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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 in 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 the *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.

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1. Introduction

The *ARMC5* gene is mutated in almost 50% of patients with primary macronodular adrenocortical hyperplasia (PMAH) (Alencar et al., 2014; Assie et al., 2013; Drougat et al., 2015; Duan et al., 2014; Faucz et al., 2014,Gagliardi et al., 2014; Espiard 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 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 et al., 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., 2013 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 determined the respective expression of the four known *ARMC5* isoforms in a set of 46 human normal tissues including

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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	

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 the *Ensembl* database (http://www.ensembl.org/index.html) (Yates et al., 2016). Although *ARMC5* transcripts sequences were aligned using Clustal Omega (Sievers et al., 2011; Goujon et al., 2010), the alignment of ARMC5 protein sequences and the phylogenic tree were generated using Geneious version 4.8 (Kearse 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

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
	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