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Analysis of *ARMC5* expression in human tissues

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Abstract

Mutations in *ARMC5* gene have been recently identified as the main cause of Primary Macronodular Adrenocortical Hyperplasia (PMAH). PMAH patients have an *ARMC5* germline mutation and, in addition, somatic tissue-specific mutations. This is consistent with the two-hit hypothesis of tumorigenesis and suggests that *ARMC5* may be a tumor suppressor gene. As its function is still unclear, we analyzed the expression of the four *ARMC5* isoforms in 46 normal human tissues. This showed that at least one *ARMC5* isoform is ubiquitously expressed throughout the body; however, only 7 tissues expressed all isoforms, including the adrenal gland and the brain. Interestingly, the highest expression for ARMC5 in the brain is the pituitary gland. The isoform ARMC5-003 was present in most endocrine tissues including the pituitary, adrenal glands and the pancreas. In this report, we present new data about *ARMC5* expression pattern in human tissues; its wide expression in brain, pituitary gland and other tissues suggest that mutations may be responsible for additional pathologies, beyond what is already known in PMAH and meningiomas.

Keywords

ARMC5; Adrenal; Primary Macronodular Adrenal Hyperplasia; Isoform; expression

1. Introduction

The *ARMC5* gene is mutated in almost 50% of patients with primary macronodular adrenocortical hyperplasia (PMAH) (Alencar, Lerario, Nishi et al., 2014,Assie, Libe,

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Espiard et al., 2013,Drougat, Omeiri, Lefevre et al., 2015,Duan, Gomez Hernandez and Mete, 2014,Faucz, Zilbermint, Lodish et al., 2014,Gagliardi, Schreiber, Hahn et al., 2014,Espiard, Drougat, Libe et al., 2015). These mutations are found at the germline and somatic levels consistent with the two-hit hypothesis of tumorigenesis. Functional studies suggested that *ARMC5* may be functioning as a tumor suppressor gene. In 2014, a family with a germline inactivating *ARMC5* mutation and PMAH was described in which there was also a meningioma and neuroendocrine tumor (NET); a somatic frameshift *ARMC5* mutation (p.R502fs) was found in the meningioma but not in the NET (Elbelt, Trovato, Kloth et al., 2015). Additional patients with meningiomas and *ARMC5* mutations have since been described; this discovery suggested that *ARMC5* defects may have a pathogenic role in other tissues or diseases.

Most of *ARMC5* mutations identified are frameshift (21/54, 38%) and/or nonsense (12/54, 22%) leading to a clear loss of function (Espiard and Bertherat, 2015). Missense variants, which represent approximately 31% of the identified variants (Drougat et al., 2015), are harder to classify as pathogenic because *ARMC5*'s function remains unclear. Assie *et al.* demonstrated *in vitro* that some of the missense variants identified in patients fail to induce apoptosis after transfection in a human adrenocortical cancer cell line (H295R), suggesting that these variants affected *ARMC5*'s pro-apoptotic function (Assie et al., 2013). However, if and how the inhibition of apoptosis resulting from biallelic inactivation of *ARMC5* leads to hyperplasia remains unknown.

In the existing literature, all *ARMC5* mutations have been analyzed against ARMC5-201 (NM_001105247), one of the four *ARMC5* isoforms described in the *Ensembl* database (http://www.ensembl.org/index.html). However, ARMC5-201 is not the longest isoform of this gene. Two additional coding exons at the 5' end of the gene that are present only in the ARMC5-001 transcript (NM_001288767) are, thus, often ignored. In this paper, we determine the respective expression of the four known *ARMC5* isoforms in a set of 46 human normal tissues including the adrenal gland. This information is useful for the functional interpretation of *ARMC5* defects; the broad expression of *ARMC5* in various tissues suggests that *ARMC5* may have additional functions in both physiology and perhaps pathology in a number of other tissues.

2. Materials and methods

2.1 Alignment

ARMC5 transcripts and proteins sequences have been obtained via *Ensembl* database (http://www.ensembl.org/index.html) (Yates, Akanni, Amode et al., 2016). Although *ARMC5* transcripts sequences were aligned using Clustal Omega (Sievers, Wilm, Dineen et al., 2011,Goujon, McWilliam, Li et al., 2010), the alignment of ARMC5 protein sequences and the phylogenic tree were generated using Geneious version 4.8 (Kearse, Moir, Wilson et al., 2012).

2.2. Reverse Transcription quantitative real-time PCR (RTqPCR)

RTqPCR for *ARMC5* expression was performed using SYBR Green (4309155, Applied biosystems) on cDNA from 46 human normal tissues using the Tissue Scan qPCR assays (HMRT503, Origene). The different brain tissues used for RTqPCR were also obtained from Origene (HBRT301). The sequence of primers used to discriminate *ARMC5* isoforms are provided in Table 2. For relative quantification, relative expression of *ARMC5* isoforms was calculated using $\Delta\Delta C_T$ method. All measurements were normalized to the ARMC5/beta actin ratio.

2.4. Immunohistochemistry

Paraffin-embedded normal human brain slides were provided by Alpha diagnostic international (HTS-10301). The slides were deparaffinized in Histoclear (HS-202, National diagnostics) and rehydrated through ethanol gradient. After the epitope retrieval in Vector Antigen Retrieval Solution (H3300, Vector Labs) at 95°C, ARMC5 antibody (NBP1-94024, Novus, USA) diluted 1/50 was incubated overnight at 4°C. The primary antibody was detected using an anti-rabbit antibody coupled to biotin (111-065-144, Jackson ImmunoResearch Laboratory) following by a streptavidin-HRP amplification (016-030-084, Jackson ImmunoResearch Laboratory). The HRP activity was detected with 3,3'-diaminobenzidine tetrahydrochloride (DAB) (SK-4105, Vector Labs). The slides were counterstained with hematoxylin (K8008, Dako).

3. Results

3.1 Structure of the *ARMC5* gene

ARMC5 gene orthologs are found both in vertebrates and invertebrates and their sequence identity can vary from 30 to 90% at protein level, with the highest conservation among mammals (Figure 1). Even though there is a large variability in the protein sequence conservation between species, it is important to notice that both the *Armadillo* and the BTB domain are conserved. Therefore, the structure of *ARMC5* gene is well conserved throughout evolution as homologous genes are found for instance, in fish. In humans, *ARMC5* gene (NM_001288767) consists of 8 exons. Eight transcripts resulting from alternative splicing are reported in Ensembl database but only 6 of them are predicted to encode for protein. Among these 6 isoforms, four have been confirmed in at least 4 different sources: European Bioinformatics Institute (EBI), National Center for Biotechnology Information (NCBI), the Welcome Trust Sanger Institute (WTSI) and the University of California at Santa Cruz (UCSC) and have then a Consensus CDS (CCDS) number. In this paper, we focus our attention on these four isoforms and to avoid any confusion, we are using the *Ensembl* nomenclature, ARMC5-001 (NM_001288767), ARMC5-002 (NM_024742), ARMC5-003 (NM_001301820) and ARMC5-201 (NM_001105247) (Figure 2). The isoform ARMC5-001 that is the longest isoform (3626bp, 1030aa) is divided in 8 exons (Figure 2a). The exons 3 to 6 coding for the Armadillo domains are conserved in all the isoforms. Noteworthy, the last exon of ARMC5-002 is longer than the corresponding exon in the other isoform. The transcripts varied mostly from their 5' and 3' UTR. The second protein-protein interaction domain identified in ARMC5, the BTB (Broad-Complex, Tramtrack and Bric a brac)/POZ (Poxvirus and Zinc finger) is located at the carboxyl-

terminal end of the protein. This domain is conserved in all the isoforms except ARMC5-002 (Figure 2b). In conclusion, only ARMC5-001, ARMC5-003 and ARMC5-201 are quite similar at the protein level regarding their domain organization.

3.2. *ARMC5* isoforms expression

Unfortunately, the high similarity between ARMC5-201 and the other isoforms does not allow us to design primers specific for that isoform (Supplementary Figure 1). Therefore, we are using one pair of primers recognizing both ARMC5-201 and ARMC5-002 and a second pair of primers targeting specifically ARMC5-002.

We analyzed *ARMC5* expression by RTqPCR in 46 human normal tissues. The cDNAs used for this study came from a pool of samples including both genders and different ages. The diversity of the samples analyzed has to be taken in consideration for the analysis. Although *ARMC5* is ubiquitously expressed in the set of tissues analyzed, the expression level is highly heterogeneous. *ARMC5* expression is higher in the thyroid, the ureter, the brain, the tonsil, the spinal cord, the adipose tissue, the tongue, the lung and the lymphocyte than in the adrenal gland (Figure 2c). Whereas the isoform ARMC5-002 is expressed ubiquitously (Table 2), the isoform ARMC5-003 and ARMC5-001 show more specific expression pattern as they are expressed in only 16 and 18 tissues respectively (Table 2). Only 7 out of 46 tissues expressed all 4 *ARMC5* isoforms. These tissues were thymus, pancreas, the adrenal gland, adipose tissue, trachea and lung.

3.3. *ARMC5* expression in brain

The brain was one of the sites with the highest expression of *ARMC5* in our analysis and it was also one of the tissues that expressed all 4 isoforms. Therefore, we analyzed *ARMC5* expression in 24 tissues originating from the brain using primers targeting all the isoforms (Figure 3a). Interestingly, *ARMC5* was highly expressed in the pituitary gland.

The expression of *ARMC5* in the brain was specific to some other tissues in addition to the pituitary, such as substantia nigra, choroid plexus, and the cerebellum (Figure 3a). Similarly, ARMC5 is only expressed in a subset of cells in the cerebellum as demonstrated by imunohistochemistry (Figure 3b), confirming the data available on the protein databases, where ARMC5 is reported as being highly expressed in glial and neuronal cells (Uhlen, Fagerberg, Hallstrom et al., 2015).

4. Discussion

ARMC5 is a tumor suppressor gene involved in the development of adrenal hyperplasia but its expression is not limited to the adrenal gland. Although the four *ARMC5* isoforms have a different pattern of expression, *ARMC5* is generally ubiquitously expressed. We showed that the isoform ARMC5-201, which is used as a reference sequence for the functional analysis of the missense variants identified in our patients, is expressed in all the studied tissues. However, in the adrenal gland, all four *ARMC5* isoforms are expressed suggesting that the sequencing of the first two exons of ARMC5-001 may be useful to search for somatic mutations where previously none has been identified. Indeed, no somatic *ARMC5* mutations have been identified in tissues from patients with PMAH in a number of publications to date

(Faucz, Zilbermint, Lodish et al., 2014). These patients could, in theory, carry somatic mutations in the coding sequence of the ARMC5-001 isoform.

Interestingly, the only *ARMC5* isoform that is clearly expressed ubiquitously is the one without a BTB domain, ARMC5-002, suggesting that the BTB interaction may be part of an organ-specific function of *ARMC5*. We can then hypothesize that the presence of the BTB domain may change the interacting partners leading to ARMC5 involvement in different signaling pathways in the sites where the ARMC5-002 is expressed.

On the other hand, ARMC5-003 is expressed mostly in endocrine tissues. Among the 16 tissues expressing this isoform, 9 tissues are either endocrines (pancreas, pituitary, adrenal, placental, testis, prostate) or they produce hormones (thymus, stomach, adipose tissue). Therefore, the regulatory region controlling ARMC5-003 expression may be specifically regulated by the endocrine system.

In conclusion, we present *ARMC5*'s phylogeny, transcript isoforms, and pattern of expression. This information is useful for future studies of *ARMC5*'s genetic screening and function. The data suggest that ARMC5 may have a role in the pathogenesis of diseases involving other tissues beyond the adrenal gland.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Alencar GA, Lerario AM, Nishi MY, Mariani BM, Almeida MQ, Tremblay J, Hamet P, Bourdeau I, Zerbini MC, Pereira MA, Gomes GC, De Souza Rocha M, Chambo JL, Lacroix A, Mendonca BB, Fragoso MC. ARMC5 Mutations are a Frequent Cause of Primary Macronodular Adrenal Hyperplasia. The Journal of clinical endocrinology and metabolism. 2014:jc20134237.
2. Assie G, Libe R, Espiard S, Rizk-Rabin M, Guimier A, Luscap W, Barreau O, Lefevre L, Sibony M, Guignat L, Rodriguez S, Perlemoine K, Rene-Corail F, Letourneur F, Trabulsi B, Poussier A, Chabbert-Buffet N, Borson-Chazot F, Groussin L, Bertagna X, Stratakis CA, Ragazzon B, Bertherat J. ARMC5 mutations in macronodular adrenal hyperplasia with Cushing's syndrome. The New England journal of medicine. 2013; 369:2105–2114. [PubMed: 24283224]
3. Drougat L, Omeiri H, Lefevre L, Ragazzon B. Novel Insights into the Genetics and Pathophysiology of Adrenocortical Tumors. Frontiers in endocrinology. 2015; 6:96. [PubMed: 26106367]
4. Duan K, Gomez Hernandez K, Mete O. Clinicopathological correlates of adrenal Cushing's syndrome. Journal of clinical pathology. 2014
5. Faucz FR, Zilbermint M, Lodish MB, Szarek E, Trivellin G, Sinaii N, Berthon A, Libe R, Assie G, Espiard S, Drougat L, Ragazzon B, Bertherat J, Stratakis CA. Macronodular Adrenal Hyperplasia due to Mutations in an Armadillo Repeat Containing 5 (ARMC5) Gene: A Clinical and Genetic Investigation. The Journal of clinical endocrinology and metabolism. 2014; 99:E1113–E1119. [PubMed: 24601692]

6. Gagliardi L, Schreiber AW, Hahn CN, Feng J, Cranston T, Boon H, Hotu C, Oftedal BE, Cutfield R, Adelson DL, Braund WJ, Gordon RD, Rees DA, Grossman AB, Torpy DJ, Scott HS. Armc5 Mutations Are Common in Familial Bilateral Macronodular Adrenal Hyperplasia. The Journal of clinical endocrinology and metabolism. 2014:jc20141265.
7. Espiard S, Drougat L, Libe R, Assie G, Perlemoine K, Guignat L, Barrande G, Brucker-Davis F, Doullay F, Lopez S, Sonnet E, Torremocha F, Pinsard D, Chabbert-Buffet N, Raffin-Sanson ML, Groussin L, Borson-Chazot F, Coste J, Bertagna X, Stratakis CA, Beuschlein F, Ragazzon B, Bertherat J. ARMC5 Mutations in a Large Cohort of Primary Macronodular Adrenal Hyperplasia: Clinical and Functional Consequences. The Journal of clinical endocrinology and metabolism. 2015; 100:E926–E935. [PubMed: 25853793]
8. Elbelt U, Trovato A, Kloth M, Gentz E, Finke R, Spranger J, Galas D, Weber S, Wolf C, Konig K, Arlt W, Buttner R, May P, Allolio B, Schneider JG. Molecular and clinical evidence for an ARMC5 tumor syndrome: concurrent inactivating germline and somatic mutations are associated with both primary macronodular adrenal hyperplasia and meningioma. The Journal of clinical endocrinology and metabolism. 2015; 100:E119–E128. [PubMed: 25279498]
9. Yates A, Akanni W, Amode MR, Barrell D, Billis K, Carvalho-Silva D, Cummins C, Clapham P, Fitzgerald S, Gil L, Giron CG, Gordon L, Hourlier T, Hunt SE, Janacek SH, Johnson N, Juettemann T, Keenan S, Lavidas I, Martin FJ, Maurel T, McLaren W, Murphy DN, Nag R, Nuhn M, Parker A, Patricio M, Pignatelli M, Rahtz M, Riat HS, Sheppard D, Taylor K, Thormann A, Vullo A, Wilder SP, Zadissa A, Birney E, Harrow J, Muffato M, Perry E, Ruffier M, Spudich G, Trevanion SJ, Cunningham F, Aken BL, Zerbino DR, Flicek P. Ensembl 2016. Nucleic acids research. 2016; 44:D710–D716. [PubMed: 26687719]
10. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular systems biology. 2011; 7:539. [PubMed: 21988835]
11. Goujon M, McWilliam H, Li W, Valentin F, Squizzato S, Paern J, Lopez R. A new bioinformatics analysis tools framework at EMBL-EBI. Nucleic acids research. 2010; 38:W695–W699. [PubMed: 20439314]
12. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28:1647–1649. [PubMed: 22543367]
13. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Ponten F. Proteomics. Tissue-based map of the human proteome. Science. 2015; 347:1260419. [PubMed: 25613900]
14. Faucz FR, Zilbermint M, Lodish MB, Szarek E, Trivellin G, Sinaii N, Berthon A, Libe R, Assie G, Espiard S, Drougat L, Ragazzon B, Bertherat J, Stratakis CA. Macronodular adrenal hyperplasia due to mutations in an armadillo repeat containing 5 (ARMC5) gene: a clinical and genetic investigation. The Journal of clinical endocrinology and metabolism. 2014; 99:E1113–E1119. [PubMed: 24601692]

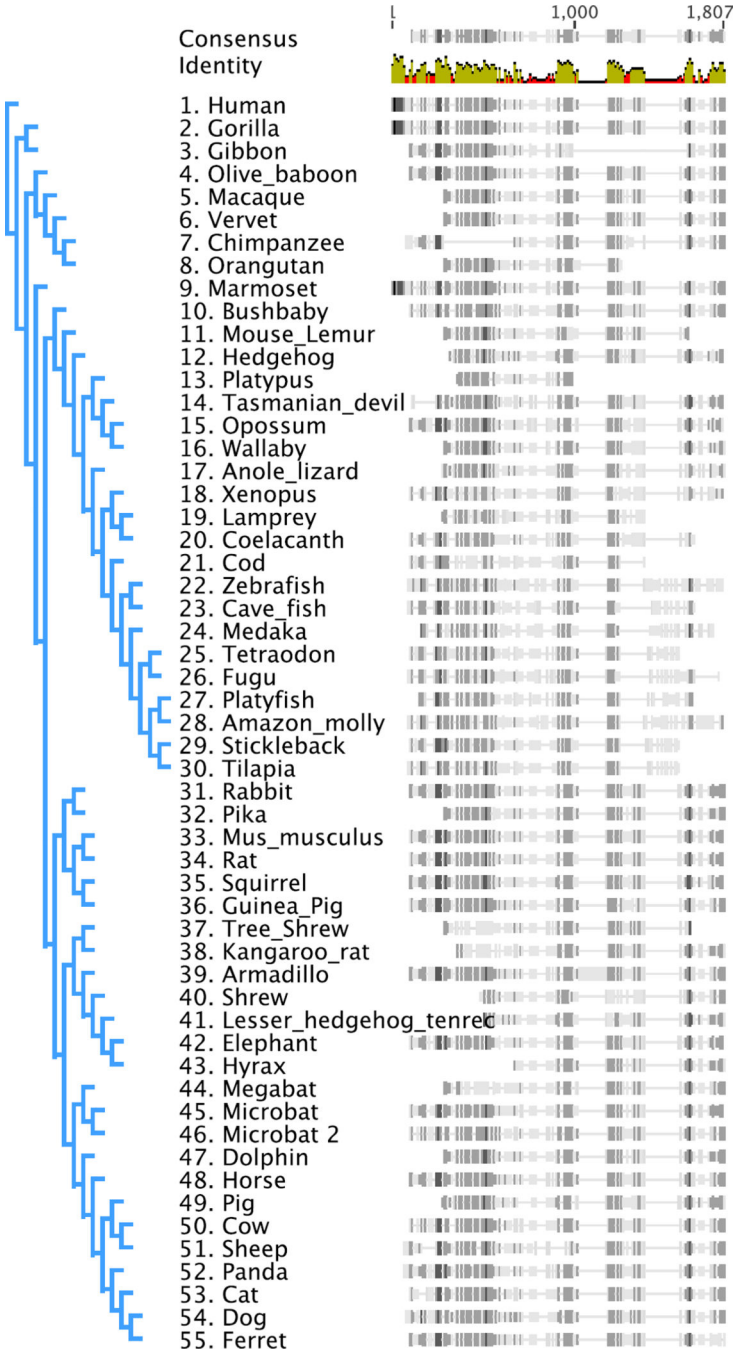

Figure 1. ARMC5 phylogeny

ARMC5 proteins sequences from 55 different species were classified by phylogenic tree.

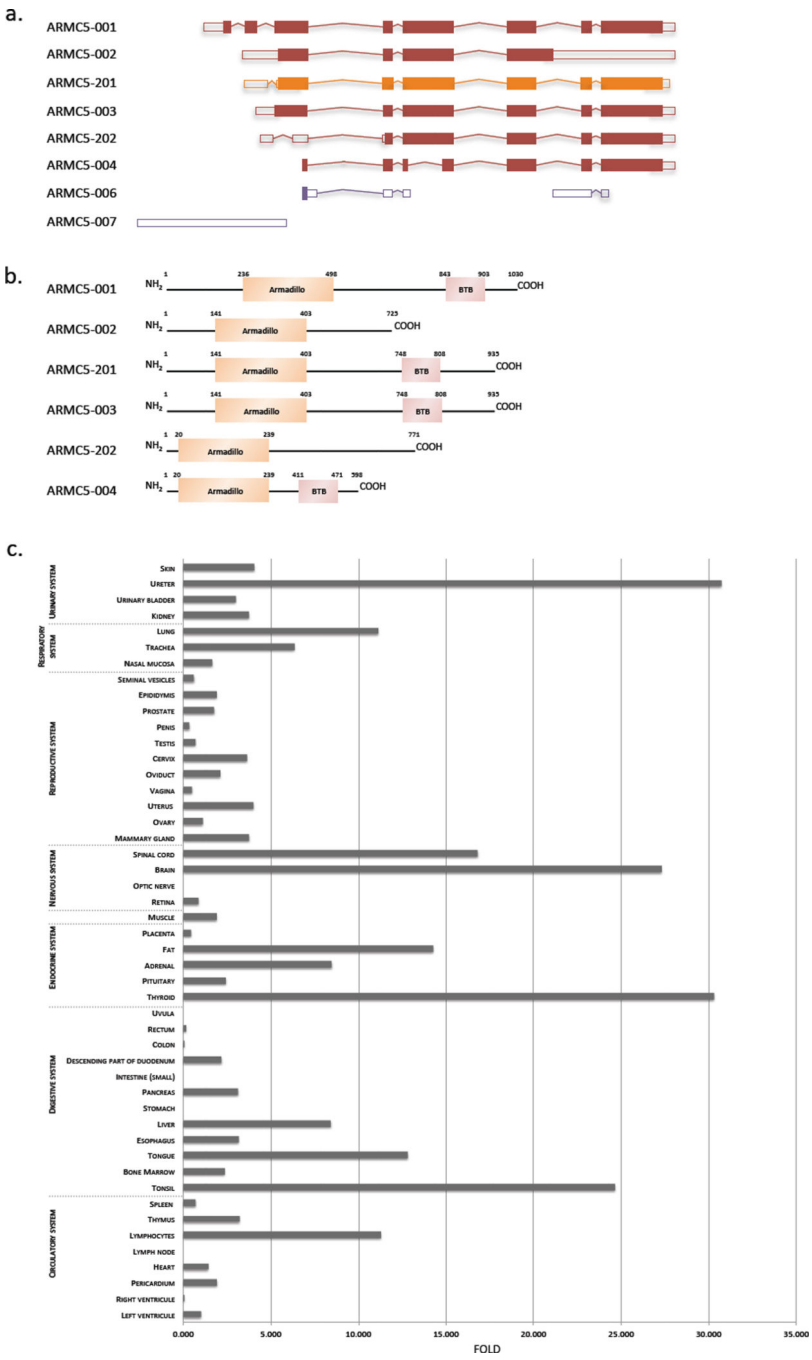

Figure 2. Expression of human *ARMC5* isoforms in 46 human normal tissues

a. Schematic representation of human *ARMC5* transcripts. The full boxes represent the coding sequences whereas the open boxes are the 5 and 3' untranslated region (UTR). b. Schematic representation of human ARMC5 proteins. The orange boxes are the Armadillo repeats and the red boxes, the BTB domain. c. *ARMC5* expression is expressed in the 46 human normal tissues analyzed by RTqPCR using primers recognizing all the isoforms. These results were normalized to βactin expression.

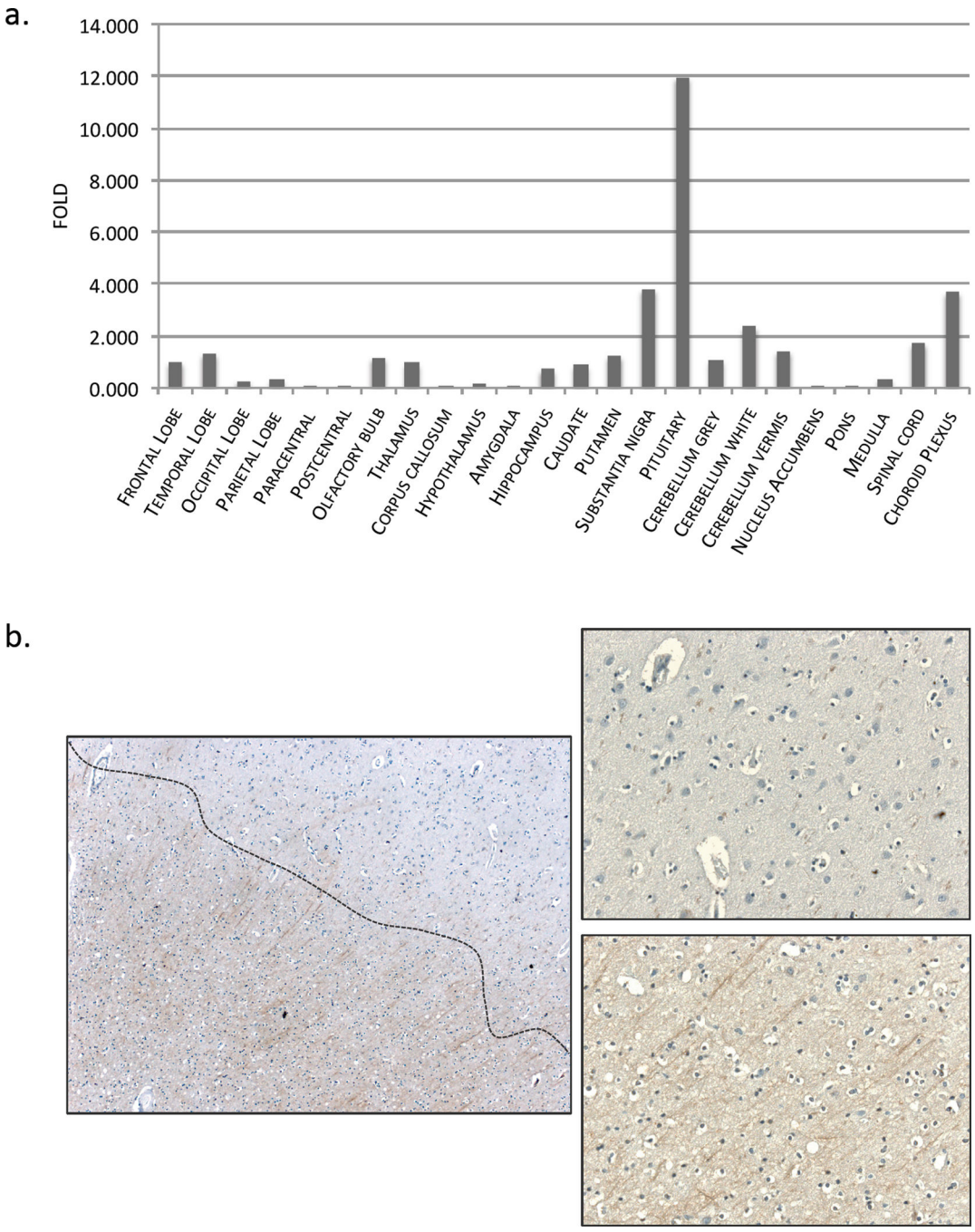

Figure 3. ARMC5 expression in human brain

a. *ARMC5* expression was analyzed in 24 different tissues of the brain by RTqPCR using primers targeting all *ARMC5* isoforms. These results were normalized to β–actin expression. b. ARMC5 analysis by immunohistochemistry shows that *ARMC5* expression is not ubiquitinously expressed in the brain.

Table 1

Sequences of the primers used to analyze *ARMC5* expression.

	Sequence 5'−3'	Amplicon size
ARMC5-001F	GGCAGCGAACGTTCTCGTTCC	80 bp
ARMC5-R1	AGGGTTGGCTTCGCAGCCG	
ARMC5-002F	GAGTGAAGAACTCCCCGTTTCC	100 bp
ARMC5-R4	GCCGCTTCCTAGAGTGACGG	
ARMC5-003F	GCGCTGCGATTAAGTTCCGC	80 bp
ARMC5-R1	AGGGTTGGCTTCGCAGCCG	
ARMC5-002-201F	CGTGTGCAAGGACAGACTTC	127 bp
ARMC5-R6	GAAGACAGGGAAATCGTCGG	
ARMC5-F1	GAACCGAACGGCCCGTGCCC	80 bp
ARMC5-R5	GTCAGGCTCTCCACAAGCAGG	

Table 2

Expression of ARMC5 isoforms on 46 human normal tissues

Primers designed to recognize specifically ARMC5-001, or ARMC5-003, or ARMC5-201/ARMC5-002, or ARMC5-002 isoforms were used to determine *ARMC5* isoforms expression. The results are presented as an induction fold.

		ARMC5-003	ARMC5-001	ARMC5-201 ARMC5-002	ARMC5-002
Circulatory system	Left ventricule	-	-	1.00	1.00
	Right ventricule	-	-	-	0.90
	Pericardium	-	-	8.92	15.86
	Heart	-	1.00	17.15	4.80
	Lymph node	-	92.80	80.95	30.40
	Lymphocytes	1.00	-	34.18	11.52
	Thymus	1.32	85.63	70.03	10.20
	Spleen	0.07	-	12.92	3.70
	Tonsil	-	800.08	9.90	14.95
	Bone Marrow	-	-	36.73	26.08
Digestive System	Tongue	-	139.01	2.54	10.79
	Esophagus	-	-	21.60	6.34
	Liver	-	1540.31	202.53	36.18
	Stomach	0.32	-	7.35	2.22
	Pancreas	0.08	70.67	13.41	11.07
	Intestine (small)	-	107.19	75.37	29.73
	Descending part	-	-	10.91	3.96
	Colon	-	41.16	13.77	14.04
	Rectum	-	-	2.33	2.02
	Uvula	0.97	-	30.06	10.00
Endocrine system	Thyroid	-	-	60.05	34.49
	Pituitary	2.61	-	79.78	14.70
	Adrenal	1.80	41.99	63.65	14.26
	Fat	0.62	53.33	37.56	33.50

		ARMC5-003	ARMC5-001	ARMC5-201 ARMC5-002	ARMC5-002
	Placenta	0.18	-	3.48	1.74
Nervous system	Muscle	-	-	9.21	3.46
	Retina	-	-	26.32	10.70
	Optic nerve	-	-	19.79	18.64
	Brain	0.51	107.19	32.58	28.44
	Spinal cord	-	-	40.03	14.26
Reproductive system	Mammary gland	-	141.24	19.95	9.87
	Ovary	-	-	0.78	7.42
	Uterus	2.65	-	81.52	40.87
	Vagina	-	-	28.07	15.35
	Oviduct	-	-	27.21	12.09
	Cervix	-	-	17.70	21.27
	Testis	0.48	-	11.22	2.59
	Penis	-	-	8.66	9.05
	Prostate	1.20	-	7.87	14.44
	Epididymis	-	-	9.77	7.26
	Seminal vesicles	-	-	2.75	13.78
Respiratory system	Nasal mucosa	-	39.75	5.84	2.94
	Trachea	1.55	271.16	40.20	14.54
	Lung	2.02	810.12	140.26	40.96
Urinary system	Kidney	-	-	18.30	7.45
	Urinary bladder	-	-	9.23	11.59
	Ureter	-	742.89	27.76	24.05
	Skin	-	32.83	15.64	10.99