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Prevalence and diversity of hypoglossal canal bridging in the Portuguese population

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RESUMEN

Este estudio evalúa las frecuencias y las posibles diferencias sexuales y de lateralidad de las distintas variaciones morfológicas del canal del hipogloso en una muestra portuguesa, Para llevar a cabo este estudio se analizaron un total de 589 cráneos (320 mujeres y 269 hombres) de la Colección de esqueletos identificados de Lisboa (Luis Lopes Collection) datada entre el siglo XIX y XX, depositados en del Museo Nacional de Historia Natural y Ciencia (MUHNAC) en Lisboa (Portugal).

Los resultados indican que el 32.4% de la muestra presenta alguna variación morfológica del canal del hipogloso diferente de la normal (un solo conducto). El 27.8% de la muestra presenta doble canal hipogloso, encontrándose tanto unilateral como bilateralmente. No obstante, ninguna de las variaciones del canal hipogloso muestra diferencias significativas respecto al sexo o de lateralidad.

La frecuencia del doble canal hipogloso en la colección de Lisboa se encuentra dentro de los rangos normales reportados en la literatura, y es ligeramente inferior a la frecuencia encontrada en los países vecinos (p.ej. Italia, Francia y Reino Unido). No obstante, estas diferencias no son estadísticamente significativas. La diversidad de variaciones y combinaciones laterales del canal del hipogloso, las diferencias en las frecuencias en el predominio lateral y sexual, sugiere que la formación del canal del hipogloso es muy compleja y/o está sometida a una gran restricción genética y ambiental. Además, en este trabajo se aportan datos de un caso con triple canal hipogloso bilateral. El doble canal hipogloso, de considerable frecuencia, unido a la ausencia de diferencias sexuales, lo hace útil tanto en estudios de distancia biológica como en estudios ambientales, mientras que la diversidad de los tipos de canales y las combinaciones de lateralidad lo hace útil en antropología forense.

ABSTRACT

This study evaluates the prevalence and potential differences according to sex and laterality in morphological variations of the hypoglossal canal in a Portuguese sample. Five hundred and eighty nine skulls (320 females and 269 males) from the Collection of identified skeletons of Lisbon (Luis Lopes Collection) dated between the 19th and 20th centuries and housed in the National Museum of Natural History and Science (MUHNAC) in Lisbon (Portugal), were used.

Results indicate that 32.4% of the sample presents a morphological variation of the hypoglossal canal compared to the normal single canal. A double hypoglossal canal, either unilaterally or bilaterally, is present in 27.8% of the sample. However, none of the morphological variations of the hypoglossal canal showed significant differences, according to sex or laterality.

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The prevalence of a double hypoglossal canal in the Lisbon collection falls within the normal ranges found in the literature and is slightly lower than the prevalence found in neighboring countries (i.e. Italy, France, and United Kingdom) although these differences are not statistically significant. The diversity of variations and lateral combinations of the hypoglossal canal and differences in prevalence according to side and sex point to the complexity behind the formation of the hypoglossal canal and/or that it is subject to great genetic and environmental constraints. In addition, this work presents the rare case of a bilateral triple hypoglossal canal. The high prevalence of double hypoglossal canals together with the absence of sexual differences makes this feature useful for both biological distance studies and environmental studies, while the diversity of canal types and various combinations of laterality makes it useful as an identification feature in forensic anthropology.

Introduction

The hypoglossal canal, also called the anterior condylar canal (Raghunath et al. 2015; Kumar & Anuradha 2017), is a bony passage anterolateral to the occipital condyles. It is traversed by the hypoglossal nerve which is the twelfth cranial nerve. The hypoglossal canal can be divided by a bony bridge or a septum. Occipital bone is formed from the union of the anterior basioccipital (basilar) with the lateral exoccipitals (condylars), the posterior supraoccipital (squamous) at the interparietal suture (Healy & Varacallo 2019). The formation of the hypoglossal canal is related to the process of occipital chondrification and occurs at Carnegie stage 17, at approximately 6 weeks of gestation. Hence, the formation of a bony bridge or septum occurs prior to the embryo reaching stage 17 (Ari et al. 2005).

In order to evaluate the morphological variations of the hypoglossal canal, Hauser & De Stefano (1985) developed a five-stage classification of the hypoglossal canal, where type I is a single canal, type II has one incomplete osseous spine, type III, has two or more incomplete osseous spines, type IV has complete osseous bridging, and type V has complete hypoglossal osseous bridging. This classification system is often simplified as two stages following Berry & Berry (1967): the absence of double hypoglossal canal (types I, II and III presented above) and the presence of hypoglossal canal (types IV and V described above). This trait is mainly unilaterally expressed and, in most cases, shows a statistically significant predominance for left side (Ari et al. 2005; Jacob et al. 2014; Kanda et al. 2015).

Aside from the clinical significance of the hypoglossal canal in several pathologies like occipital bone fractures or intracranial and extracranial neoplasms (Braun & Tournade 1977; Tanzer 1978; Canalis et al. 1993; Lang & Hornung 1993), hypoglossal canal bridging is a nonmetric morphological trait that has been mainly used for biological distance analyses between populations (Berry & Berry 1967; De Villiers 1968; Dodo 1974; Dodo & Ishida 1990; Dodo & Sawada 2010). Berry & Berry (1967) analysed 583 adult crania from several locations and time periods, finding a prevalence of 7 to 27.4% for the double hypoglossal canal. More recently Dodo & Sawada (2010), in a study based on 71 samples from around the world, found prevalence rates from 5.5% up to 41.5%. These epigenetic studies reflect the interaction between genes and environment and the resulting variation in the expression of anatomical traits (Gluckman et al. 2007). In addition, the hypoglossal canal can be used (a) to measure biological distance, for which it has been mostly used for so far (Berry & Berry 1967; De Villiers 1968; Dodo 1974; Dodo & Ishida 1990; Dodo & Sawada 2010), (b) as a stress marker (Amoroso & Garcia 2021) - in an ongoing study of the same collection, double hypoglossal canal morphology was correlated with other stress markers, therefore also exhibiting its potential to capture environmental stress -, and (c) as an identifying feature or additional element to build a biological profile in forensic anthropology, such as other nonmetric cranial traits (L'Abbé et al. 2011). Therefore, it is of great importance to study and understand the morphological variability of the hypoglossal canal in different populations.

The objective of this study is to evaluate the morphological variability of the hypoglossal canal, its prevalence, and potential differences according to sex and laterality in the Portuguese population. This study is the first to provide information about the morphological variability of the hypoglossal canal in a Portuguese sample.

Materials and methods

To carry out this study, 589 skulls from Lisbon Identified Skeletal Collection (Luis Lopes Collection), stored at National Museum of Natural History and Science (MUHNAC), Lisbon, Portugal, were analysed. The Lisbon Collection is dated between the 19th and 20th centuries and accurately captures population diversity as a result of massive rural exodus to the capital throughout that time (Cardoso 2006). Furthermore, living conditions in Lisbon deteriorated greatly during that period, resulting in additional environmental stress that could have enhanced variability in epigenetic traits. Although this collection has more than 700 individuals available, only those with known sex and age-at-death and a well-preserved skull base were used. The sample comprises 320 females (54.3%) and 269 males (45.7%). Age-at-death ranges between two and 98 years old. Individuals were born between 1806 and 1949 and died between 1880 and 1970. One hundred and eighty-seven individuals (31.7%) were born in Lisbon, 346 (58.7%) were born elsewhere, and 56 (9.5%) had no information about their place of birth. A summary of the sample demographics is presented in Table 1.

Table 1. Sample, according to sex and age group.

Age group (years)	Sex		Total
	Females	Males	
≤ 9	6	2	8
10-19	20	17	37
20-29	23	23	46
30-39	11	20	31
40-49	18	37	55
50-59	46	55	101
60-69	47	38	85
70-79	80	42	122
≥ 80	69	35	104
Total	320	269	589

A simplified version of Hauser & De Stefano (1985) methodology (Types I to V) was used to record of the morphology of the hypoglossal canal with the addition of a new category for triple hypoglossal canal. This resulted in four different groups for variants, from a to d: a) single hypoglossal canal (Type I), b) partial osseous bridging (Types II and III), c) double hypoglossal canal (Types IV and V), and d) triple hypoglossal canal. This simplification was chosen to reduce inter-observer error in differentiating between Type II and Type III, and Type IV and Type V, respectively.

The prevalence of variants was measured: a) in the entire sample, and b) by sex. Prevalence was calculated by dividing the number of individuals with a double hypoglossal canal (either unilaterally or bilaterally expressed) by the total number of individuals observed. Prevalence of double hypoglossal canal by side and sex was also calculated. Chi-square tests - or Fischer´s test whenever assumptions were not met- were used to test for sex differences. The z-score test for proportions was used to test side differences using the following formula:

$$\frac{(\bar{p}_1 - \bar{p}_2) - 0}{\sqrt{\bar{p}(1 - \bar{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Differences between the prevalence of double hypoglossal canal bridging observed here and in other studies were tested using Chi-square or Fischer´s test whenever assumptions for the former were not met.

Results

Table 2 shows the prevalence of the morphological variants of the hypoglossal canal recorded in the present study in the entire sample and by sex. This table also indicates the level of statistical significance of sex differences for each variant by Chi-Square/Fischer's test. No statistically significant differences were found according to sex in the prevalence of hypoglossal canal variants.

One remarkable find was individual MB61-00353, a 77-year old female, who was born in 1867 and died in 1944, who presents a very rare case of bilateral triple hypoglossal canal (Figure1).

Table 2. Prevalence of hypoglossal canal variants and Chi-Square/Fisher's test results for sex differences.

SHC		PARTIAL		DHC		THC		Males		Females		p-value	Total	
L	R	L	R	L	R	L	R	n	%	n	%		n	%
x	x							178	66.17	220	68.75	0.505	398	67.57
	x			x				36	13.38	29	9.06	0.096	65	11.04
x					x			24	8.92	25	7.81	0.627	49	8.32
				x	x			17	6.32	21	6.56	0.905	38	6.45
	x	x						5	1.86	10	3.13	0.331	15	2.55
x			x					3	1.12	3	0.94	1	6	1.02
		x	x					2	0.74	1	0.31	0.595	3	0.51
			x	x				2	0.74	5	1.56	0.463	7	1.19
		x			x			2	0.74	1	0.31	0.595	3	0.51
x							x	-	-	2	0.63	0.503	2	0.34
						x	x	-	-	1	0.31	1	1	0.17
					x	x		-	-	1	0.31	1	1	0.17
				x			x	-	-	1	0.31	1	1	0.17
Total								269		320			589	

SHC: single hypoglossal canal; PARTIAL: partial bridging in hypoglossal canal; DHC: double hypoglossal canal; THC: triple hypoglossal canal; L: left; R: right.

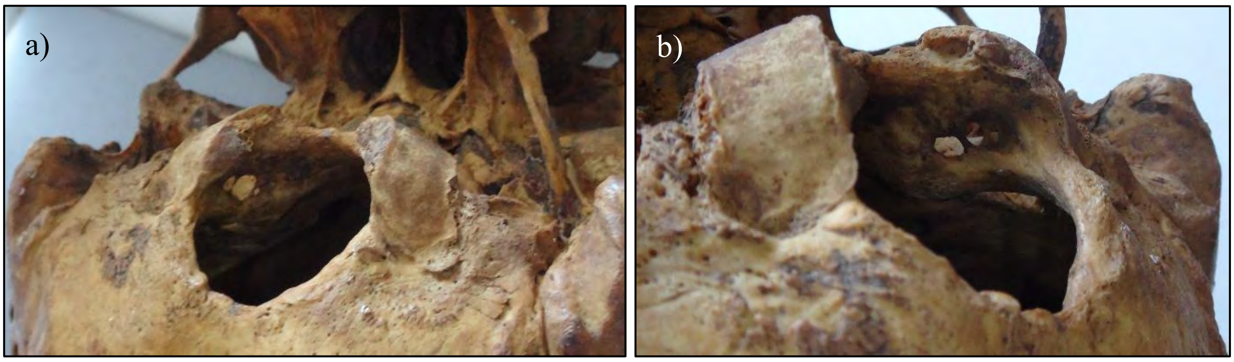

Figure 1: Bilateral triple hypoglossal canal: right (a) and left (b). MB61-00353 Luis Lopes Collection, MUHNAC, University of Lisbon (photograph S. Garcia, courtesy University of Lisbon).

In order to compare the results of this study related to complete double hypoglossal canal, expressed either laterally or bilaterally with those of other authors Table 3 was constructed. This table shows the different values of the prevalence of hypoglossal canal bridging, sorted from highest to lowest that were found in the literature. As we previously said, values only represent complete double hypoglossal canals, expressed either laterally or bilaterally. Chi-square/Fisher's exact test results for prevalence differences between published data and the present study is also indicated.

According to our results (Table 2), in the Lisbon sample, the prevalence of overall variants of the hypoglossal canal includes partial and complete double and triple hypoglossal canal, both unilaterally and bilaterally expressed is 32.4%. Particularly, partial bridging of the left hypoglossal canal has a prevalence of 3.6% (n=21), whereas this prevalence for the right side is 2.7% (n=16) (Table 2). There are no statistically significant differences according to side for partial bridging of the hypoglossal canal (z=0.835; p=0.401). Neither females (z=0.666; p=0.503), nor males (z=0.508; p=0.610) have statistically significant side differences in partial bridging of the hypoglossal canal (Table 2).

In relation to left double hypoglossal canal, in Lisbon sample (Table 2), a prevalence of 18.8% (n=111) were observed (Table 2), whereas the right side has a prevalence of 15.4% (n=91) (Table 2). Our results indicate, there are no statistically significant differences in double hypoglossal canal (z=1.546; p=0.121) according to its side. Neither females (z=0.857; p=0.390), nor males (z=1.340; p=0.180) present a statistically significant side difference in the double hypoglossal canal (Table 2). The prevalence of double hypoglossal canals, either unilaterally or bilaterally expressed is 27.8% (Table 3).

Triple hypoglossal canal on left side of the Lisbon sample has a prevalence of 0.3% (n=2), and a prevalence of 0.7% (n=4) for the right side (Table 2). Triple hypoglossal canal has no statistically significant differences (z=0.818; p=0.412) according to side. There are no statistically significant differences in triple hypoglossal canal according to sex (z=0.820; p=0.412; see Table 2).

According our results, when combined together, left side hypoglossal canal variants have a prevalence

Table 3. Prevalence of double hypoglossal canal, expressed laterally and bilaterally and differences with the present study.

Continent	Country/Collection	n	%	Reference	p_value
Americas	Peru	53	54.7	Berry & Berry, 1967*	**<0.001**
Americas	British Colombia	50	48.0	Berry & Berry, 1967*	**0.003**
Americas	Peru	176	41.5	Dodo & Sawada, 2010	**0.001**
Americas	Tierra del Fuego/Patagonia	61	39.3	Dodo & Sawada, 2010	0.059
Americas	Northeast America	76	36.8	Dodo & Sawada, 2010	0.103
Americas	Northwest America	72	36.1	Dodo &Sawada, 2010	0.143
Asia	India (Punjab)	53	35.8	Berry &Berry, 1967*	0.216
Asia	Hokkaido Ainu	242	35.5	Dodo & Sawada, 2010	**0.028**
Europe	Russia	116	34.5	Dodo & Sawada, 2010	0.150
Europe	Ensay, UK	111	33.3	Dodo & Sawada, 2010	0.241
Africa	Egypt	247	33.2	Berry & Berry, 1967*	0.121
Africa	26th-30th Dynastic Egypt	184	33.2	Dodo & Sawada, 2010	0.166
Arctic	Asian Eskimo	125	32.8	Dodo & Sawada, 2010	0.266
Arctic	Ekven	98	32.7	Dodo & Sawada, 2010	0.329
Asia	Tagar	121	32.2	Dodo & Sawada, 2010	0.331
Europe	France	93	31.2	Dodo & Sawada, 2010	0.507
Americas	Northwest Coast	90	31.1	Dodo & Sawada, 2010	0.522
Asia	Kazakh	120	30.8	Dodo & Sawada, 2010	0.508
Africa	Pre-Dynastic Egypt	231	30.7	Dodo & Sawada, 2010	0.410
Africa	Nubia	214	29.9	Dodo & Sawada, 2010	0.567
Europe	East Europe	124	29.8	Dodo & Sawada, 2010	0.654
Europe	Italy	180	29.4	Dodo & Sawada, 2010	0.676
Europe	Scandinavia	65	29.2	Dodo & Sawada, 2010	0.813
Europe	Finland/Ural	79	29.1	Dodo & Sawada, 2010	0.813
Asia	Jomon	159	28.9	Dodo & Sawada, 2010	0.787
Arctic	Aleut	104	28.8	Dodo & Sawada, 2010	0.834
Asia	Neolithic Baikal	70	28.6	Dodo & Sawada, 2010	0.898
Europe	**Portugal**	**589**	**27.8**	**Present study**	**-**

Africa	Kerma/Sudan	191	27.7	Dodo & Sawada, 2010	0.980
Europe	Spitafields, UK	346	27.2	Dodo & Sawada, 2010	0.823
Asia	Northwest India	173	26.6	Dodo & Sawada, 2010	0.745
Arctic	Greenland Inuit	166	26.5	Dodo &Sawada, 2010	0.733
Europe	Poundbury, UK	151	26.5	Dodo & Sawada, 2010	0.740
Arctic	Chukchi	73	26.0	Dodo & Sawada, 2010	0.743
Asia	Yakut	64	25.0	Dodo & Sawada, 2010	0.629
Europe	Greece	60	25.0	Dodo & Sawada, 2010	0.639
Asia	East India	123	24.4	Dodo & Sawada, 2010	0.434
Asia	South India	181	23.3	Dodo & Sawada, 2010	0.218
Asia	Amur Basin	163	23.3	Dodo & Sawada, 2010	0.248
Africa	Nigeria (Ashanti)	56	23.2	Berry & Berry, 1967*	0.458
Asia	Korea	71	22.5	Dodo & Sawada, 2010	0.343
Asia	Buryat	149	22.1	Dodo & Sawada, 2010	0.160
Africa	South Africa	132	22.0	Dodo & Sawada, 2010	0.168
Asia	Borneo	137	21.9	Dodo & Sawada, 2010	0.157
Asia	Assam/Sikkim	65	21.5	Dodo & Sawada, 2010	0.278
Asia	Japan	590	21.4	Kanda et al., 2015	**0.010**
Asia	North China	165	20.6	Dodo & Sawada, 2010	0.062
Asia	Java	133	20.3	Dodo & Sawada, 2010	0.075
Oceania	Society	64	20.3	Dodo & Sawada, 2010	0.198
Oceania	Maori	175	20.0	Dodo & Sawada, 2010	**0.038**
Asia	India	50	20.0	Kumar & Anuradha, 201´	0.232
Asia	Philippines	207	19.8	Dodo & Sawada, 2010	**0.023**
Asia	Burma	51	19.6	Berry & Berry, 1967*	0.205
Asia	Mongol	180	19.4	Dodo & Sawada, 2010	**0.024**
Asia	Myanmar	188	19.1	Dodo & Sawada, 2010	**0.018**
Africa	Erigavo/Somalia	67	17.9	Dodo & Sawada, 2010	0.082
Oceania	Southern Island Melanesia	188	17.0	Dodo & Sawada, 2010	**0.003**
Asia	Main-Island Japan	172	16.9	Dodo & Sawada, 2010	**0.004**
Southeast Asia	Andaman/Nicobar	119	16.8	Dodo & Sawada, 2010	**0.012**
Asia	India	100	16.0	Kaur et al., 2012*	**0.013**
Europe	Germany	70	15.7	Dodo & Sawada, 2010	**0.030**
Oceania	Torres Strait	97	15.5	Dodo & Sawada, 2010	**0.010**
Africa	Kenya	133	15.0	Dodo & Sawada, 2010	**0.002**
Asia	Tibet/Nepal	121	14.9	Dodo & Sawada, 2010	**0.003**
Africa	Ibo/southern Nigeria	155	14.8	Dodo & Sawada, 2010	**0.001**
Africa	Khoisan	63	14.3	Dodo & Sawada, 2010	**0.021**
Asia	Mainland Southeast Asia	184	14.1	Dodo & Sawada, 2010	**<0.001**
Africa	Fernand Vas River/Gabon	143	14.0	Dodo & Sawada, 2010	**<0.001**
Asia	Palestine (Laschish)	50	14.0	Berry & Berry, 1967*	**0.034**
Asia	Thai	130	13.8	Dodo & Sawada, 2010	**0.001**

Oceania	Northern Island Melanesia	293	13.3	Dodo & Sawada, 2010	**<0.001**
Oceania	Moriori	105	13.3	Dodo & Sawada, 2010	**0.002**
Oceania	Hawaii	153	13.1	Dodo & Sawada, 2010	**<0.001**
Oceania	Marquesas	99	13.1	Dodo & Sawada, 2010	**0.002**
Asia	Lesser Sunda	64	12.5	Dodo & Sawada, 2010	**0.008**
Oceania	Papua New Guinea	312	12.2	Dodo & Sawada, 2010	**<0.001**
Oceania	East Australia	126	11.9	Dodo & Sawada, 2010	**<0.001**
Africa	Ghana (Ashanti)	127	11.8	Dodo & Sawada, 2010	**<0.001**
Asia	South China	88	11.4	Dodo & Sawada, 2010	**0.001**
Oceania	Southwest Australia	267	9.0	Dodo & Sawada, 2010	**<0.001**
Asia	Palestine (modern)	18	8.3	Berry & Berry, 1967*	**0.036**
Africa	Tanzania	90	5.6	Dodo & Sawada, 2010	**<0.001**
Oceania	Easter	145	5.5	Dodo & Sawada, 2010	**<0.001**

* The results were calculated from the data in the article

of 22.8% (n=134), whereas right side variants have a prevalence of 18.8% (n=111), showing no statistically significant side differences (z=1.651; p=0.099). Neither females (z=0.882; p=0.379), nor males (z=1.276; p=0.201) have statistically significant differences in hypoglossal canal variants according to laterality.

Discussion

This study is the first to identify several variants of the hypoglossal canal in a Portuguese sample. Prevalence of overall variants of the hypoglossal canal includes partial and complete double and triple hypoglossal canal, both unilaterally and bilaterally expressed is 32.4% (Table 2). Prevalence of double hypoglossal canals, either unilaterally or bilaterally expressed, is 27.8% (Table 3). None of the hypoglossal canal variants showed any statistically significant difference according to sex or laterality. Published prevalence rates for double hypoglossal canals range from 5.5%, observed in a sample of 145 skulls of Recent Easter Islanders (Oceania) (Dodo & Sawada 2010), to 54.7% observed for Amerindians in Peru, South America (Berry & Berry 1967), see Table 3. There are statistically significant differences in double hypoglossal canal prevalence between the Lisbon sample and the 36 other samples showed in Table 3. These samples are from Africa (6), Americas (3), Asia (15), Europe (1) and Oceania (11). Among these samples, there is only one sample from Europe, which is Germany (Table 3). For most of the statistically significant differences (32 samples), the Lisbon sample had higher prevalence (Table 3). Prevalence of the double hypoglossal canal (27.8%) fits within the normal range of variation of this trait (5.5 to 54.7%) (Table 3).

According to O'Rahilly & Müller (1984), who were the first to analyse human embryonic development of the hypoglossal nerve and canal from day 24 to day 54 of gestation, the morphology of the hypoglossal canal is connected with occipital chondrification and the development of the hypoglossal nerve, occurring during Carnegie stage 17 (at approximately week 6 of embryonic development). This means any disturbances in normal development of the hypoglossal canal from which might originate a hypoglossal canal variant has to occur prior to stage 17, i.e. up to week 6 of embryonic development. Paraskevas et al. (2009) state that disturbance of the ossification process during that period could not only result in the formation of a double hypoglossal canal, but also a posterior condylar canal and jugular foramen bridging. The aetiology of double hypoglossal canal is unknown, although a combined gene-environment causation is plausible (Saunders & Rainey 2008).

Results presented here show there is much more variability in hypoglossal canal expression than what is usually reported in the literature, mainly because most studies do not report detailed information about all variations, especially concerning bilateral combinations. Additionally, several individuals show different variations of single/normal hypoglossal canal between each side. Therefore, the normal formation of hypoglossal canal during occipital chondrification during the 6th week of embryonic development appears to be a rather complex process in which any disturbances/disruptions might result in a wide variety of hypoglossal canal variants and side combinations.

Reports from countries geographically-close to Portugal such as Italy and France showed a prevalence of 29.4% in a sample of recent Italian soldiers housed at the Natural History Museum, and a 31.2% prevalence in a sample of soldiers of the army of Napoleon and recent French people, housed at the Natural History Museum, Musée de l'Homme (Dodo & Sawada 2010). In both cases, prevalence is just slightly higher than what was calculated in the Portuguese sample, but differences are not statistically significant.

Our results indicate that are no sex differences in the expression of the double hypoglossal canal, which is agreement with the studies of Berry & Berry 1967; Paraskevas et al. 2009; Dodo & Sawada 2010; Kaur et al. 2012, and constitutes a normal and important characteristic of non-metric traits used in population distance studies. Even so, statistically significant differences between sexes were found by Kanda et al. (2015) in a Japanese sample of 590 patients, who underwent cerebral computed tomographic (CT) angiography and suffered from Central Nervous System anomalies, with male predominance of a double hypoglossal canal. The authors argue that male predominance might be a characteristic of the Japanese population or might result from females having smaller hypoglossal canals that could be reflected on the thickness of the bridge and in some cases might not be seen in CT, biasing results.

Statistically significant side differences with predominance in the left side were found by Kanda et al. (2015), whereas in Paraskevas et al. (2009) these differences were predominant for the right side. Other studies, including the present one, did not find statistically significant side differences (Ari et al. 2005; Kaur et al. 2012). This variance of predominance according to side might be a form of anti-symmetry. Although this concept is used for continuous traits, it could perhaps be extended to non-metric traits. According to Palmer & Strobeck (1992) anti-symmetry has a possible genetic origin, where some individuals have a predisposition to develop a left or a right bias. Therefore, this type of bilateral asymmetry is probably not a product of developmental noise.

Although there are no statistically significant sex differences for the presence of a triple hypoglossal canal, perhaps due to a very low prevalence of this variant, all 5 cases recorded in the sample for this variant are females, with ages ranging from 5 to 79 years old. All five died as a result of very distinct pathologies (5 years old: tuberculous meningitis; 54 years old: gastric neoplasia; 68 years old: liver neoplasia; 77 years old: cerebral thrombosis; 79 years old: cerebral hemorrhage). From such a low prevalence and with very few cases of triple hypoglossal canal reported in the literature, it is not possible to know if there is a tendency towards females presenting this rare variant of the hypoglossal canal, and if so, the reasons behind this possible difference (e.g. hormonal differences). A very rare case of triple hypoglossal canal, bilaterally expressed, was recorded in this study in a 77-year-old female. To our knowledge, only Raghunath et al. (2015) reported a similar case, on the dry skull of an adult male of approximately 60 years old from the Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University (Varanasi, Uttar Pradesh, India). Raghunath et al. (2015) present three possible causes for this triplication: a) the presence of two roots in the hypoglossal nerve, forming a double canal and a third canal for the emissary vein; b) each canal serves a distinct pathway for the hypoglossal nerve, the ascending pharyngeal artery, and the emissary vein; and c) a single canal for the hypoglossal nerve and a double one for the emissary vein. Wysocki et al. (2004) also reported a bilateral triple hypoglossal canal in a male rhesus monkey (*Macacus rhesus*), in a study comprising humans, rhesus monkeys, European bison, mongrel dogs, foxes, cats, hares and rats. Triple hypoglossal canals are considered a rare trait, with very few reports found in the literature (Murlimanju et al. 2014), and is therefore expected to be expressed unilaterally, like other rare traits (Hallgrímsson et al. 2005).

Conclusions

The prevalence of the double hypoglossal canal in the present study is within the ranges reported in the literature (from 5.5% to 54.7%), and very close to prevalence rates reported in neighboring countries (i.e. Italy, France, and the United Kingdom). Results also showed greater diversity of hypoglossal canal variants and side combinations than what is normally reported in the literature, likely because these studies did not report all types of variants (e.g. partial bridging). Diversity of hypoglossal canal variants and side combinations, differences in prevalence rates, side, and sex predominance, suggest that the formation of hypoglossal canal is highly complex and/or is susceptible to great genetic and environmental constraints. A very rare case of bilateral triple hypoglossal canal is reported.

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