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Non-metric variations in individuals who died during the perinatal period in past populations: recording protocol and comparative data

Variations anatomiques non métriques des individus décédés en période périnatale dans les populations anciennes : protocole d'enregistrement et données de comparaison

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Abstract – Non-metric variations, also referred to as "epigenetic variations" or asymptomatic bone variants, are bone or dental phenotypic variants of unknown origin, with no known pathological etiology. They are classically identified during osteological analysis to develop an individual profile, analyse biological affinities between individuals and outline possible genetic relatedness clusters in burial spaces. However, although numerous studies have focused on their occurrence in adult archaeological samples, data on their development, expression and frequency in skeletally immature individuals are still scarce. The aim of this study was therefore to document the expression and frequencies of a selection of non-metric variations in an archaeological sample of 116 individuals who died during the perinatal period, in order to outline a diachronic reference database for comparative analysis. The individuals analysed are from an ancient Sudanese necropolis and two medieval/modern cemeteries and churches in the Paris Basin. Altogether, the occurrence of 22 selected variants were scored directly from the examination of the bones. No significant population-based difference was found between the Nubian and the Western European samples. Frequencies in the perinatal sample were compared with those of samples including juvenile and adult individuals from other chrono-geographical contexts.

Keywords – anatomical variants, biological profile, discrete traits, infants, immature individuals

Résumé – Les variations anatomiques non-métriques, également appelées variants épigénétiques ou variations osseuses asymptomatiques, sont des variants phénotypiques osseux ou dentaires sans origine déterminée et sans étiologie pathologique. Elles sont classiquement utilisées pour développer un profil individuel, analyser les affinités biologiques entre les individus et identifier de possibles proximités génétiques au sein de l'espace funéraire. Même si de nombreuses études se sont attachées à décrire leurs fréquences dans des collections archéologiques constituées de sujets adultes, les données disponibles relatives à leur développement, leur expression et leurs fréquences chez les sujets ostéologiquement immatures restent limitées. L'objectif de notre étude était de documenter les modalités d'expression et les fréquences d'une sélection de variations anatomiques dans un corpus de 116 sujets décédés durant la période périnatale, afin d'esquisser une base de données de référence diachronique disponible pour les analyses comparatives. Les individus analysés proviennent d'une nécropole soudanaise antique et de deux cimetières et églises médiévales et modernes du bassin parisien. La présence de 22 variations sélectionnées a été cotée via observation directe. Aucune différence interpopulationnelle significative n'a été identifiée entre l'échantillon nubien et l'échantillon médiéval/moderne européen. Les fréquences observées dans l'échantillon périnatal ont été comparées à celles observées dans des corpus d'individus immatures et adultes provenant d'autres contextes chrono-géographiques.

Mots clés – variations anatomiques non métriques, profil biologique, caractères discrets, nourrissons, sujets immatures

Introduction

Non-metric variations (NMV), also referred to as "discrete traits", anatomical variants, asymptomatic bone variants, discontinuous morphological variations or "epigenetic variations", are minor asymptomatic phenotypic variations

in bones or teeth without a pathological origin and usually scored as present or absent (e.g. Le Double, 1912; Berry and Berry, 1967; Ossenberg, 1969; Saunders, 1978; Hauser and De Stefano, 1984; Crubézy, 1991; Verna, 2022). The first descriptions of what are considered to be NMV date back to Antiquity with the work of Hippocrates (5th-4th centuries BC) on sutural bones (Le Double, 1903) and Galen (2nd-3rd centuries AC) on supernumerary cervical ribs (Boudon-Millot, 2012).

According to the categories defined by Ossenberg (1969) and modified by Verna (2022), NMVs may be classified as hyperostotic (e.g. excessive ossification of cartilaginous structures or ligaments), hypostotic (e.g. incomplete ossification of a bony element), articular facet variations or supernumerary bones. Since the origin of NMVs appears to be multifactorial, resulting from genetic, epigenetic and/or environmental factors (Crubézy and Sellier, 1990; Konigsberg et al., 1993), studies have challenged their relevance as familial markers (Gemmerich Pfister, 1999) and point towards their use for the analysis of micro-evolutionary phenomena (Crubézy et al., 1999). Case reports where combinations of several rare non-metric variations have been identified in a single individual may indicate the involvement of a common DNA coding area that influences these traits (Verna and Villotte, 2016). In forensic and medical contexts, anatomical variants are also used for biological profiling and identification of individuals (Verna et al., 2013; Verna, 2022).

Although numerous studies have focused on the occurrence and frequencies of NMV in adults from archaeological and medical contexts (e.g. Le Double, 1912; Berry and Berry, 1967; Ossenberg, 1969; Sjovold, 1977; Saunders, 1978; Hauser and De Stefano, 1984; Turner et al., 1991; Braga, 1995; Verna, 2022), data on their expression and frequencies in osteologically immature individuals, and particularly infants, remain scarce. Transposing the scoring protocols established for adults to non-adult individuals, especially infants, can be difficult (in some cases, impossible) due to the state of ossification, so that their inclusion in non-adult osteological studies is limited. Moreover, it now appears that "classic" variations such as supernumerary cervical ribs, which are rare among adults (see the review in Partiot et al., 2020, table 1), are more frequent in non-adults, their occurrence being in fact related to early developmental disruption with an altered expression of Hox genes (Galis et al., 2006; Bots et al., 2011). Therefore, these supernumerary cervical ribs may be considered as a morbidity indicator in foetal and perinatal individuals recovered from the archaeological record (Partiot et al., 2020), although the informative potential of other anatomical variations in infants and children is yet to be explored.

The aims of this study were to develop a practical recording protocol for observable NMV in individuals who died during the perinatal period, to record the frequencies of these NMV in this age group in two chrono-geographically distinct populations, and to investigate lateral variations in traits as well as population-based differences.

Material and methods

The archaeological sample analysed consists of 116 individuals who died during the perinatal period *sensu lato* (Partiot, 2020), with estimated ages-at-death between 26 and 48 gestational weeks. The sample was made up from three collections: 54 individuals from the 8B-51 Classic Kerma necropolis on the island of Sai in the River Nile (Sudan, 1580-1680 calBC; Murail et al., 2004; Maureille et al., 2006; Partiot, 2018), 27 from the cemetery of the church of Blandy-les-Tours (France, Paris Basin, 10th-12th centuries AC; Delattre, 2008), and 35 from the church and parish cemetery of Provins (France, Paris Basin, 13th-18th centuries AC; Guillon et al., 2002; Guillon and Portat, 2016; Portat and Guillon, 2016; Portat, 2018). Ages-at-death were estimated in gestational weeks based on the length of long bones (femur, tibia, humerus, radius, ulna, see Partiot 2018 for further details on the methodology) using the Fazekas and Kósa method (1978) revised by Sellier (published in Schmitt and Georges, 2008). This method was selected because it provides formulae for several long bones from foetal up to four years of age, with confidence intervals also provided (Sellier et al., 1997). To assess population-based differences, the 54 ancient Nubian individuals (Sai Island, Sudan) were compared to the 62 mediaeval and contemporary French individuals from the Paris basin (Provins and Blandy-les-Tours).

Thirteen cranial and ten infra-cranial NMVs were selected for analysis based on a review of the scientific literature on adult and immature variants and macroscopic observation of the remains (table 1). Due to the age group considered in this study and the very low mineralisation level of tooth germs, dental variants were not considered. Unilateral and bilateral occurrences of the traits were identified through macroscopic visual examination and recorded as absent or present when the areas of interest were sufficiently well preserved. Statistical analyses were conducted using the free software R* (version 3.3., R Core Team, 2017). Examples of the NMV traits that could be observed in the archaeological remains are given in figure 1. The full illustrated recording protocol is presented in Partiot (2018, Appendix 9, p. 673).

However, due to the variable completeness of the spinal columns and ribs in the sample analysed, the sequential presence or absence of the bipartite transverse foramen and supernumerary lumbar rib could not be recorded systematically, and thus prevented us from calculating frequencies. For the same reasons, costal grill reduction (i.e., agenesis of one or more ribs) could also not be identified.

Results

Non-metric variations of the cranial skeleton

The Kerckring process (figure 1A) is an occasional projecting tongue of bone located at the inferior margin of the squamous portion of the occipital, and on the posterior

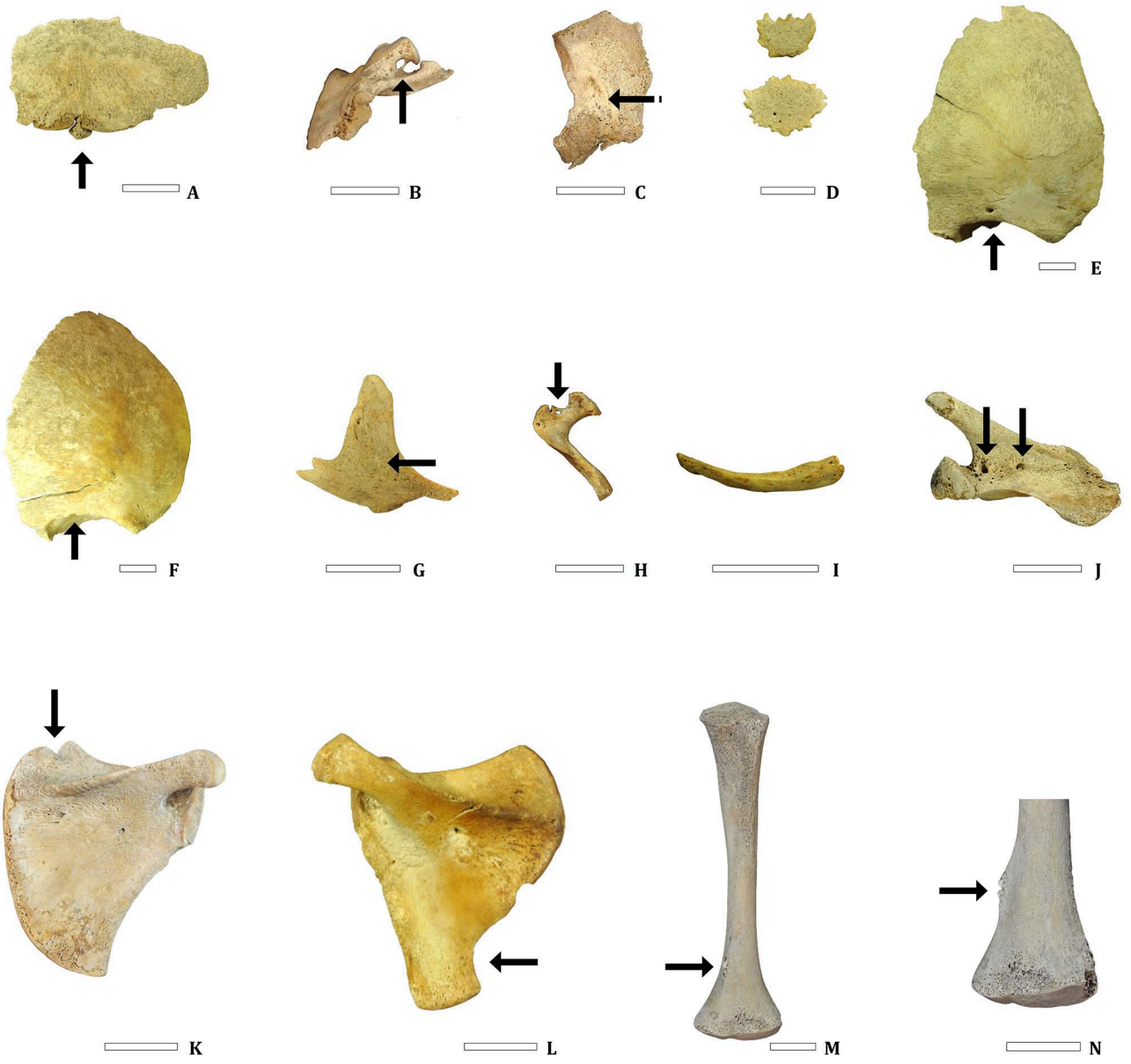

Figure 1. Illustrations of the non-metric variations observed in the perinatal archaeological individuals analysed. A: Kerckring process, fragment of occipital squama, ectocranial view, individual P266A; B: bipartite hypoglossal canal, left pars lateralis, infero-lateral view, individual S10; C: absent condylar canal, right pars lateralis, inferior view, individual S51; D: Wormian bones, individual P288; E: Supra-orbital foramen, left hemi-frontal, anterior view, individual P292; F: supra-orbital notch, left hemi-frontal, anterior view, individual P248; G: absence of zygomatic foramen, right zygomatic bone, anterior view, individual S55; H: accessory transverse foramen of the vertebral hemi-arch, C6, superior view, individual S67; I: possible lumbar rib, inferior view, individual S22; J: double foramens of the supraspinous fossa, right scapula, superior view, individual P220; K: epispinous notch, right scapula, posterior view, individual S63; L: constriction of the scapula, left scapula, posterior view, individual S12-2; M: possible supra-condylar process, let humerus, anterior view, individual P289; N: possible supra-condylar process of individual P289, detail, antero-lateral view, scale: 1 cm / *Illustrations des variations anatomiques non métriques observées dans le corpus archéologique de sujets analysés décédés durant la période périnatale. A : Processus de Kerckring, fragment d'écaille occipitale, vue exocrânienne, individu P266A ; B : canal hypoglosse bipartite, pars lateralis gauche, vue inféro-latérale, individu S10 ; C : canal condylaire absent, pars lateralis droite, vue inférieure, individu S51 ; D : Os wormiens, individu P288 ; E : foramen supra-orbitaire, hémi-frontal gauche, vue antérieure, individu P292 ; F : encoche supra-orbitaire, hémi-frontal gauche, vue antérieure, individu P248 ; G : absence de foramen zygomatique, os zygomatique droit, vue antérieure, individu S55 ; H : foramen transverse accessoire de l'hémi-arc vertébral de, C6, vue supérieure, individu S67 ; I : possible côte lombaire, vue inférieure, individu S22 ; J : double foramens de la fosse supra-épineuse, scapula droite, vue supérieure, individu P220 ; K : epispinous notch omoplate droite, vue postérieure, individu S63 ; L : constriction de la scapula, scapula gauche, vue postérieure, individu S12-2 ; M : possible processus supra-condylaire, humérus gauche, vue antérieure, individu P289 ; N : possible processus supra-condylaire de l'individu P289, détail, vue antéro-latérale, échelle : 1 cm*

Anatomical localization	Bone/s	Non-metric variation
Cranial skeleton	occipital	Kerckring process
	vault	sutural bone / Wormian bone
	temporal	bipartite temporal
	parietal	bipartite parietal
	pars lateralis	bipartite condylar facet
		bipartite hypoglossal canal
		incomplete condylar canal (canal only present on the superior or inferior side of the pars lateralis)
		absent condylar canal
	hemi-frontal	supra-orbital foramen
		supra-orbital notch
	zygomatic	bipartite zygomatic bone
		zygomatic foramen
	hemi-mandible	bipartite mandibular condyle
Thoracic cage	spine	bipartite facet of the atlas
		bipartite transverse foramen of the vertebral hemi-arches /accessory transverse foramen
	ribs	supernumerary lumbar rib or 13th rib
		costal grill reduction
		bifid rib
		cervical rib
Pectoral girdle	scapula	foramen of the supraspinous fossa
		epispinous notch
		inferior constriction
Upper limb	Humerus	supra-condylar process

Table 1. List of cranial and infra-cranial non-metric variations assessed /
Liste des variations anatomiques non-métriques crâniennes et infra-crâniennes recherchées dans le corpus

margin of the foramen magnum (Weinberg et al., 2005). This trait, also called the "manubrium squamae occipitalis" (Virchow, 1857) or "opisthial process" (O'Rahilly and Meyer, 1956), was first described by the anatomist Kerckring in 1717 (Scheuer and Black, 2004). It is also referred to as the "Kerckring bone" when identified as a separate midline ossicle. This ossicle is reported to appear in the 4th or 5th foetal month, fusing with the occipital squama before birth, or between 2 postpartum months and 1 year (Scheuer and Black, 2004). Therefore, its embryological origin and development remain unclear. In archaeological contexts, it may be very difficult to identify a separate Kerckring bone in situ with certainty, or subsequently among cranial bone fragments. Since no separate Kerckring bone was observed in the skeletal individuals assessed for this study, the term 'Kerckring process' has been used throughout. Altogether, the Kerckring process was identified in more than a quarter of the observable individuals (n=8/30, table 2). The population-based differences are not significant according to Fisher's exact test (p=0.37).

For the *pars lateralis* (table 2), no occurrence of a bipartite condylar facet (Hauser and De Stefano, 1984) was identified in the individuals assessed (Nr=0/79, Nl=0/79). The bipartite hypoglossal canal (figure 1B) shows very low frequencies (between 0 and 3%), with 0/79 individuals showing NMV on the right element, and 2/77 on the left side. Similarly, incomplete condylar canals (Nr=5/77, Nl=5/77, figure 1C) or absent condylar canals (Nr=4/79, Nl=7/77) were rare (i.e. respectively 3% and 6% for incomplete condylar canals, 5% and 9% for absent condylar canals).

Regarding other NMV in the cranial vault, four cranial sutural bones or Wormian bones (supernumerary ossicles mostly located on the coronal or lambdoid suture [Hauser and De Stefano, 1984]) belonging to 3 individuals were identified, with one individual (P288) having two of them (figure 1D). However, due to taphonomic fragmentation, the state of preservation of each cranial bone could not be recorded with precision and the presence or absence of these ossicles could not be systematically recorded. The non-preservation of bone contours also prevented us from deducing their presence from the identification of a potential 'notch' in the bone contour, and frequencies could not be calculated.

No bipartite temporal bones (nl=0/20, 0%; nr=0/26, 0%) or parietal bones (nl=0/4, 0; nr=0/7, 0%) were identified among the cranial bones that were well enough preserved to be observed in their entirety.

Regarding hemi-frontal bones, the supra-orbital foramen (figure 1E, table 2) appears to be relatively frequent in the sample, since the trait was observed in 10/27 individuals (37%; left side) and 13/28 individuals (46%; right side) (table 2). The supra-orbital foramen was bilateral in approximately half of the cases (N=6/11, table 3). Rates of occurrence of this NMV between the samples were not significant according to Fisher's exact test (pr=0.87 and pl=0.187). Notably, the supra-orbital notch (figure 1F) was less frequent than the supraorbital foramen (Nl=4/24, 17% and Nr=2/24, 8%, table 2).

In adult skeletal individuals, the zygomatic foramen is scored as an anatomical variation when it is absent or multiple (Buikstra and Ubelaker, 1994). In these perinatal individuals (figure 1G), only foramina determined to be open, on both anterior and orbital surfaces, were scored as such. On the right side, 4/43 individuals (9%) had no zygomatic foramen, 17/43 (40%) had 2 zygomatic foramina, and 5/43 individuals (11%) had more than 2 zygomatic foramina (table 4). Among the 27 individuals that had both left and right zygoma, 17 had the same number of foramina on each side. In cases of asymmetry, the discrepancy between the

Non-metric variations	Side	Corpus			Saï			Provins			Blandy		
		n	n. obs	%	n	n. obs	%	n	n. obs	%	n	n. obs	%
Cranial skeleton													
Kerckring process	/	8	30	27	1	8	13	7	19	37	0	3	0
Bipar tite condylar facet	L	0	79	0	0	0	0	0	0	0	0	0	0
	R	0	79	0	0	0	0	0	0	0	0	0	0
Bipartite hypoglossal canal	L	2	77	3	1	39	3	1	26	4	0	13	0
	R	0	79	0	0	41	0	0	26	0	0	13	0
Incomplete condylar canal	L	5	77	6	4	39	10	1	24	4	0	15	0
	R	2	79	3	0	42	0	2	24	8	0	14	0
Absence of condylar canal	L	7	77	6	5	39	13	2	24	8	0	15	0
	R	4	79	5	3	42	7	0	24	0	1	14	7
Supra-orbital foramen	L	10	27	37	4	18	33	2	12	14	4	7	36
	R	13	28	46	5	9	36	6	15	29	2	4	33
Supra-orbital notch	L	4	24	17	0	7	0	2	13	13	2	6	25
	R	2	24	8	1	8	11	1	13	7	0	3	0
Bipartite mandibular condyle	L	0	12	0	0	0	0	0	0	0	0	0	0
	R	0	22	0	0	0	0	0	0	0	0	0	0
Infra-cranial skeleton													
Epispinous notch	L	4	29	14	3	17	18	1	11	9	0	1	0
	R	5	33	15	2	21	10	2	9	22	1	2	50
Inferior constriction of the scapula	L	3	53	6	3	27	11	0	20	0	0	20	0
	R	3	50	6	3	25	12	0	18	0	0	8	0

Table 2. Rates of occurrence and frequency for the selected non-metric variations recorded as present or absent in all three collections. Results are presented combined (corpus) and for each individual site (Sai; Provins; Blandy). L: left side, R: right side; n: total of occurrences, n.obs: total of observable individuals; %: corresponding frequency of each anatomical variant / *Occurrences et fréquences des variations anatomiques non-métriques sélectionnées cotées présentes ou absentes dans le corpus global, ainsi que dans les trois collections. L : côté gauche ; R : côté droit ; n : nombre de sujets porteurs de la variation anatomique ; n.obs : nombre de sujets observables ; % : fréquence correspondante pour chaque variation anatomique*

	N bilat.	N unilat.		
		Total	N unilat. left	N Unilat. right
supra-orbital foramen	6	5	2	3
supra-orbital notch	0	1	1	0

Table 3. Right-left distribution of hemi-frontal anatomic variations in the individuals assessed where both sides were observable. N bilat.: total of bilateral occurrences; N unilat.: total of unilateral occurrences; N unilat left: total of unilateral occurrences on the left side; N unilat right: total of unilateral occurrences on the right side / *Distribution droite-gauche des variations anatomiques de l'hémi-frontal sur les individus observables des deux côtés. N bilat. : nombre de sujets présentant la variation de manière bilatérale ; N unilat. : nombre de sujets présentant la variation de manière unilatérale ; N unilat left : nombre de sujets présentant la variation de manière unilatérale du côté gauche ; N unilat right : nombre de sujets présentant la variation de manière unilatérale du côté droit*

side	Total observable	N.ZF	n	%
L	40	0	3	8
		1	15	38
		2	17	43
		>2	5	13
R	43	0	4	9
		1	17	40
		2	17	40
		>2	5	12

Table 4. Occurrence of zygomatic foramina in the total skeletal sample. L: left side, R: right side, NZF: number of zygomatic foramina; n: total of individuals showing that number of foramina; %: corresponding frequency of each case with respect to the total of observable individuals / *Expression du foramen zygomatique dans le corpus. L : côté gauche, R : côté droit, NZF : nombre de foramens zygomatiques ; n : total des individus présentant ce nombre de foramens ; % : fréquence correspondante de chaque cas de figure par rapport au total observable*

right and left sides was typically only of ±1 foramen, except for one individual, who had one foramen on the left side and more than 2 foramina on the right (table 5). There was also no occurrence of a bipartite zygomatic bone in the individuals assessed (Nr=0/43 and Nl=0/40; Hauser and De Stefano, 1984), or of a bipartite mandibular condyle (Nr=0/22 and Nl=0/12, table 1).

Non-metric variations of the thoracic cage

Regarding the spine, no occurrence of the bipartite facet of the atlas (Saunders, 1978) was identified during analysis (nr=0/40; nl=0/43), unlike the bipartite transverse foramen of the vertebral hemi-arches, or so-called accessory transverse foramen (Saunders, 1978), which was identified in 15 individuals (figure 1H). This trait was only identified in the lower part of the cervical spine, between C4 and C6, and one individual had this variation on the left side of C4, C5 and C6.

For the ribs, the frequencies of supernumerary cervical ribs (figure 2), which is very high in the Sai collection (unilateral or bilateral ribs in 27/64 individuals, i.e. at least 42% of the sample), have been the subject of a specific article (Partiot et al., 2020), since their occurrence has previously been identified as being significantly associated with anomalies in developmental Hox genes. One individual from the Provins collection (PJ215) was also identified as having a unilateral supernumerary lumbar rib on the right side, and one rib (initially identified as a cervical rib in a Sai individual (S22) could also be a lumbar rib (figure 1I). However, unlike supernumerary cervical ribs, the presence of supernumerary lumbar ribs is not statistically associated with deleterious effects on the probability of survival (Galis et al., 2006). No bifid ribs were observed.

However, due to taphonomic alterations and limited preservation of the spinal columns and ribs in the sample analysed, the sequential presence or absence of the bipartite transverse foramen, supernumerary lumbar rib and bifid rib could not be recorded systematically, and thus prevented us from calculating frequencies. Costal grill reduction (i.e., agenesis of one or more ribs) could also not be identified.

Non-metric variations of the pectoral girdle

The foramen of the supraspinous fossa on the scapula (figure 1J, table 6) is single in about half of the observable elements (nr=29/60, nl=30/60), double in 40-45% of the cases (nr=24/60, nl=27/60), and triple in 5-12% of them (nr=7/60, nl=3/60). Symmetry between the number of foramina on the left and right supraspinous fossae (table 7) was observed in nearly 80% of the observable cases (n=35/44).

Two other variants were scored in the scapula (table 2): the epispinous notch (Hrdlička, 1942a), located on the medial-superior angle (figure 1K), and the inferior constriction (figure 1L) identified among possible variants of the bone by Hrdlička (1942b). The epispinous notch was observed on 14-15% of the observable elements (Nl=4/29 and

Zygomatic foramen		on the right			
		0	1	2	> 2
on the left	0	0	0	0	0
	1	1	7	3	1
	2	0	3	7	1
	> 2	0	0	1	3

Table 5. Expression of zygomatic foramen between left and right sides / *Expression du foramen zygomatique entre le côté gauche et le côté droit*

Figure 2. Example of a supernumerary cervical rib, individual S11, 8B-51 Classic Kerma necropolis from Sai Island, Sudan / *Exemple de côte surnuméraire cervicale, individu S11, nécropole 8B-51 du Kerma classique, île de Saï, Soudan*

side	Total observable	N.FSF	n	%
L	60	0	0	0
		1	30	50
		2	27	45
		3	3	5
R	60	0	0	0
		1	29	48
		2	24	40
		3	7	12

Table 6. Occurrence of foramina to the supraspinous fossa. L: left side, R: right side, total observable: total observable on each side; N.FSF: number of zygomatic foramina; n: total of individuals showing each number of foramina; %: corresponding frequency of each case with respect to the total of observable individuals / *Expression du foramen de la fosse supra-épineuse dans le corpus. L : côté gauche, R : côté droit, total observable : total d'individus observables du côté considéré ; N.FSF : nombre de foramen de la fosse supra-épineuse ; n : total des individus présentant chaque nombre de foramens ; % : fréquence correspondante de chaque cas de figure par rapport au total observable*

Number of foramens		on the right		
		1	2	3
on the left	1	15	1	2
	2	4	18	1
	3	1	0	2

Table 7. Expression of the foramina of the supraspinous fossa between left and right sides / *Expression du foramen de la fosse supra-épineuse entre le côté gauche et le côté droit*

Nr=5/33), and the constriction on about 6% (Nl=3/53 and Nr=3/50, table 2). For individuals where both left and right sides were observable, both variations always occurred bilaterally. The epispinous notch is observed in both the Sudanese and French samples, with no significant population-based difference between the Nubian and the West European samples according to Fisher's exact test (pr=0.31, pl=0.109). Despite the constriction of the scapula being observed only in the Sudanese sample, this population-based difference was not statistically significant according to Fisher's exact test (pr=0.11, pg=0.061).

Non-metric variations of the upper limb

The supra-condylar process, a bony spur located on the anteromedial portion of the distal third of the humerus (Verna, 2014) is rarely identified in non-adults in the archaeological record who died around birth (Partiot, 2018; Palamenghi et al., 2020; Dorado-Fernández et al., 2022). However, a bony outgrowth, projecting medially, that may correspond to this developing variation was observed on the left humerus of one individual (n=1/60, 1.7%) from the French collection of Provins (figure 1M-N).

Discussion

Investigation of population-based differences in the perinatal age group

The existence of population-based morphological variations in the perinatal skeleton has been studied primarily for forensic purposes and in medically-derived samples. However, the traits analysed in these studies are very often different from the classic variants studied in archaeologically-derived samples, and are sometimes difficult to apply to the latter (e.g. temporal squamous shape, vomer shape, subnasal margin definition, Weinberg et al., 2005). As a result, comparative data for the perinatal age group in general, and for population variation studies in particular, remain scarce (Weinberg et al., 2005; Partiot, 2018).

In general, none of the variants examined in our perinatal sample show significant population-based differences between the Kerma and the Mediaeval/Modern samples. However, the Kerckring process has been found to be absent by Kósa (1995, cited by Weinberg et al., 2005) in African perinatal samples, but it is found more frequently by Weinberg and colleagues (2005). With a process present in n=19/32 individuals from the African sample (30%), vs. in n=14/31 of the European sample (14%), this difference is still statistically not significant according to the Fisher's exact test (p=0.317).

Regarding the epispinous notch, no data for comparison were available for the perinatal age group, since the trait is only mentioned as "an occasional cleft" in the princeps publication (Hrdlička, 1942a).

Investigation of age-based differences

Furthermore, it appears that the frequencies of non-metric anatomical variations in the immature sample correspond, in 12/16 of the cases that could be compared (table 8), to the frequencies observed in comparative adult and juvenile samples, such as the Neolithic Cerny population (Parisian Basin, Thomas, 2011), or the Aleut and Egyptian samples from Hrdlička (1942a and 1942b). In 4/16 of the comparable cases, the NMV was either significantly more or less frequent in the perinatal sample compared to the other age groups. One of them is a facial trait (the supra-orbital foramen), one is on the base of the skull (bipartite hypoglossal canal), one on the upper limb (the supra-condylar process), and one on the rib cage (cervical rib).

The supra-orbital foramen is the most frequent trait in the perinate sample, since it is found in 46% of the individuals on the right side. It also is observed in 23% of the Cerny adult sample, and in 13% of the Cerny immature sample (table 8). The distribution differences between the perinate sample and the adult Cerny sample (p=0.04), as well as between the perinate sample and the immature Cerny sample (p=0.04), are statistically significant according to the Fisher's exact test. This discrepancy could be associated with bone maturation, with a trait possibly evolving as a supra-orbital notch as the bone grows. This hypothesis could be investigated, for example, in extant medical samples through longitudinal studies.

Another important discrepancy in frequencies between adult and perinatal samples concerns the supernumerary cervical rib. This variant is reported in 42% of the Kerma infant sample (with only one possible occurrence in the French perinatal sample, probably for taphonomic reasons, see Partiot et al., 2020 for more details), in 0.1% (n=19/38 105, medical sample, Steiner, 1943) and up to 3.4% (n=19/560, medical sample, Davran et al. 2017) of the adult samples (see review in Partiot et al., 2020, table 1). This statistically significant difference compared to adult frequencies ($p<0.1^{10-5}$) appears, however, to be related to the fact that this supernumerary rib can be associated with developmental anomalies and is considered as a morbidity marker in human perinatal and infant age groups (Galis et al., 2006; Bots et al., 2011; Partiot et al, 2020). The occurrence of the trait in the Kerma sample is, to our knowledge, the first to be documented in the archaeological record in this age group, and has therefore been the subject of a separate article (Partiot et al., 2020).

Regarding the NMVs with frequencies ranging from 10 to 40% in the perinate sample and the epispinous notch (Hrdlička, 1942a), the distribution differences between the perinate sample and the Aleut adult sample (p=0.49), as well as between the perinate sample and the Aleut juvenile sample (p=0.296), are not statistically significant according to the Fisher's exact test, thus no age-based difference is observed.

Global % in the perinatal sample	Nmv	% in the perinate sample	Cerny adult sample (Thomas, 2011)	Cerny juvenile sample (Thomas, 2011)	Turkish Adult sample* (Davran et al. 2017)	Adult Aleut sample (Hrdlička, 1942a)	Juvenile Aleut sample (Hrdlička, 1942a)	Adult Egyptian sample (Hrdlička, 1942b)	Juvenile Egyptian sample Hrdlička (1942b)	p-value
>40%	Supra-orbital foramen	13/28, 46%	13/56, 23%	2/15, 13%	/	/	/	/	/	<0.05
	Cervical rib	27/60, 42%	/	/	19/560, 3.4%	/	/	/	/	<0.05
10-40%	Kerckring process	8/30, 27%	/	/	/	/	/	/	/	/
	Epispinous notch	5/33, 15%	/	/	/	41/189, 21.7%	5/18, 27.8%	/	/	>0.05
1-10%	Supra-orbital notch	2/24, 8%	8/57, 14%	0/15, 0%	/	/	/	/	/	>0.05
	Inferior constriction	3/50, 6%	/	/	/	/	/	5/47, 10.7%	2/22, 9.1%	>0.05
	Absence of condylar canal	4/79, 5%	/	/	/	/	/	/	/	/
	Incomplete condylar canal	2/79, 3%	/	/	/	/	/	/	/	/
	Supra-condylar process (left side)	1/60, 1.7%	3/60, 5%	0/20, 0%	/	/	/	/	/	<0.05
	Bipartite hypoglossal canal (left side)	2/77, 3%	11/38, 29%	8/20, 40%	/	/	/	/	/	<0.05
0%	Bipartite facet of the atlas	0/40, 0%	4/43, 9%	1/12, 8%	/	/	/	/	/	**>0.05**
	Bipartite parietal	0/7, 0%	1/52, 2%	0/10, 0%	/	/	/	/	/	>0.05
	Bipartite condylar facet	0/79, 0%	1/36, 3%	0/9, 0%	/	/	/	/	/	>0.05
	Bipartite temporal	0/26, 0%	0/28, 0%	0/5, 0%	/	/	/	/	/	/
	Bipartite zygomatic bone	0/43, 0%	0/43, 0%	0/13, 0%	/	/	/	/	/	/
	Bipartite mandibular condyle	0/22, 0%	0/55, 0%	0/14, 0%	/	/	/	/	/	/

Table 8. Comparison of non-metric variation frequencies observed in the perinatal corpus with frequencies observed in samples with adult and immature individuals. Statistically significant differences are highlighted in grey; %: frequency; *: extant medical cohort; the comparative data from Hrdlička (1942a, 1942b) has been recalculated based on the frequencies indicated / *Comparaison entre les fréquences des variations anatomiques non-métriques observées dans le corpus périnatal et les fréquences observées dans des corpus de sujets adultes et immatures. Les différences statistiquement significatives sont surlignées en gris ; % : fréquence ; * : cohorte médicale actuelle ; les données comparatives de Hrdlička (1942a, 1942b) ont été recalculées sur la base des pourcentages indiqués*

Regarding the NMVs with frequencies ranging from 1 to 10% in the perinate sample, the supra-orbital notch is found in 8% on the right side; it is observed in 14% of the adult Neolithic Cerny sample (Thomas, 2011), and in none of the 15 immature individuals from Cerny (table 8). The distribution differences between the perinate sample and the adult Cerny sample (p=0.72), as well as between the perinate sample and the immature Cerny sample (p=0.51), are not statistically significant according to the Fisher's exact test, thus no age-based difference is observed.

Regarding the constriction of the scapula, which corresponds to the "type 3" concave shape of the inferior border described by Hrdlička (1942b), the distribution differences between the perinate sample and the Egyptian adult sample (Hrdlička, 1942b), as well as between the perinate sample and the Egyptian juvenile sample (p=0.638), are not statistically significant according to the Fisher's exact test (p=0.478), thus no age-based difference is observed.

On the humerus, the supra-condylar process (table 8), which was identified in 1/60 (1.7%) individuals from the

perinatal collection, occurred in 5% (n=3/60) of the adult Cerny sample and 0% of the immature Cerny sample (n=0/20) (Thomas, 2011). The distribution differences between the perinate sample and the adult Cerny sample (p=0.62), as well as between the perinate sample and the immature Cerny sample (p=1), are not statistically significant according to the Fisher's exact test, thus no age-based difference is observed. According to the review of the literature by Verna (2022, see table 1.24), this variant is also very rare, with frequencies in adult individuals ranging from 0 (American population, 8th-10th centuries, Saunders, 1978) to 4.1% (French population, 19th-20th centuries, Verna, 2014).

For the bipartite hypoglossal canal, identified on the left side in 2/77 individuals (3%) from the perinatal sample, in 11/38 (29%) from the adult Cerny sample and in 8/20 (40%) of the juvenile Cerny sample, the distribution differences are statistically significant according to the Fisher's exact test (respectively $p=10^{-4}$ and p=0). This disparity may be due to the gradual modification of this trait as the bone matures.

The frequencies of 3 NMV (i.e. the Kerckring process, absence of the condylar canal and incomplete condylar canal) could not be compared to the frequencies in adult samples, since these features are no longer present as the bone matures. The frequencies of 6 other NMVs (sutural bone, accessory transverse foramen, supernumerary lumbar rib, costal grill reduction, bifid rib, foramen of the supraspinous fossa) could not be calculated in the perinatal sample due to the highly disparate conservation status between the individuals, and for methodological reasons.

Among these NMVs, the accessory transverse foramen of the vertebral hemi-arches (Saunders, 1978) or bipartite transverse foramen (Thomas, 2011) could only be identified in 15 of the perinatal individuals assessed in this study, but due to the highly disparate state of preservation of these individuals and for methodological reasons, we were not able to calculate accurate frequencies. This trait is observed in 0 to 16% of the adult Cerny sample depending on the spine level (Thomas, 2011, see table 238). Verna's (2022) review of the literature shows also that the occurrence of this variant appears to be very variable, depending on the population and on the spine level. For example, it is reported in 0.3% of the 2nd cervical vertebra from a Swiss sample from the late modern period (Gemmerich, 1999) and in 52.4% of the 6th cervical vertebra from a French sample dating also from the late modern period (see Verna, 2014). As in our immature sample and compared with Verna's (2022) review of the literature on adults from different chrono-geographical contexts, accessory transverse foramina of the vertebral hemi-arches are more frequently identified in the lower part of the cervical spine, most frequently in C5 and C6 (for more details, see Verna, 2022, table 3.22).

The NMVs which were not identified in the perinatal sample (bipartite facet of the atlas, bipartite temporal bone, bipartite parietal bone, bipartite condylar facet, bipartite zygomatic bone, bipartite mandibular condyle) are, similarly, also non identified or very rare (table 8) in the comparative sample of adult and juvenile individuals from the Neolithic Cerny population (Thomas, 2011).

Conclusion and perspectives

At the individual and population levels, the study of NMV in immature individuals is central to improving the accuracy of biological profiling and investigating biological affinities at local and global scales, similarly to what is classically done for adults in archaeological samples and forensic contexts (e.g. Crubézy et al., 1999; Scott et al., 2018). The attributes of some of the NMVs (especially the dental traits), such as their possible heritability, their evolutionarily conservative nature and their limited sexual dimorphism make them very useful to assess patterned geographic variation, biodistances and ancestry (e.g. Scott et al., 2018; Irish et al., 2020).

At the ontogenic and developmental levels, exploring these NMVs in very young individuals as early as the foetal stage is also fundamental to understand the origin of these osteological variants and to distinguish between congenital, environmental or behavioural determinants. This distinction can be investigated by analysing their association with morbidity and individual health status (Verna and Villotte, 2016; Partiot et al., 2020), with patterned geographic variations (e.g. Irish et al. 2020), population endogamy or even cultural practices and daily activities (e.g. Partiot, 2018). Analyses of NMVs in young children can thus be incorporated into osteological and/or genetic multiproxy analyses, with the aim of examining the role of congenital, environmental and behavioural factors. This approach could be implemented, on the one hand, through transversal analyses of samples with individuals of all age groups and from the same population and, on the other hand, through analyses of extant documented medical cohorts.

Longitudinal data are also lacking, since we still do not know whether some variations, such as an accessory transverse foramen, will correspond to the same variation in osteologically mature individuals, or if an immature accessory transverse foramen will disappear due to the bone growth process. Investigations of these aspects of biological diversity in infants are therefore likely to contribute to analyses conducted for older age groups.

In view of the recent identification of morbidity-related variants such as supernumerary cervical ribs in the archaeological record (Partiot, 2020), we would like to emphasise that special attention needs to be given to atypical osteological features in the specific age group of infants. Systematic scoring of NMVs would allow the identification of recurrent combinations and their potential links to specific syndromes (Verna and Villotte, 2016). Therefore, these preliminary data will need to be completed through studies of other populations from different chrono-geographical contexts, while the reliability of the protocol will have to be tested and possibly improved by setting up intra and inter-observer tests.

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Data availability statement

The remains are available for study on request at the CNRS UMR 5199 PACEA (Bâtiment B8, Allée Geoffroy Saint Hilaire, CS 50023 33615 PESSAC CEDEX France).

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