is the breaking of the symmetry of brain structures as a result of an intrinsic genetic programme. Sun et al. (2005) and several other scholars (Guarneri et al., 1988; Larisch & Klimke, 1988; Moskal, Kroes, Otto, Rahimi, & Claiborne, 2006; Orman & Stewart, 2007; Ribasés et al., 2009; Samara et al., 2011) explored this hypothesis performing large, serial gene expression screening in the human, mouse and rat brains at different stages of development. These studies revealed that more than 500 genes are differentially expressed in the left and right brain in mammalian species. Among them, there are genes involved in the generation of left-side identity during vertebrate brain development, such as *id2* and *ndr2* (Carl et al., 2007; Concha et al., 2000; Essner et al., 2005; Hüsken & Carl, 2013; Liang et al., 2000; Raya et al., 2003; Regan et al., 2009) but also intracellular inhibitors of *Wnt* signalling cascade such as *dapper1* (Zhang, Gao, Wen, Ning, & Chen, 2006). The list also included three serotonin receptors (*htr3a*, *htr3b* and *htr4*) with a leftward asymmetric distribution in different areas of the mammalian brain (Andersen & Teicher, 1999; Fink et al., 2009; Sun et al., 2005), and during chick and frog embryonic development (Fukumoto, Keme, & Levin, 2005); the gene *pkcβ* which is leftward asymmetrical distributed in the mammalian amygdala and involved in different lateralized behaviours such as fear (Orman & Stewart, 2007), pain (Ji & Neugebauer, 2009) and olfactory discrimination (Cohen, Putrino, & Wilson, 2015); the gene *lmo4* which is one of the first genes described as differentially distributed in human and mouse cortex (Sun et al., 2005) and play a pivotal role in the definition of the shape of lateralized cortical functional areas in mouse brain (Huang et al., 2009; Li et al., 2013); and several other genes linked to autism, some leftward asymmetrical distributed, such as *auts2* and *baiap2* (Bedogni et al., 2010; Grabrucker et al., 2018; Toma et al., 2011) and others rightward asymmetrical distributed, such as *arrb2, fez1*, *gap43* (Bedogni et al., 2010; Toma et al., 2011) and *robo1* (Anitha et al., 2008).

Asymmetry of gene expression in telencephalic structures of zebrafish adult brain has not been explored. Pallial asymmetries have been examined in the scale-eating cichlid *Perissodus microlepis*. A study reported an asymmetry in the expression of 173 genes after food preference behavioural tests (Lee et al., 2017), whereas another study identified only three genes, belonging to signal transduction cascades, as differentially expressed in the left and right hemisphere in fish individually housed from capture to tissue extraction (Takeuchi, Ishikava, Oda, & Kitano, 2018).

Here, we performed RT-qPCR experiments (reverse transcription–quantitative polymerase chain reaction) in order to investigate whether any left–right asymmetrical distribution in the expression of pallial genes could be observed in the dorsal telencephalon of adult zebrafish. Given the recent interest for the neural correlates of number (quantity) estimation using zebrafish as an animal model (Potrich, Sovrano, Stancher, & Vallortigara, 2015; Potrich, Rugani, Sovrano, Regolin, & Vallortigara, 2019; Messina et al., 2020; see Nieder, 2019 for a general review on neural mechanisms of numerical cognition), we also checked for the expression of eight genes (*baz1b*, *fzd9*, *limk1*, *tubgcp5*, *cyfip1*, *grik1a*, *nipa1* and *nipa2*) which have been associated with developmental dyscalculia in humans (Chai et al., 2003; Docherty, Yulia Kovas, Petrill, & Plomin, 2010; Maver, Čuturilo, Kovanda, Miletić, & Peterlina, 2019; Tassabehji, 2003), a specific learning disability that affects the natural predisposition to estimate quantity (Shalev, 2004) and that has been suggested to be linked with abnormality in brain asymmetry as well (Shalev et al., 1995). We also checked for the expression of three thalamic genes which have been well-characterized and that are known to be asymmetrically distributed in zebrafish (Concha & Wilson, 2001; Gamse et al., 2003, 2005) as a control for our procedures.

2 | MATERIALS AND METHODS

2.1 | Ethical regulations

All husbandry and experimental procedures complied with the European Legislation for the Protection of Animals used for Scientific Purposes (Directive 2010/63/EU) and were approved by the Scientific Committee on Animal Health and Animal Welfare (Organismo Preposto al Benessere Animale, OPBA) of the University of Trento and by the Italian Ministry of Health (Protocol n. 893/2018-PR and Protocol n. 135/2020-PR).

2.2 | Animals

Eleven adult (one year old) wild-type commercial mixed strain male zebrafish were used. Fish were maintained in an automated aquarium system (ZebTEC Benchtop, Tecniplast), with 7-L plastic tanks, in standard conditions (28°C, light/dark cycle of 12 hr/12 hr, fed two times a day with artemia and one time with dry food) in accordance with the guidelines of animal welfare.

The zebrafish were killed by putting them in a bath of ice-cold water, and their brains were dissected in phosphate-buffered saline solution (PBS; Fisher BioReagents).

2.3 | Right and left brain tissue dissection and total RNA extraction

Before starting our analyses of left–right distribution of selected gene markers in the dorsal telencephalon of adult zebrafish, the left and right adult pallia were separately collected and the ventral subpallium was accurately removed from each side.

The left and right dorsal pallium, subpallium and left and right anterior thalamus were separately processed, and total RNA extraction was performed using the RNeasy Mini Kit (QIAGEN) according to manufacturer's instructions. Briefly, brain-derived tissues were homogenized in lysis buffer and loaded onto RNeasy spin columns, treated with DNase (RNase-Free DNase Set; QIAGEN) and eluted in RNase/DNase-free water. Collected total RNA was quantified using NanoDrop™ (Thermo Fisher Scientific). Reverse transcription was performed using the SuperScript™ VILO™ cDNA Synthesis Kit (Invitrogen, Thermo Fisher Scientific) according to manufacturer's instructions.

2.4 | Reverse transcription–quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR experiments were performed using specific primer pairs commercially synthesized (Sigma-Aldrich/Merck). Primers were listed in Table 1. Triplicate reactions/sample were performed using the PowerUp™ SYBR™ Green Master Mix (2×) and a CFX96™ Real-Time System (Bio-Rad). The ΔCq method was used for expression quantification (Messina et al., 2020). Data were normalized on the expression of the *18S* reference gene (ΔCq), and the relative expression (to the reference gene) of each target was calculated.

2.5 | Index of laterality

An index of laterality (LI) was calculated as previously reported (de Kovel, Lisgo, Fisher, & Francks, 2018). In short, the RT-qPCR normalized data deriving from the left hemisphere for each gene were compared with the amount deriving from the quantification of the same gene in the right hemisphere using the following formula:

TABLE 1 List of primers

Gene name	Gene ID		Primers	Amplicon size	Efficiency (%)
18 S	100037361	For	TCGCTAGTTGGCATCGTTTATG	85 pb	93.0
		Rev	CGGAGGTTCGAAGACGATCA		
kctd12.1/lov	373865	For	AGTTCTTTCAGCTGCGGGACCTTA	112 pb	98.3
		Rev	GCGACGGACAGTGTGCGAGAG		
kctd8/dex	568933	For	CATGCCATCAATTACGCAAC	112 pb	101.9
		Rev	CGGATCCCGACTTTCATTTA		
kctd12.2/ron	553403	For	GCCACTCAACTTTGCTCTCC	191 pb	99.7
		Rev	GAGGCTCGCTTTCTCTTTCTCTTTGA		
eomesa	64603	For	CTTATTGATCTCCGCCTTGC	147 pb	99.4
		Rev	TATTGGTGCTTTCGGAGGAC		
emx3	30536	For	TTCACTCCATCATCGGGTTC	145 pb	93.7
		Rev	GCGTTTGACGAATTGGAGTC		
emx2	30537	For	TTTCACATCCTCCCAACTCC	199 pb	94.6
		Rev	GGACTCGTTTCGTTTCCTTG		
prox1a	30679	For	TTACGAAGACGCTGTGATGC	195 pb	92.0
		Rev	AATGGTGAAAGGCACTCCTG		
dlx2a	30574	For	TTCAGCCACCACTTCATCAC	193 pb	95.0
		Rev	AACAGTGTCACGCCCAAATC		
dlx5a	30569	For	TCATACTCCACAGCGTATCACC	148 pb	90.0
		Rev	AGTAAATGGTTCGGGGCTTC		
dapper1	405799	For	TTCAGCGAGTGTTTGTCCAG	127 pb	99.1
		Rev	AATCACACTCGGCACAACTG		
htr3a	571641	For	TCGCTCAGCACAATGAGAAG	86 pb	101.7
		Rev	TTCACTGAGCAATCCACCAC		
htr3b	571632	For	AGTGAGCGAAGTGGATTTGG	83 pb	90.0
		Rev	GCTCCCATTCTCCATCATTC		
htr4	101882850	For	TAGTTTGCGCTGACAGCAAC	83 pb	97.2
		Rev	AAGCTTTACCGGGTTTACGG		
id2	266599	For	GGCGTGTGAATGAGAAGATG	182 pb	97.6
		Rev	GATACCGGCAGTCCAATTTC		
ndr2	30292	For	CATGCTGCTGCTGTTTTCAG	183 pb	101.8
		Rev	TTGTGCAGTGGTGTTCTCTG		
lmo4a	114412	For	TTTGGTCACAGTGGAGCTTG	164 pb	98.2
		Rev	ATGGTGCCGTTGACATAGTG		
pkcbeta	393953	For	CAGGACGATGATGTCGAATG	182 pb	96.0
		Rev	TCCTTAAACTTGCCGACCTG		
arrb2	394099	For	CCCTTCAACTTCACGATTCC	127 pb	93.0
		Rev	CATCCACAGTTTTGGCACAG		
auts2a	368890	For	TGGCTCAAACCTGGAGAATC	181 pb	98.5
		Rev	TAACAGGAAGTGGGGATTGG		
baiap2a	678518	For	CAAAAGCAAAGGCGAGAGTC	183 pb	99.2
		Rev	GAAAGCGCTGTTTTGTAGCC		
fez1	406705	For	AAACACTCAACGGCAACCTC	159 pb	95.2
		Rev	CCGGTGAGTTTTCCATCATC		

(Continues)

TABLE 1 (Continued)

Gene name	Gene ID		Primers	Amplicon size	Efficiency (%)
gap43	30608	For	GACACATCACCCGGAAAAAG	151 pb	96.4
		Rev	TCATTTGCTGGGGAGTTAGG		
robo1	30769	For	ATCTCAATCCCGAAGTGCTG	118 pb	97.5
		Rev	TTTCCTGCTCACAGACGATG		
baz1b	571727	For	GCGTATCAGGAGGCAAAAAG	171 pb	96.3
		Rev	TCGTGAACCCATCCTTTCTC		
fzd9b	58023	For	TTCTGGACCCTCATCGTTTC	145 pb	99.9
		Rev	TACAACTCGCCATTCTCACG		
limk1a	735292	For	TACAGCCCTGAACACAAACG	114 pb	103.5
		Rev	AACGTTCTGGATTGGCGTTC		
tubgcp5	553191	For	ACTTTGTCAACAGCCTGCAC	166 pb	102.4
		Rev	TGGCCTCCTTGACAAAACTG		
cyfip1	336613	For	AACCAGGGTGCAAACATCTC	134 pb	99.3
		Rev	TAAAGCGCAGAAGACACACG		
grik1a	798001	For	ACGAGAAGATGTGGGCATTC	147 pb	99.7
		Rev	TGCAGTTTCTCTGGGTGATG		
nipa1	450042	For	AGTCTTGTTTGGTGCCGTTC	119 pb	101.4
		Rev	TGGGTGAGTGAATGATGAGC		
nipa2	406399	For	CTTTGTGGTGTTTGCGACAG	176 pb	98.5
		Rev	CAGCGATGGCCTCTTTAATC		

$$\mathrm{LI} = \frac{\text{Left Hemisphere (L)} - \text{Right Hemisphere (R)}}{\text{Left Hemisphere (L)} + \text{Right Hemisphere (R)}}$$

2.6 | Statistical analyses

Statistical analyses on RT-qPCR data were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics; IBM). RT-qPCR comparisons between left and right dorsal pallium expressions were performed using a two-tailed *t* test for paired samples. Departures from chance level in the laterality index were estimated by one-sample two-tailed *t* tests. The Bonferroni–Holm correction was used for multiple testing comparisons.

3 | RESULTS

3.1 | Thalamic markers

The thalamic markers *kctd12.1/leftover* (*lov*), *ktcd8/dexter* (*dex*) and *ktcd12.2/right-on* (*ron*) were used to test brain asymmetry of the subjects examined in the present study. A predominant expression of *kctd12.1/leftover* on the left side of the adult thalamus and a predominant expression of *ktcd12.2/right-on* the right side were observed, whereas *ktcd8/dexter* expression was symmetrically distributed (Figure 1b). See also Figure 1c for the index of laterality and Table 2 for the statistical analyses. Our results thus confirm the left–right patterning of genes in the thalamic structures of zebrafish brains which have been described in previous studies.

3.2 | Dissection of dorsal pallium and ventral subpallium of adult zebrafish brain

The samples collection (Figure 2a) and the results relative to dissections validation are shown in Figure 2b,c, in which total RNAs deriving from left and right dorsal (pallia) and ventral (subpallium) telencephalon were separately pooled and RT-qPCR was performed to test the distribution of six previously described telencephalic-positioning markers in the dissected area, that is *eomesa*, *emx2, emx3*, *prox1, dlx2a* and *dlx5a* (Ganz et al., 2011, 2014). *Eomesa* identifies the extent of pallium in tetrapods and fish (Brox, Puelles, Ferreiro, & Medina, 2004; Bulfone et al., 1999; Ganz et al., 2014); *emx2, emx3* and *prox1* label the different pallial subdivisions of zebrafish brain (Ganz et al., 2014), whereas *dlx2a* and *dlx5a* are markers of subpallium in fish (Ganz et al., 2011). Results showed a predominant expression of *eomesa*, *emx2, emx3* and *prox1* in the dorsal tissues of telencephalon but not

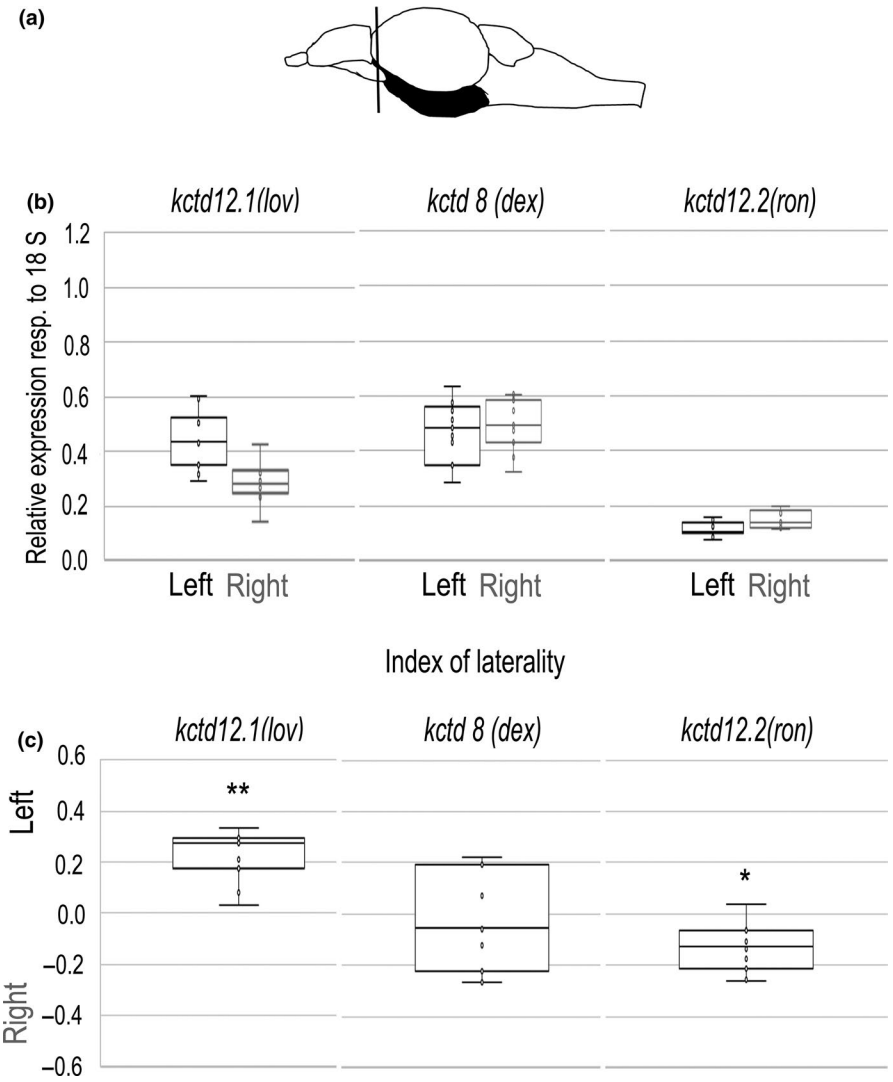

FIGURE 1 RT-qPCR in the left and right thalamus of zebrafish. (a) Schematic representation of thalamic regions selected for the analyses. (b) RT-qPCR results for *kctd12.1/lov*, *kctd8/dex* and *kctd12.2/ron* in the left and right thalamus of zebrafish. (c) Index of laterality for *kctd12.1/lov*, *kctd8/dex* and *kctd12.2/ron*

of *dlx2a* and *dlx5a* (Figure 2b), and a predominant expression of *dlx2a* and *dlx5a* in the subpallial tissues with a marginal expression of the pallial markers *eomesa*, *emx2*, *emx3* and *prox1* (Figure 2c), showing that the pallium and subpallium were properly collected and separated.

A comparison between the expression of the four pallial markers (*eomesa*, *emx2*, *emx3* and *prox1*) in the left and right pallium was shown in Figure 2d. No differences between the left and right hemisphere were observed (see Table 2 for the statistical analyses).

3.3 | Asymmetric distribution of genes expressed in zebrafish pallium

A leftward bias in the expression of *dapper1*, *htr3a*, *htr3b*, *htr4*, *id2*, *ndr2* and *pkcβ* and a rightward bias in the expression of *lmo4* were observed (Figure 3 and Table 2).

A leftward bias for *auts2* and *baiap2* and a predominant right bias for *arrb2*, *fez1, gap43* and *robo1* were observed (Figure 4 and Table 2).

3.4 | Developmental dyscalculia-associated genes

As to genes associated with human developmental dyscalculia, we found that the genes *nipa1* and *nipa2* were predominantly expressed in the right hemisphere and the gene *grik1a* in the left hemisphere, whereas the genes *baz1b*, *cyfip1*, *fzd9*, *limk1* and *tubgpc5* were bilaterally distributed in the zebrafish pallium (Figure 5 and Table 2).

3.5 | Summary of left-right distribution of all tested genes

A summary of the results is shown in Figure 6, with the lateralization index of all twenty-six telencephalic genes that we characterized in our experiments. We found a leftward lateralization for ten genes (*dapper1, htr3a, htr3b, htr4, id2, ndr2, pkcß, auts2, baiap2 and grik1a*) and a rightward lateralization for seven genes (*lmo4, arrb2, fez1, gap43, robo1, nipa1 and nipa2*); the others genes were bilaterally

TABLE 2 RT-qPCR results (group means with *SEM* are shown) of left- and right-side gene expression with statistical analyses and effect size

Gene	Left hemisphere	Right hemisphere	Paired *t* test	Effect size (Cohen's)
Thalamus				
kctd12.1/lov	0.453 ± 0.031	0.288 ± 0.021	$t(10) = 7.526$ $p = .003$	2.269174379
kctd8/dex	0.235 ± 0.016	0.249 ± 0.014	$t(10) = -0.556$ $p > .999$	−0.167640308
kctd12.2/ron	0.116 ± 0.007	0.152 ± 0.009	$t(10) = -4.693$ $p = .027$	−1.41499274
Telencephalon				
General dorsal markers in mammals				
eomesa	0.581 ± 0.064	0.598 ± 0.051	$t(10) = -0.307$ $p > .999$	−0.092563983
emx3	0.319 ± 0.047	0.322 ± 0.032	$t(10) = -0.157$ $p > .999$	−0.047337281
emx2	0.151 ± 0.015	0.158 ± 0.018	$t(10) = -0.354$ $p > .999$	−0.106735016
prox1	0.193 ± 0.017	0.195 ± 0.011	$t(10) = -0.147$ $p > .999$	−0.044322168
Differentially distributed				
dapper1	0.321 ± 0.028	0.215 ± 0.022	$t(10) = 4.099$ $p = .044$	1.235895001
htr3a	0.111 ± 0.008	0.062 ± 0.009	$t(10) = 6.297$ $p = .002$	1.898616937
htr3b	0.144 ± 0.021	0.087 ± 0.015	$t(10) = 7.380$ $p = .002$	2.225153723
htr4	0.805 ± 0.024	0.486 ± 0.032	$t(10) = 7.689$ $p = .002$	2.318320728
id2	0.428 ± 0.031	0.304 ± 0.026	$t(10) = 8.7150$ $p = .002$	2.627671368
ndr2	0.427 ± 0.033	0.322 ± 0.031	$t(10) = 6.620$ $p = .002$	1.996005101
lmo4a	0.154 ± 0.015	0.250 ± 0.015	$t(10) = -4.879$ $p = .016$	−1.47107385
pkcbeta	0.581 ± 0.035	0.458 ± 0.031	$t(10) = 8.072$ $p = .002$	2.434101085
Autism-related genes				
arrb2	0.314 ± 0.029	0.447 ± 0.039	$t(10) = -4.931$ $p = .014$	1.48675244
auts2a	0.659 ± 0.046	0.535 ± 0.027	$t(10) = 5.832$ $p = .001$	1.758414162
baiap2a	0.807 ± 0.034	0.655 ± 0.043	$t(10) = 3.970$ $p = .036$	1.197000038
fez1	0.357 ± 0.021	0.515 ± 0.037	$t(10) = -6.790$ $p = .001$	−2.04726203
gap43	0.517 ± 0.041	0.638 ± 0.055	$t(10) = -5.270$ $p = .001$	−1.588964786
robo1	0.785 ± 0.025	1.008 ± 0.034	$t(10) = -7.388$ $p = .001$	−2.227565814
Dyscalculia-related genes				
baz1b	0.666 ± 0.042	0.728 ± 0.055	$t(10) = -1.095$ $p > .999$	−0.330154922
fzd9	0.491 ± 0.066	0.485 ± 0.068	$t(10) = 0.187$ $p > .999$	0.056382621
limk1a	0.607 ± 0.032	0.563 ± 0.067	$t(10) = 0.629$ $p > .999$	0.189650636
tubgcp5	0.192 ± 0.014	0.219 ± 0.021	$t(10) = -1.703$ $p = .595$	−0.51347382
cyfip	0.685 ± 0.037	0.824 ± 0.053	$t(10) = -2.795$ $p = .076$	−0.842724208
grik1a	0.633 ± 0.062	0.399 ± 0.051	$t(10) = 4.780$ $p = .004$	1.441224227
nipa1	0.066 ± 0.003	0.085 ± 0.004	$t(10) = -4.344$ $p = .003$	−1.309765281
nipa2	0.228 ± 0.015	0.292 ± 0.023	$t(10) = -4.997$ $p = .001$	−1.506652189

distributed (*eomesa, emx2, emx3, prox1, baz1b, cyfip1, fzd9, limk1 and tubgpc5*).

4 | DISCUSSION

Asymmetry of gene expression in the thalamus has been documented in zebrafish (Barth et al., 2005; Carl et al., 2007; Concha et al., 2000; Dreosti et al., 2014; Essner et al., 2005; Hüsken & Carl, 2013; Liang et al., 2000; Raya et al., 2003; Regan et al., 2009). Here, we confirmed a predominant expression of *kctd12.1/leftover* on the left side (Gamse et al., 2003) and *ktcd12.2/right-on* on the right side of the adult thalamus (Gamse et al., 2005). On the contrary, *ktcd8/dexter* appeared symmetrically distributed in the adult thalamus (thought it seems to show a rightward bias during zebrafish embryonic development; Gamse et al., 2005).

We found a symmetrical expression of the general pallial marker *eomesa* (Brox et al., 2004; Bulfone et al., 1999; Ganz et al., 2014), but also of *emx2* (marker of dorsocentral

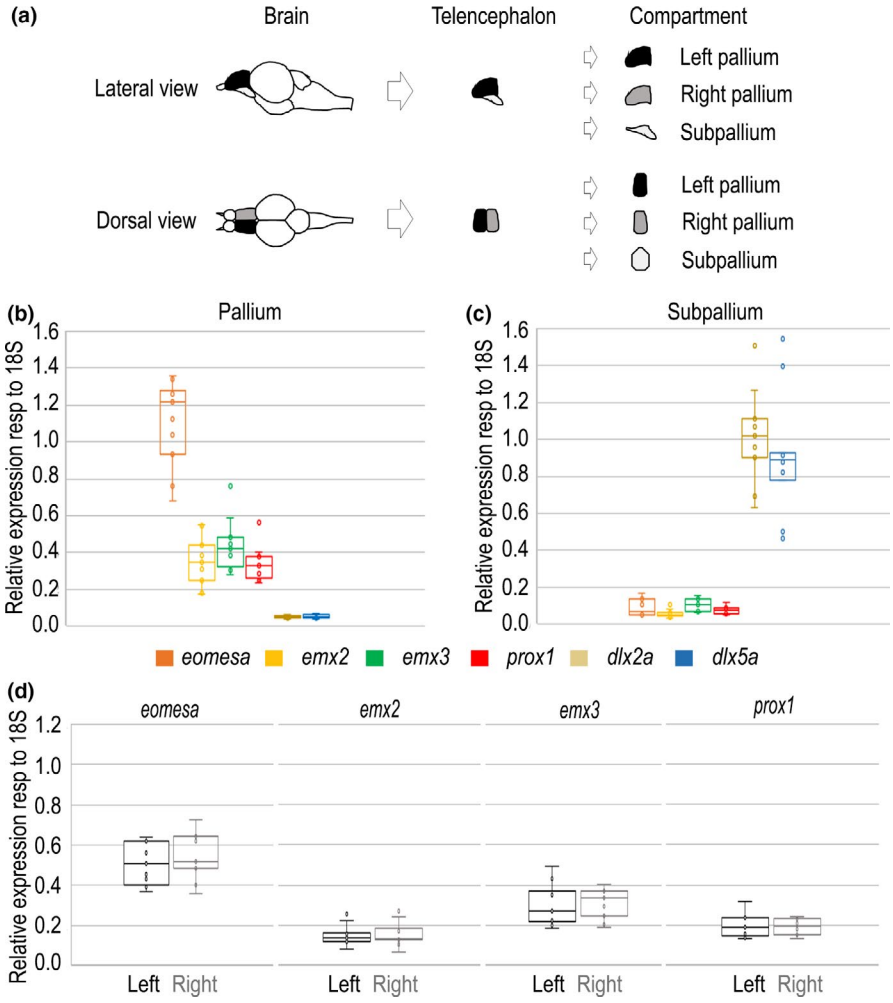

FIGURE 2 RT-qPCR in the telencephalon of zebrafish. (a) Schematic representation of the dissected telencephalic areas selected for the analyses: lateral view (top) and dorsal view (bottom). (b, c) RT-qPCR results for *eomesa, emx2, emx3, prox1, dlx2a* and *dlx5a* in (b) pallial (dorsal telencephalon) and (c) subpallial (ventral telencephalon) regions. (d) RT-qPCR results for *eomesa, emx2, emx3, prox1 and dlx2a* in the left and right pallium [Colour figure can be viewed at wileyonlinelibrary.com]

pallium), *emx3* (marker of dorsomedial and dorsolateral pallium) and *prox1* (marker of dorsolateral pallium; Ganz et al., 2014).

A leftward bias in the expression of *id2*, *ndr2* and *dapper1* was observed in zebrafish, as reported in mammals (Sun et al., 2005). The asymmetric expression of these genes in the

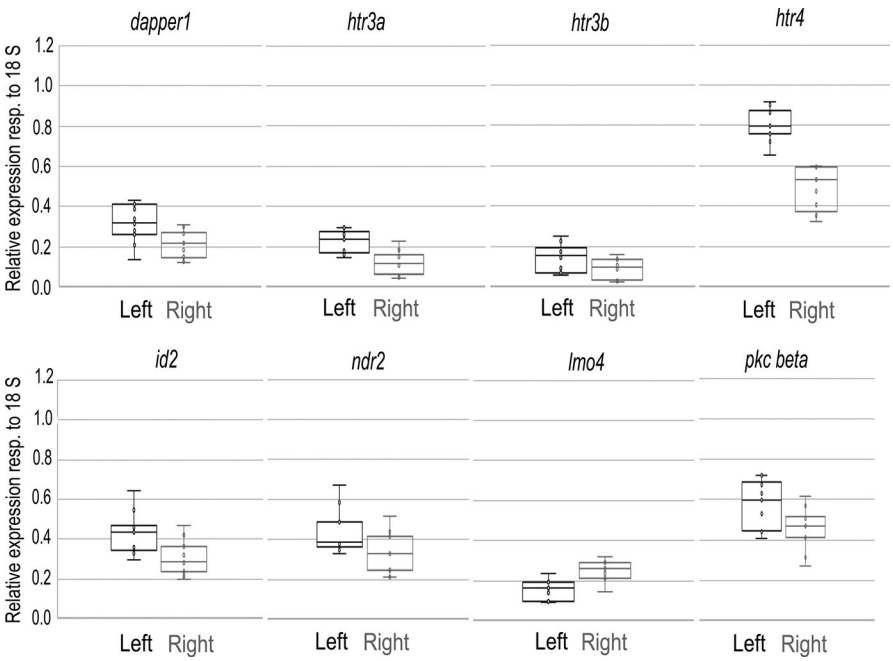

FIGURE 3 RT-qPCR left and right expression of zebrafish pallial genes: *dapper1* (an inhibitor of Wnt signalling); *htr3a, htr3b* and *htr4* (serotonin receptors); *id2* and *ndr2* (two genes known to be involved in vertebrates laterality); *lmo4* (known to be involved in mammalian cortical development); and *pkcβ* (known to be differentially expressed in the mammalian amygdala)

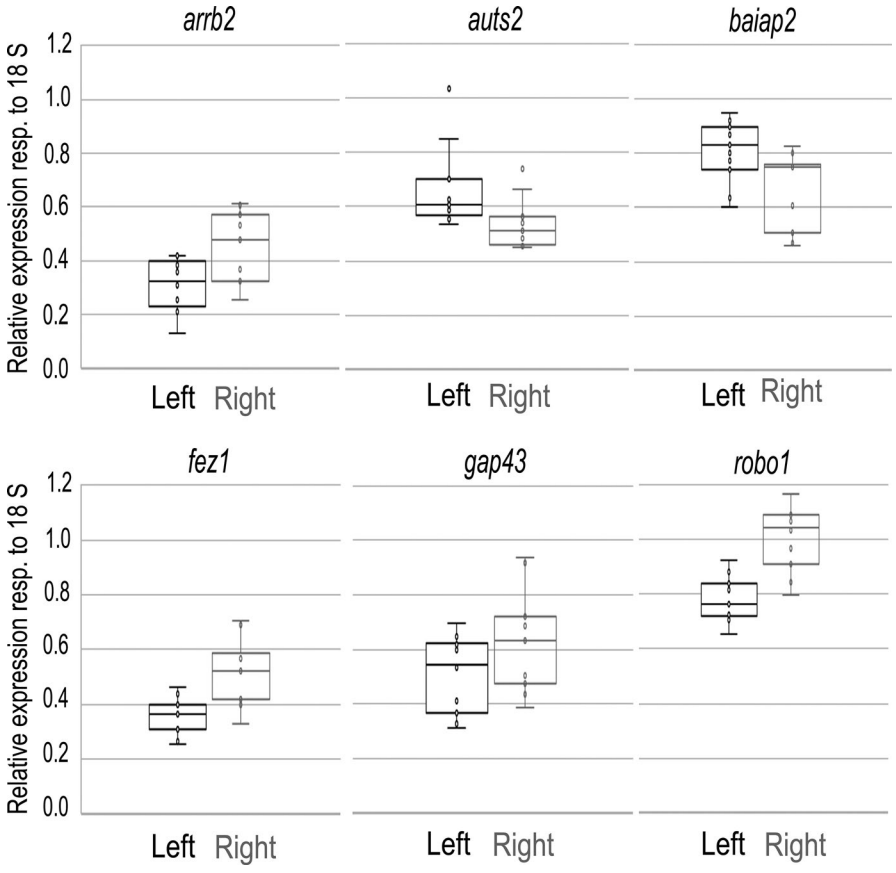

FIGURE 4 RT-qPCR left and right expression of zebrafish pallial genes *arrb2*, *auts2*, *baiap2*, *fez1*, *gap43* and *robo1* (these genes are left and right differentially distributed in the mammalian cortex and linked to autism)

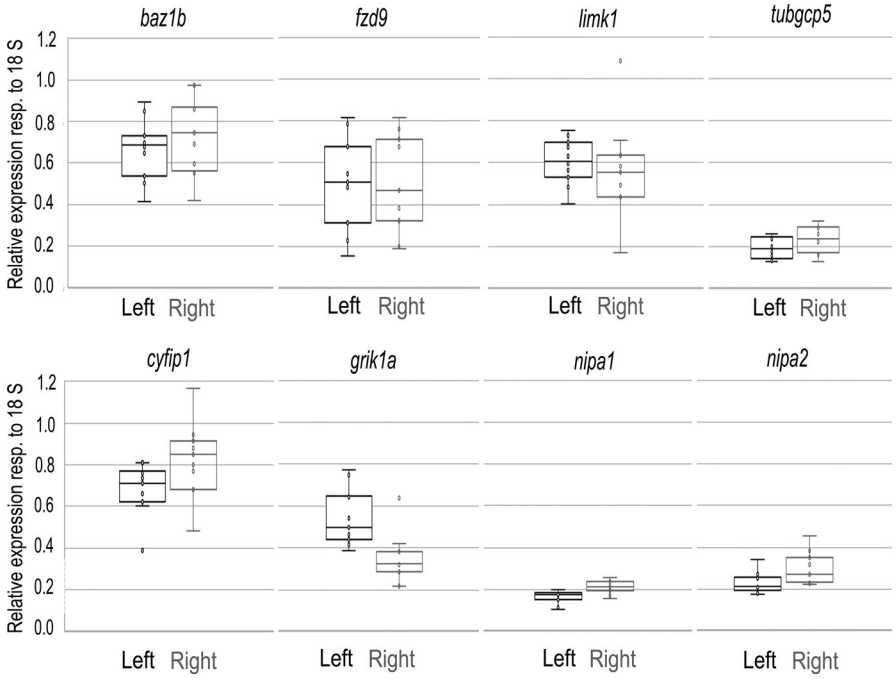

FIGURE 5 RT-qPCR left and right expression of zebrafish pallial genes *baz1b*, *fzd9*, *limk1*, *tubgcp5*, *cyfip1*, *grik1a*, *nipa1* and *nipa2* (these genes have been shown to be associated with human developmental dyscalculia and dyslexia)

adults opened the possibility that the *nodal* (*id2* and *ndr2*) and *Wnt (dapper1)* signalling pathway could play a role not only for the left–right patterning during early embryogenesis, but also in the maintenance of left–right asymmetry in the adult zebrafish brain (Carl et al., 2007; Concha et al., 2000; Essner et al., 2005; Hüsken & Carl, 2013; Liang et al., 2000; Raya et al., 2003; Regan et al., 2009; Zhang et al., 2006).

A leftward asymmetric expression was found also for *htr3a*, *htr3b* and *htr4* similar to that observed in other taxonomic groups (human, Sun et al., 2005; chick and frog,

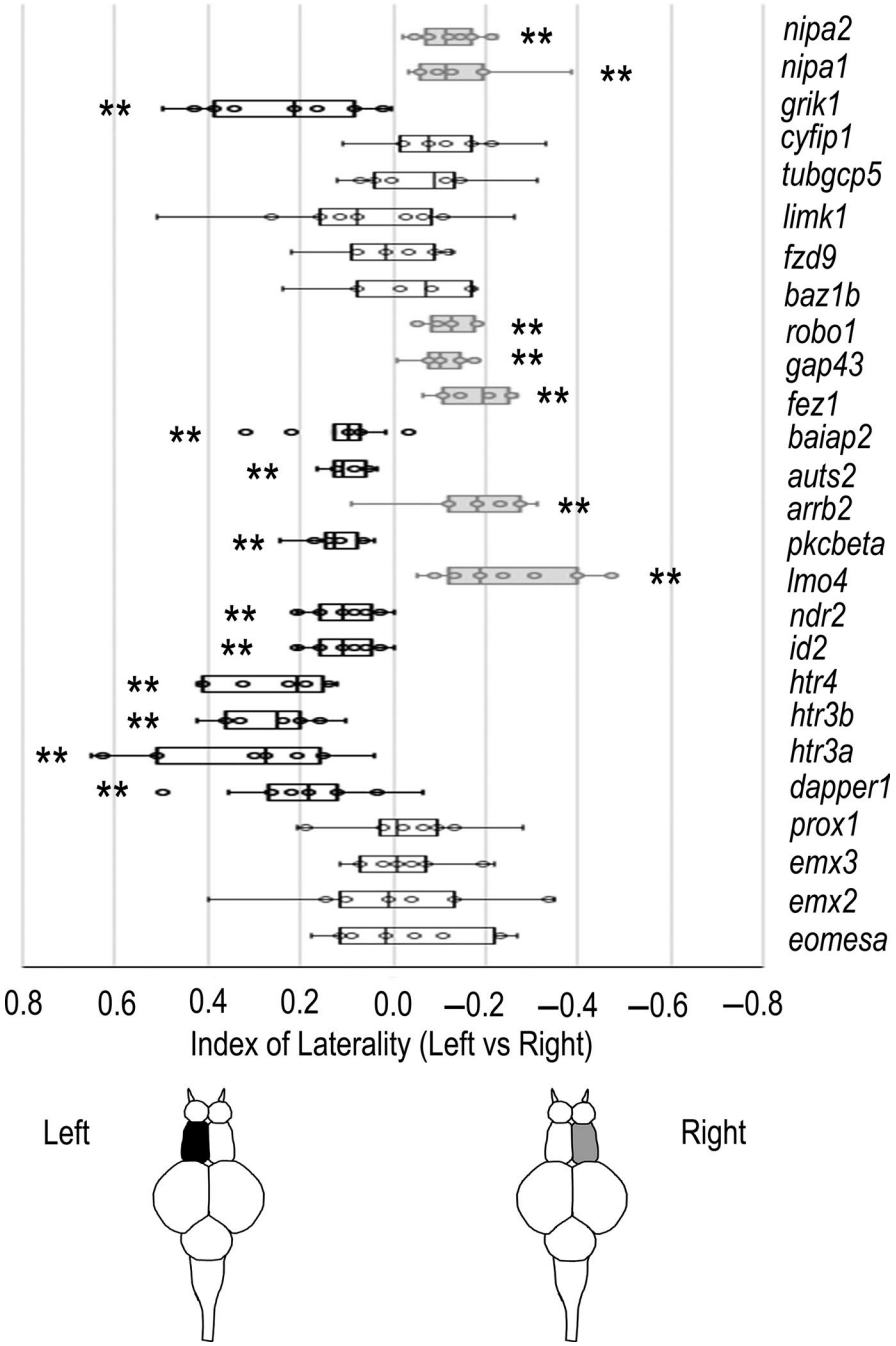

FIGURE 6 Laterality index for the genes tested in the left and right pallium of zebrafish (asterisks indicate significant departures from random distribution, $*p < .05$, $**p < .01$ two-tailed one-sample *t* tests)

Fukumoto et al., 2005). htr3 and htr4 receptors, but not other serotonin cascade members, guide brain laterality during early development of vertebrates, and the selective inhibition of these serotonin receptors leads to a randomization of brain laterality (Fukumoto et al., 2005). The conservative asymmetric distribution of serotonin receptors in different area of mammalian (Andersen & Teicher, 1999; Fink et al., 2009; Sun et al., 2005), chick and frog (Fukumoto et al., 2005) and fish brain could become a useful marker to study the molecular basis of the laterality of the vertebrate telencephalon. Moreover, since *htr4* receptor was linked to aggressive behaviour (Kudryavtseva et al., 2017) and aggressive behaviour appears to be lateralized in different species (Hews, Catsellano, & Hara, 2004; Hedayatirad, Nematollahi, Forsatkar, & Brown, 2017; review in Vallortigara, Rogers, & Bisazza, 1999), the magnitude of asymmetry in the distribution of this serotonin receptor could represent key index to monitor this behaviour.

We also observed a leftward asymmetrical distribution of *pkcβ* in the telencephalon of zebrafish, an asymmetry similar to that described in the mammalian amygdala (Orman & Stewart, 2007) and a rightward asymmetrical distribution of *lmo4* similar to that describe for the mammalian cortex (Huang et al., 2009; Li et al., 2013; Sun et al., 2005). In particular, *lmo4* represents one of the first genes described as differentially distributed in human and mouse cortex (Sun

et al., 2005) and seems to play a pivotal role in the definition of the shape of lateralized cortical functional areas during mouse brain development (Huang et al., 2009; Li et al., 2013). The asymmetrical distribution of *lmo4* in zebrafish brain could represent a new tool to study the molecular mechanism through which anatomical brain asymmetries could be linked to lateralized functions.

Since in the last years the zebrafish became one of the animal model mostly used to study the molecular bases of autism, we checked for the distribution of some of the autism-related markers in the left and right hemisphere. We found a striking correspondence with the results obtained in humans (Sun et al., 2005; Sun & Walsh, 2006), that is a leftward distribution of *auts2* and *baiap2* and a rightward distribution for *arrb2*, *fez, gap43* and *robo1*. Differences were apparent with respect to mouse results (Grabrucker et al., 2018), in which *auts2* and *baiap2* revealed only a trend for a left bias, while *arrb2*, *fez1* and *gap43* were lateralized to the left and not to the right as it happens in zebrafish (present work) and humans (Sun et al., 2005; Sun & Walsh, 2006). The asymmetric distribution of some autism-related genes in vertebrates, including fish, represents an important support to the hypothesis of an involvement of brain laterality in the occurrence of autism spectrum disorders.

Finally, given the current interest for the neural correlates of quantity estimation in zebrafish (Messina et al., 2020; Potrich et al., 2015, 2019), we also checked for the expression of eight genes associated with developmental dyscalculia in human. We found clear asymmetries in the expression of *grik1a*, *nipa1* and *nipa2* genes in the left and right pallium of zebrafish, which confirm a possible link between brain laterality and the presence of such a disability as it has been described in humans (Shalev et al., 1995).

Recently, *robo1* was also associated with developmental dyslexia and dyscalculia (Mascheretti et al., 2014; Tran et al., 2014). The asymmetric distribution of *robo1,* with a greater magnitude with respect to other tested genes, could be another support to the hypothesis of an involvement of lateralized molecular mechanism in the insurgence of these disabilities. *robo1* is a factor downstream *baz1b,* another dyscalculia-associated gene (Tassabehji, 2003; Zanella et al., 2019), but appears as symmetrically distributed in our analyses. The inconsistency between *robo1* and *baz1b* data in our results could be related to the high variability in the expression levels of *baz1b* in the tested samples or to a role of *baz1b* as chromatin remodeler. Further analyses will be needed to clarify *baz1b* distribution between the left and right hemisphere of zebrafish and its relationships with *robo1* expression.

In conclusion, our results provide further evidence of a conservative distribution of pallial genes among vertebrates, which extend also to their pattern of asymmetric expression in the left and right side of the brain.

ACKNOWLEDGEMENTS

This research was supported by grants from the European Research Council Grant (ERC AdG 833504-SPANUMBRA), and PRIN 2017 (2017PSRHPZ) to G.V.

CONFLICT OF INTERESTS

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

A.M. and G.V. conceived and designed the experiments. A.M., A.B. performed the experiments. A.M., A.B. and G.V. analysed and interpreted the data. G.V. contributed reagents/materials. All authors contributed to the manuscript writing.

DATA AVAILABILITY STATEMENT

Data are available in a submitted Supporting Information.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ejn.14914

ORCID

Andrea Messina https://orcid.org/0000-0001-7363-4301

REFERENCES

Andersen, S. L., & Teicher, M. H. (1999). Serotonin laterality in amygdala predicts performance in the elevated plus maze in rats. *NeuroReport*, *10*, 3497–3500. https://doi.org/10.1097/00001756-199911260-00006

Anitha, A., Nakamura, K., Yamada, K., Suda, S., Thanseem, I., Tsujii, M., … Norio Mori, N. (2008). Genetic analyses of roundabout (ROBO) axon guidance receptors in autism. *American Journal of Medical Genetics*, *147*, 1019–1027.

Barth, K. A., Miklosi, A., Watkins, J., Bianco, I. H., Wilson, S. W., & Andrew, R. J. (2005). fsi zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses. *Current Biology*, *15*, 844–850.

Bedogni, F., Hodge, R. D., Nelson, B. R., Frederick, E. A., Shiba, N., Daza, R. A., & Hevner, R. F. (2010). Autism susceptibility candidate 2 (Auts2) encodes a nuclear protein expressed in developing brain regions implicated in autism neuropathology. *Gene Expression Patterns*, *10*, 9. https://doi.org/10.1016/j.gep.2009.11.005

Brox, A., Puelles, L., Ferreiro, B., & Medina, L. (2004). Expression of the genes Emx1, Tbr1, and Eomes (Tbr2) in the telencephalon of xenopus laevis confirms the existence of a ventral pallial division in all tetrapods. *The Journal of Comparative Neurology*, *474*, 562–577.

Bulfone, A., Martinez, S., Marigo, V., Campanella, M., Basile, A., Quaderi, N., … Ballabio, A. (1999). Expression pattern of the Tbr2 (eomesodermin) gene during mouse and chick brain development. *Mechanisms of Development*, *84*, 133–138.

Carl, M., Bianco, I. H., Bajoghli, B., Aghaallaei, N., Czerny, T., & Wilson, S. W. (2007). Wnt/Axin1/beta-catenin signaling regulates asymmetric nodal activation, elaboration, and concordance of CNS asymmetries. *Neuron*, *55*, 393–405.

Chai, J. H., Locke, D. P., Greally, J. M., Knoll, J. H. M., Ohta, T., Dunai, J., … Nicholls, R. D. (2003). Identification of four highly

conserved genes between breakpoint hotspots BP1 and BP2 of the Prader-Willi/Angelman syndromes deletion region that have undergone evolutionary transposition mediated by flanking duplicons. *American Journal of Human Genetics*, *73*, 898–925. https://doi.org/10.1086/378816

Cohen, Y., Putrino, D., & Wilson, D. A. (2015). Dynamic cortical lateralization during olfactory discrimination learning. *Journal of Physiology*, *593*, 1701–1714.

Concha, M. L., Burdine, R. D., Russell, C., Schier, A. F., & Wilson, S. W. (2000). A nodal signaling pathway regulates the laterality of neuroanatomical asymmetries in the zebrafish forebrain. *Neuron*, *28*, 399–409. https://doi.org/10.1016/S0896-6273(00)00120-3

Concha, M. L., & Wilson, S. W. (2001). Asymmetry in the epithalamus of vertebrates. *Journal of Anatomy*, *199*, 63–84.

de Kovel, C. G. F., Lisgo, S. N., Fisher, S. E., & Francks, C. (2018). Subtle left-right asymmetry of gene expression profiles in embryonic and foetal human brains. *Scientific Reports*, *8*, 12606.

Docherty, S. J., Yulia Kovas, Y., Petrill, S. A., & Plomin, R. (2010). Generalist genes analysis of DNA markers associated with mathematical ability and disability reveals shared influence across ages and abilities. *BMC Genetics*, *11*(61), 1–10. https://doi.org/10.1186/1471-2156-11-61

Dreosti, E., Vendrell Llopis, N., Carl, M., Yaksi, E., & Wilson, S. W. (2014). Left- right asymmetry is required for the habenulae to respond to both visual and olfactory stimuli. *Current Biology*, *24*, 440–445.

Duboc, V., Dufourcq, P., Blader, P., & Roussigné, M. (2015). Asymmetry of the brain: Development and implications. *Annual Review of Genetics*, *49*, 647–672.

Essner, J. J., Amack, J. D., Nyholm, M. K., Harris, E. B., & Yost, H. J. (2005). Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. *Development*, *132*, 1247–1260. https://doi.org/10.1242/dev.01663

Fink, M., Wadsak, W., Savli, M., Stein, P., Moser, U., Hahn, A., … Lanzenberger, R. (2009). Lateralization of the serotonin-1A receptor distribution in language areas revealed by PET. *NeuroImage*, *45*, 598–605. https://doi.org/10.1016/j.neuroimage.2008.11.033

Fukumoto, T., Keme, I. P., & Levin, M. (2005). Serotonin signaling is a very early step in patterning of the left-right axis in chick and frog embryos. *Current Biology*, *15*, 794–803.

Gamse, J. T., Kuan, Y. S., Macurak, M., Brösamle, C., Thisse, B., Thisse, C., & Halpern, M. E. (2005). Directional asymmetry of the zebrafish epithalamus guides dorsoventral innervation of the midbrain target. *Development*, *132*, 4869–4881. https://doi.org/10.1242/dev.02046

Gamse, J. T., Thisse, C., Thisse, B., & Halpern, M. H. (2003). The parapineal mediates left-right asymmetry in the zebrafish diencephalon. *Development*, *130*, 1059–1068. https://doi.org/10.1242/dev.00270

Ganz, J., Kroehne, V., Freudenreich, D., Machate, A., Geffarth, M., Braasch, I., … Brand, M. (2011). Subdivisions of the adult zebrafish subpallium by molecular marker analysis. *The Journal of Comparative Neurology*, *3*, 308.

Ganz, J., Kroehne, V., Freudenreich, D., Machate, A., Geffarth, M., Braasch, I., … Brand, M. (2014). Subdivisions of the adult zebrafish pallium based on molecular marker analysis. *The Journal of Comparative Neurology*, *3*, 308.

Ghirlanda, S., Frasnelli, E., & Vallortigara, G. (2009). Intraspecific competition and coordination in the evolution of lateralization. *Philosophical Transactions of the Royal Society of London*, *364*, 861–866.

Ghirlanda, S., & Vallortigara, G. (2004). The evolution of brain lateralization: A game theoretical analysis of population structure. *Proceedings of the Royal Society of London*, *271*, 853–857.

Grabrucker, S., Haderspeck, J. C., Sauer, A. K., Kittelberger, N., Asoglu, H., Abaei, A., … Grabrucker, A. M. (2018). Brain lateralization in mice is associated with zinc signaling and altered in prenatal zinc deficient mice that display features of autism spectrum disorder. *Frontiers in Molecular Neuroscience*, *10*, 450. https://doi.org/10.3389/fnmol.2017.00450

Guarneri, P., Guarneri, R., La Bella, V., Scondotto, S., Scoppa, F., & Piccoli, F. (1988). Lateral differences in GABA binding sites in rat brain. *Neurochemical Research*, *13*, 209–211.

Güntürkün, O., & Ocklenbrug, S. (2017). Ontogenesis of lateralization. *Neuron*, *94*, 249–263. https://doi.org/10.1016/j.neuron.2017.02.045

Hashimoto, M., & Hibi, M. (2012). Development and evolution of cerebellar neural circuits. *Development, Growth & Differentiation*, *54*, 373–389.

Hedayatirad, M., Nematollahi, M. A., Forsatkar, M. N., & Brown, C. (2017). Prozac impacts lateralization of aggression in male Siamese fighting fish. *Ecotoxicology and Environmental Safety*, *140*, 84–88. https://doi.org/10.1016/j.ecoenv.2017.02.027

Herget, U., Wolf, A., Wullimann, M. F., & Ryu, S. (2014). Molecular neuroanatomy and chemoarchitecture of the neurosecretory preoptic-hypothalamic area in zebrafish larvae. *The Journal of Comparative Neurology*, *522*, 1542–1564.

Hews, D. K., Catsellano, M., & Hara, E. (2004). Aggression in females is also lateralized: Left-eye bias during aggressive courtship rejection in lizards. *Animal Behavior*, *5*, 1201–1207.

Huang, Z., KAwase-Koga, Y., Zhang, S., Visvader, J., Toth, M., Walsh, C. A., & Sun, T. (2009). Transcription factor Lmo4 defines the shape of functional areas in developing cortices and regulates sensorimotor control. *Developmental Biology*, *327*, 132–142.

Hüsken, U., & Carl, M. (2013). The Wnt/beta-catenin signaling pathway establishes neuroanatomical asymmetries and their laterality. *Mechanisms of Development*, *130*, 330–335.

Ji, G., & Neugebauer, V. (2009). Hemispheric lateralization of pain processing by amygdala neurons. *Journal of Neurophysiology*, *102*(4), 2253–2264. https://doi.org/10.1152/jn.00166.2009

Kinkhabwala, A., Riley, M., Koyama, M., Monen, J., Satou, C., Kimura, Y., … Fetcho, J. (2011). A structural and functional ground plan for neurons in the hindbrain of zebrafish. *Proceedings of the National Academy of Sciences*, *108*, 1164–1169.

Kudryavtseva, N. N., Smagin, D. A., Kovalenko, I. L., Galyamina, A. G., Vishnivetskaya, G. B., Babenko, V. N., & Orlov, Y. L. (2017). Serotoninergic genes in the development of anxiety/depression-like state and pathology of aggressive behavior in male mace: RNA-seq data. *Molecular Biology*, *51*, 251–262.

Larisch, R., & Klimke, A. (1988). Clinical impact of cerebral dopamine-D2 receptor scintigraphy. *Nuklearmedizin*, *37*, 245–250.

Lee, H. J., Schneider, R. F., Manousaki, T., Kang, J. H., Lein, E., Franchini, P., & Meyer, A. (2017). Lateralized feeding behavior is associated with asymmetrical neuroanatomy and lateralized gene expressions in the brain in scale-eating cichlid fish. *Genome Biology and Evolution*, *9*, 3122–3136. https://doi.org/10.1093/gbe/evx218

Li, Q., Bian, S., Liu, B., Hong, J., Toth, M., & Sun, T. (2013). Establishing brain functional laterality in adult mice through

unilateral gene manipulation in the embryonic cortex. *Cell Research*, *23*, 1147–1149.

Liang, J. O., Etheridge, A., Hantsoo, L., Rubinstein, A. L., Nowak, S. J., Izpisúa Belmonte, J. C., & Halpern, M. E. (2000). Asymmetric nodal signaling in the zebrafish diencephalon positions the pineal organ. *Development*, *127*, 5101–5112.

MacNeilage, P. F., Rogers, L. J., & Vallortigara, G. (2009). Origins of left & right brain. *Scientific American*, *301*, 60–67.

Maschereti, S., Riva, V., Giorda, R., Beri, S., Francesca, L., Lanzoni, E., … Marino, C. (2014). KIAA0319 and ROBO1: Evidence on association with reading and pleiotropic effect in language and mathematics abilities n developmental dyslexia. *Journal of Human Genetics*, *59*, 189–197.

Maver, A., Čuturilo, G., Kovanda, A., Miletić, A., & Peterlina, B. (2019). Rare missense TUBGCP5 gene variant in a patient with primary microcephaly. *European Journal of Medical Genetics*, *62*(12), 103598. https://doi.org/10.1016/j.ejmg.2018.12.003

Messina, A., Potrich, D., Schiona, I., Sovrano, V. A., Fraser, S. E., Brennan, C. H., & Vallortigara, G. (2020). Response to change in the number of visual stimuli in zebrafish: A behavioural and molecular study. *Scientific Reports*, *10*, 1.

Miletto Petrazzini, M. E., Sovrano, V. A., Vallortigara, G., & Messina, A. (2020). Brain and behavioral asymmetry: A lesson from fish. *Frontiers in Neuroanatomy*, *14*, 11.

Milne, R. D., Syngeniotis, A., Jackson, G., & Corballis, M. C. (2002). Mixed lateralization of phonological assembly in developmental dyslexia. *Neurocase*, *8*, 205–209. https://doi.org/10.1093/neucas/8.3.205

Moskal, J. R., Kroes, R. A., Otto, N. J., Rahimi, O., & Claiborne, B. J. (2006). Distinct patterns of gene expression in the left and right hippocampal formation of developing rats. *Hippocampus*, *16*, 629–634. https://doi.org/10.1002/hipo.20198

Mueller, T. (2012). What is the thalamus in zebrafish? *Frontiers in Neuroscience*, *6*, 64.

Nieder, A. (2019). *A brain for numbers: The biology of the number instinct*. Cambridge, MA, London, UK: The MIT Press.

Orman, R., & Stewart, M. (2007). Hemispheric differences in protein kinase C betaII levels in the rat amygdala: Baseline asymmetry and lateralized changes associated with cue and context in a classical fear conditioning paradigm. *Neuroscience*, *144*, 797–807.

Panula, P., Chen, Y. C., Priyadarshini, M., Kudo, H., Semenova, S., Sundvik, M., & Sallinen, V. (2010). The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. *Neurobiology of Diseases*, *40*, 46–57.

Paquet, A., Golse, B., Girard, M., Olliac, B., & Vaivre-Douret, L. (2017). Laterality and lateralization in autism spectrum disorder, using a standardized neuro- psychomotor assessment. *Developmental Neuropsychology*, *42*, 39–54.

Potrich, D., Rugani, R., Sovrano, V. A., Regolin, L., & Vallortigara, G. (2019). Use of numerical and spatial information in ordinal counting by zebrafish. *Scientific Reports*, *9*, 18323.

Potrich, D., Sovrano, V. A., Stancher, G., & Vallortigara, G. (2015). Quantity discrimination by zebrafish (*Danio rerio*). *Journal of Comparative Psychology*, *129*, 88–93.

Raya, A., Kawakami, Y., Rodriguez-Esteban, C., Buscher, D., Koth, C. M., Itoh, T., … Belmonte, J. C. (2003). Notch activity induces Nodal expression and mediates the establishment of left-right asymmetry in vertebrate embryos. *Genes & Development*, *17*, 1213–1218.

Regan, J. C., Concha, M. L., Roussigne, M., Russell, C., & Wilson, S. W. (2009). An Fgf8-dependent bistable cell migratory event establishes CNS asymmetry. *Neuron*, *61*, 27–34. https://doi.org/10.1016/j.neuron.2008.11.030

Ribasés, M., Bosch, R., Hervás, A., Ramos-Quiroga, J. A., Sánchez-Mora, C., Bielsa, A., … Cormand, B. (2009). Case-control study of six genes asymmetrically expressed in the two cerebral hemispheres: Association of BAIAP2 with attention-deficit/hyperactivity disorder. *Biological Psychiatry*, *66*, 926–934. https://doi.org/10.1016/j.biopsych.2009.06.024

Rogers, L. J., Rigosi, E., Frasnelli, E., & Vallortigara, G. (2013). A right antenna for social behaviour in honeybees. *Scientific Reports*, *3*, 2045.

Rogers, L., & Vallortigara, G. (2015). When and why did brains break symmetry? *Symmetry*, *7*, 2181. https://doi.org/10.3390/sym7042181

Rogers, L. J., Vallortigara, G., & Andrew, R. J. (2013). *Divided brains: The biology and behaviour of brain asymmetries*, Cambridge, United Kingdom: Cambridge University Press. https://www.cambridge.org/it/academic/subjects/life-sciences/animal-behaviour/divided-brains-biology-and-behaviour-brain-asymmetries?format=HB&isbn=9781107005358

Rogers, L. J., Zucca, P., & Vallortigara, G. (2004). Advantages of having a lateralized brain. *Proceedings of the Royal Society of London*, *271*, 420–422.

Samara, A., Vougas, K., Papadopoulou, A., Anastasiadou, E., Baloyanni, N., Paronis, E., … Tsangaris, G. T. (2011). Proteomics reveal rat hippocampal lateral asymmetry. *Hippocampus*, *21*, 108–119. https://doi.org/10.1002/hipo.20727

Schweitzer, J., & Driever, W. (2009). Development of the dopamine systems in zebrafish. *Advances in Experimental Medicine and Biology*, *651*, 1–14.

Shalev, R. S. (2004). Developmental dyscalculia. *Journal of Child Neurology*, *19*, 765–771.

Shalev, R. S., Manor, O., Amir, N., Wertman-Eiad, R., & Gross-Tsur, V. (1995). Developmental dyscalculia and brain laterality. *Cortex*, *31*, 357–365. https://doi.org/10.1016/S0010-9452(13)80368-1

Sun, T., Patoine, C., Abu-Khalil, A., Visvader, J., Sum, E., Cherry, T. J., … Walsh, C. A. (2005). Early asymmetry of gene transcription between embryonic human left and right cerebral cortex. *Science*, *308*, 1794–1798.

Sun, T., & Walsh, C. A. (2006). Molecular approaches to brain asymmetry and handedness. *Nature Reviews Neuroscience*, *7*, 655–662.

Takeuchi, Y., Ishikava, A., Oda, Y., & Kitano, J. (2018). Lateralized expression of left-right axis formation genes is shared by adult T brains of lefty and righty scale-eating cichlids. *Comparative Biochemistry and Physiology*, *28*, 99–106.

Tassabehji, M. (2003). Williams-Beuren syndrome: A challenge for genotype-phenotype correlations. *Human Molecular Genetics*, *12*, 229–237.

Toma, C., Hervas, A., Balmana, N., Viella, E., Aguilera, F., Cusco, I., … Bayes, M. (2011). Association study of six candidate genes asymmetrically expressed in the two cerebral hemispheres suggests the involvement of BAIAP2 in autism. *Journal of Psychiatric Research*, *45*, 280–282. https://doi.org/10.1016/j.jpsychires.2010.09.001

Tran, C., Wigg, K. G., Cate-Carter, T. D., Kerr, E., Field, L. L., Kaplan, B. J., Lovett, M. W., & Barr, C. L. (2014). Association of the ROBO1 gene with reading disabilities in a family-based analysis. *Genes Brain Behav.*, *13*, 430–438. https://doi.org/10.1111/gbb.12126.

Vallortigara, G. (2006). The evolutionary psychology of left and right: Costs and benefits of lateralization. *Developmental Psychobiology*, *48*, 418–427.

Vallortigara, G., & Rogers, L. J. (2020). A function for the bicameral mind. *Cortex*, *124*, 274–285. https://doi.org/10.1016/j.cortex.2019.11.018

Vallortigara, G., Rogers, L. J., & Bisazza, A. (1999). Possible evolutionary origins of cognitive brain lateralization. *Brain Research Reviews*, *30*, 164–175. https://doi.org/10.1016/S0165-0173(99)00012-0

Vallortigara, G., & Versace, E. (2017). Laterality at the neural, cognitive, and behavioral levels. In J. Call (Ed.), *APA handbook of comparative psychology: Vol. 1. Basic Concepts, methods, neural substrate, and behavior* (pp. 557–577). Washington, DC: American Psychological Association.

Wullimann, M. F. (1994). The teleostean torus longitudinalis: A short review on its structure, histochemistry, connectivity, possible function and phylogeny. *European Journal of Morphology*, *32*, 235–242.

Wullimann, M. F., Thomas Mueller, T., Distel, M., Babaryka, A., Grothe, B., & Köster, R. W. (2011). The long adventurous journey of rhombic lip cells in jawed vertebrates: A comparative developmental analysis. *Frontiers in Neuroanatomy*, *5*, 27. https://doi.org/10.3389/fnana.2011.00027

Wurst, W., & Bally-Cuif, L. (2001). Neural plate patterning: Upstream and downstream of the isthmic organizer. *Nature Reviews Neuroscience*, *2*, 99–108.

Zanella, M., Vitriolo, A., Abdirko, A., Martin, P. T., Sturm, A., O'Rourke, T., … Testa, G. (2019). Dosage analysis of the 7q11.23 Wialliam region identifies BAZ1B as a major human gene patterning the modern human face and underlying self-domestication. *Science Advances*, *5*(12), 1–16.

Zhang, L., Gao, X., Wen, J., Ning, Y., & Chen, Y. G. (2006). Dapper 1 antagonize Wnt signaling by promoting dishevelled degradation. *Journal of Biological Chemistry*, *281*, 8607–8612.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Messina A, Boiti A, Vallortigara G. Asymmetric distribution of pallial-expressed genes in zebrafish (*Danio rerio*). *Eur J Neurosci*. 2021;53:362–375. https://doi.org/10.1111/ejn.14914