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Association of Leu432Val (rs1056836) polymorphism of the *CYP1B1* gene with lipid profile in hypertensive Slovak women

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Abstract: Leu432Val (rs1056836) polymorphism of the *CYP1B1* gene was examined in relationship with lipid profile in hypertensive Slovak women according to their menopausal status. The entire study sample comprised 255 women suffering from hypertension aged from 39 to 65 years who were recruited from different localities in the western, southern, and middle parts of Slovakia. The participants provided a saliva or blood sample for DNA genotyping and a blood sample for biochemical analysis. The Leu432Val genotypes demonstrated statistically significant associations with all monitored atherogenic indices – total cholesterol-to-HDL-Cholesterol (AI1), Non-HDL-Cholesterol (AI2), LDL-Cholesterol-to-HDL-Cholesterol (AI3), and the logarithm of the ratio of plasma concentration of triglycerides to HDL-cholesterol (AIP log) in hypertensive pre/perimenopausal women. The mean values were significantly lower in women carrying the Val/Val genotype. In early postmenopausal hypertensive women the Leu432Val genotypes were statistically significant and associated with LDL-cholesterol (LDL-C) and AI2. The mean values of LDL-C and AI2 were significantly lower in women carrying the Leu/Leu genotype. In conclusion, the Leu432Val polymorphism may be associated with the atherogenic indices and LDL-C in hypertensive women.

Key words: hypertension, Leu432Val polymorphism, menopausal status, lipids

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Introduction

Cardiovascular disease (CVD) has the highest mortality rate in the world. The incidence of CVD is related to gender, and premenopausal women have a lower incidence of hypertension, atherosclerosis, myocardial dysfunction, ventricular hypertrophy, heart failure, and myocardial ischemia than age-matched men (Kander et al. 2017; Somani et al. 2019). Following menopause and loss of endogenous estradiol (major ovarian estrogen), these gender-based differences narrow (Patel et al. 2018). This fact suggests that estradiol protects the cardiovascular system. Estradiol induces vasoprotective effects by multiple mechanisms, including alterations in plasma concentrations of lipoproteins (decrease in low-density lipoprotein cholesterol (LDL-C) levels, decrease in oxidized LDL formation, increase in high-density lipoprotein cholesterol levels (HDL-C)), hemostatic factors, glucose, and insulin (Dubey and Jackson 2001). Estrogen deficiency after menopause is the main reason for deterioration of the serum lipid profiles (Rexrode et al. 2003; Fonseca et al. 2017).

This finding suggests that, hormonal interplay with lipid metabolism could have a significant role to play in modulating CVD risk (Dubey et al. 2005; Mc Auley and Mooney 2014). Individual genetic variability of estradiol metabolism has been described as a significant contributor to the hormone-dependent disorder susceptibility with variations depending on ethnic background. Among others, the variations of many genes encoding the cytochrome P450 (CYP) superfamily of enzymes, including variations in the *CYP1B1* gene, are considered to play an important role in this regard (Huber, Schneeberger and Tempfer 2002). The human *CYP1B1* gene has been mapped to chromosome 2 and encompasses three exons. The mRNA is 5.2 kilobases and encodes a protein of 543 amino acids (Faiq et al. 2014). Several genetic polymorphisms have been identified in the *CYP1B1* gene, and one of them, the Leu432Val polymorphism, (rs1056836; 4326C > G), located in a catalytically important heme-binding domain in exon 3 results in altered *CYP1B1* enzyme activity (Shimada et al. 1999). The *CYP1B1* 432Val allele encodes an enzyme with higher activity to 17ß-estradiol than the 432Leu variants (Tang et al. 2000). In recent years, multiple lines of evidence from both humans and mice have shown a significant role for *CYP1B1* enzyme in the cardiovascular system (Conway et al. 2009; Kaur-Knudsen et al. 2009; Song et al. 2016; Li et al. 2017; Mikstacka and Dutkiewicz 2021), development of hypertension and associated pathophysiological changes (Malik et al. 2012; White et al. 2012; Shah et al. 2019). Also, *CYP1B1* polymorphisms were associated with different types of cancers: Endometrial (Sliwinski et al. 2010; Zhang et al. 2021), breast (Matyjasik et al. 2007; Almeida et al. 2021; Martínez-Ramírez et al. 2021) and colorectal cancer (Hlavata et al. 2010; Trubicka et al. 2010). The *CYP1B1* Leu432Val polymorphism was also found to be significantly associated with the effect of hormone therapy on bone mineral density and LDL-C in postmenopausal Japanese women (Jinhua et al. 2009). In our previous pilot study (Luptakova et al. 2012), *CYP1B1* Leu432Val polymorphism appeared to modify the plasma levels of triglycerides (TG), the values of the atherogenic indices: TC-to-HDL-C ratio, and log(TG-to-HDL-C) ratio in Slovak women in their reproductive period. The mean values

were significantly lower in women carrying the Val/Val genotype.

In this cross-sectional study, we attempted to clarify the association between *CYP1B1* Leu/Val polymorphism and differences in serum lipid profile (TG, TC, LDL-C, HDL-C, atherogenic indices) and another biochemical variables in Slovak midlife women with essential hypertension in pre-/perimenopausal and early postmenopausal period of life.

Subjects and methods

This study was based on data collected during a cross-sectional survey in Slovakia to analyze the effect of genetic variants of some candidate genes on health biomarkers in Slovak women. The investigated sample comprised 255 sample of midlife women suffering from hypertension of European origin aged from 39 to 65 years, who were recruited from different localities in the western, southern, and middle parts of Slovakia. All participants were interviewed during a medical examination in the morning and were investigated with respect to their medical, anthropometric and lifestyle factors at local Health Centres. Women were approached and recruited using a non-random procedure based on volunteering and convenience. Each woman provided written informed consent for this study which adhered to the Declaration of Helsinki principles. Those who were unable to give a response due to serious physical or mental illness and with whom anthropometry and blood measurements could not be performed were excluded from the study. Data concerning lifestyle habits including physical activity, smoking, health status and menstrual cycle characteristics were investigated via a questionnaire. Women recovering from acute disorders such as cancer, myocardial infarction or stroke were also excluded from the survey. Women were divided according to their menopausal status into pre-, peri- and postmenopausal groups. Due to the low number of perimenopausal women, this group was amalgamated with premenopausal women.

Biochemical analysis

Biochemical levels of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were analyzed from fasting plasma samples by routine laboratory methods in the Department of Clinical Laboratories of the Bratislava Alpha Medical. Low-density lipoprotein cholesterol was calculated from the total cholesterol, HDL-C, and triglyceride values by the Friedewald equation if triglycerides were 4.5 mmol/L. If the serum triglyceride concentration was above this limit, LDL-C was treated as absent. The atherogenic indices were calculated as follows: AI1 = TC (mmol/l) / HDL-C (mmol/l), AI2 (non-HDL-C) = TC (mmol/l) – HDL-C (mmol/l) and AI3 = LDL-C (mmol/l) / HDL-C (mmol/l). Atherogenic index of plasma (AIP) was calculated as a logarithmically transformed ratio of molar concentrations of TG to HDL-C (mmol/l).

Anthropometric and blood pressure measurements

All anthropometric parameters were measured by professional anthropologists and the same instruments were used on all women. Anthropometric measurements were taken using the standard anthropometric technique. Body height was measured with a Sieber and Hegner anthropometer at the head level with the participant standing barefoot and with feet together, with 0.5 cm accuracy. Body weight was then measured on a personal

balance scale with the participant being barefoot and in underwear, with an accuracy of 0.1 kg. Waist and hip circumferences were measured according to the NHLBI Obesity Education Initiative (2000) and WHO (2008). Body mass index (BMI) was calculated as body weight divided by height squared. Waist-to-hip ratio (WHR) was calculated as the circumference of the waist divided by the circumference of the hips. Waist-to-height ratio (WHtR) was calculated as the circumference of the waist divided by height squared.

Resting systolic and diastolic blood pressures were obtained after a 5-minute rest, with the participant in a semi-recumbent position. Incident hypertension was defined as either by SBP ≥ 140 or DBP ≥ 90 mmHg at follow-up health examinations, a self-report of receiving treatment for high BP, and/or a physician's diagnosis of hypertension during the follow-up period. The women who underwent effective blood pressure lowering treatment were also included in our measurements. Consequently, blood pressure values in our study may have been skewed and lower than before starting the treatment.

Genetic analysis

DNA was extracted from peripheral blood samples, or saliva samples, using the SiMax™ Genomic DNA Extraction Kit; and the *CYP1B1* Leu432Val variant was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the method previously described by Luptakova et al. (2012).

Data analysis

Statistical analyses were performed using IBM SPSS for Windows (Statistical Package for the Social Science, version 20.0, Chicago, Illinois) and continuous data was expressed as mean ± SD and a two-tailed P value equal to or less than 0.05 was considered significant. Genotype distribution was analyzed using the $\chi 2$ test, and allele frequencies were assessed by the $\chi 2$ test with Yates correction. The goodness of fit evaluated whether the genotypic distribution of the *CYP1B1* Leu432Val variant matched with the Hardy–Weinberg equilibrium in hypertensive women. The normality assumption hypothesis was tested by the one-sample Kolmogorov–Smirnov test. Simple comparison of selected variables between the genotype groups, assuming an additive (AM; Leu/Leu, Leu/Val, Val/Val), dominant (DM; Leu/Val + Val/Val vs. Leu/Leu) and recessive model (RM; Val/Val vs. Leu/Val + Leu/Leu), was analyzed using the One Way ANOVA for data with normal distribution and Kruscal-Wallis Test was used for not normally distributed data.

Results

The study group of women were mostly married (69.80%), and the place of birth in towns (52.16%) prevailed. Additional baseline description such as the anthropometric, life style characteristics and distribution of the studied *CYP1B1* variant in hypertensive women are summarized in Table 1.

The participants were mostly non-smokers (70.20%), did not perform sports activities regularly (87.80%) and gained secondary education level (63.10%). The genotype distribution of the *CYP1B1* Leu432Val polymorphism was 19.30% (n = 45), 59.70% (n = 139), and 21.00% (n = 49) in hypertensive Slovak women for the Leu/Leu, Leu/Val, and Val/Val genotypes. The distribution of genotype frequencies of the polymorphism in the study women deviated from the Hardy–Weinberg equilibrium ($\chi 2 = 8.723$; df = 2; P = 0.003).

Table 1. Baseline characteristics of the study women

Variables	N	Mean		SD
Age, years	255	52.40	±	6.10
Height, cm	255	163.00	±	6.00
Weight, kg	255	79.30	±	16.30
Waist circunference, cm	255	93.09	±	14.47
Hip circumference, cm	255	108.46	±	11.83
BMI, kg/m^2	255	29.50	±	6.10
WHR	255	0.90	±	0.10
WHtR	255	0.60	±	0.10
	N			%
***CYP 1B1* (rs1056836; Leu432Val)**				
Leu/Leu	45			19.30
Leu/Val	139			59.70
Val/Val	49			21.00
Leu	229			0.49
Val	237			0.51
Smoking status				
Smokers	76			29.80
Non-smokers	179			70.20
Regular sport activity				
Yes	39			12.20
No	281			87.80
Menopausal status				
Pre-/perimenopausal	94			36.90
Early postmenopausal	161			63.10
Education				
Basic	52			20.40
Secondary	161			63.10
University	42			16.50

Notes: N, number of participants; SD, standard deviation; BMI, body mass index; WHR, waist to hip ratio; WHtR, waist to height ratio

Table 2 compares the mean values of selected biochemical variables and atherogenic indices according to the *CYP1B1* Leu432Val genotypes in the whole group of women in univariate analysis between the additive, dominant, and recessive models. No statistically significant differences were observed between Leu432Val genotypes and the monitored parameters under the different models.

Table 2. Selected biochemical variables according to the *CYP 1B1 Leu432Val (rs1056836)* genotypes in hypertensive women

	Hypertensive women									*AM*	*DM*	*RM*
	CYP 1B1 Leu/Leu			*CYP 1B1 Leu/Val*			*CYP 1B1 Val/Val*					
	N	Mean	SD	N	Mean	SD	N	Mean	SD	p	p	p
Total cholesterol (TC), (mmol/L)	38	5.21	± 0.99	120	5.44	± 1.04	38	5.57	± 0.93	0.287	0.155	0.312
Triglycerides (TG), (mmol/L)	38	1.61	± 0.74	120	1.60	± 1.31	38	1.59	± 1.02	0.678	0.378	0.825
HDL-cholesterol (HDL-C), (mmol/L)	35	1.43	± 0.33	115	1.41	± 0.43	33	1.50	± 0.31	0.548	0.918	0.277
LDL-cholesterol (LDL-C), (mmol/L)	35	3.08	± 0.94	115	3.41	± 1.03	33	3.53	± 0.85	0.127	0.051	0.318
AI1 (TC/HDL-C)	35	3.80	± 0.93	115	4.10	± 1.18	33	3.96	± 1.25	0.383	0.216	0.747
AI2 (TC-HDL-C)	35	3.80	± 0.93	115	4.02	± 1.03	33	4.13	± 1.09	0.374	0.196	0.410
AI3 (LDL-HDL-C)	35	2.24	± 0.76	115	2.60	± 1.02	33	2.53	± 1.04	0.175	0.066	0.933
AIP log (TG/HDL-C)	35	0.01	± 0.25	115	-0.01	± 0.32	33	-0.04	± 0.31	0.789	0.639	0.549

Note: N, number of participants; SD, standard deviation; AI, Atherogenic index; p, value of statistical significance; AM, aditive model (Leu/Leu, Leu/Val, Val/Val); DM, dominant model (Leu/Val + Val/Val vs. Leu/Leu); RM, recesive model (Val/Val vs. Leu/Val + Leu/Leu)

Table 3. Selected biochemical variables according to the *CYP 1B1 Leu432Val (rs1056836)* genotypes and menopausal status in hypertensive women

Pre/perimenopausal status	**Hypertensive women**									*AM*	*DM*	*RM*
	CYP 1B1 Leu/Leu			*CYP 1B1 Leu/Val*			*CYP 1B1 Val/Val*					
	N	Mean	SD	N	Mean	SD	N	Mean	SD	p	p	p
Total cholesterol (TC), (mmol/L)	12	5.43	± 0.96	38	5.50	± 0.99	13	5.11	± 0.47	0.415	0.916	0.189
Triglycerides (TG), (mmol/L)	12	1.82	± 0.94	38	1.69	± 1.07	13	1.17	± 0.54	0.112	0.211	0.053
HDL-cholesterol (HDL-C), (mmol/L)	12	1.44	± 0.37	36	1.42	± 0.42	10	1.64	± 0.31	0.292	0.821	0.117
LDL-cholesterol (LDL-C), (mmol/L)	12	3.17	± 0.92	36	3.36	± 0.87	10	2.97	± 0.56	0.402	0.699	0.242
AI1 (TC/HDL-C)	12	3.92	± 0.77	36	4.17	± 1.14	10	3.19	± 0.72	**0.032**	0.911	**0.012**
AI2 (TC-HDL-C)	12	3.99	± 0.73	36	4.12	± 0.99	10	3.42	± 0.62	0.102	0.924	**0.035**
AI3 (LDL-HDL-C)	12	2.25	± 0.60	36	2.55	± 0.89	10	1.90	± 0.61	0.069	0.564	**0.041**
AIP log (TG/HDL-C)	12	0.08	± 0.28	36	0.01	± 0.34	10	-0.24	± 0.26	0.059	0.273	**0.021**

Early postmenopausal status	Hypertensive women									*AM*	*DM*	*RM*
	CYP 1B1 Leu/Leu			*CYP 1B1 Leu/Val*			*CYP 1B1 Val/Val*					
	N	Mean	SD	N	Mean	SD	N	Mean	SD	p	p	p
Total cholesterol (TC), (mmol/L)	26	5.10 ±	1.00	82	5.41 ±	1.07	25	5.80 ±	1.03	0.062	0.089	0.047
Triglycerides (TG), (mmol/L)	26	1.51 ±	0.63	82	1.56 ±	1.41	25	1.80 ±	1.15	0.652	0.801	0.248
HDL-cholesterol (HDL-C), (mmol/L)	23	1.42 ±	0.31	79	1.41 ±	0.43	23	1.44 ±	0.30	0.966	0.985	0.802
LDL-cholesterol (LDL-C), (mmol/L)	23	3.03 ±	0.96	79	3.44 ±	1.10	23	3.77 ±	0.84	0.056	**0.046**	0.082
AI1 (TC/HDL-C)	23	3.75 ±	1.01	79	4.08 ±	1.21	23	4.30 ±	1.29	0.285	0.169	0.282
AI2 (TC-HDL-C)	23	3.69 ±	1.02	79	3.98 ±	1.05	23	4.44 ±	1.11	0.054	0.117	**0.033**
AI3 (LDL-HDL-C)	23	2.24 ±	0.85	79	2.62 ±	1.09	23	2.81 ±	1.08	0.164	0.080	0.264
AIP log (TG/HDL-C)	23	-0.02 ±	0.24	79	-0.01 ±	0.31	23	0.05 ±	0.29	0.629	0.782	0.335

Note: N, number of participants; SD, standard deviation; AI, Atherogenic index; p, value of statistical significance; AM, aditive model (Leu/Leu, Leu/Val, Val/Val); DM, dominant model (Leu/Val + Val/Val vs. Leu/Leu); RM, recesive model (Val/Val vs. Leu/Val + Leu/Leu)

Table 3 shows similar associations to Table 2 between the *CYP1B1* Leu432Val genotypes and the studied variables, but according to the menopausal status of hypertensive women. The Leu432Val genotypes demonstrated statistically significant associations with all atherogenic indices: AI1 (P = 0.032 in the additive model, and P = 0.012 in the recessive model), AI2 (P = 0.035 in the recessive model), AI3 (P = 0.041 in the recessive model) and AIP log (P = 0.021 in the recessive model) in hypertensive pre/perimenopausal women. The mean values of these atherogenic indices were significantly lower in women carrying the Val/Val genotype. (Apart from??) Between other biochemical variables and Leu432Val genotypes there were no observed statistically significant differences in hypertensive pre/perimenopausal women. On the other hand, Leu432Val genotypes in early postmenopausal hypertensive women were statistically significant associated with LDL-C (P = 0.046 in the dominant model) and AI2 (P = 0.033 in the recessive model). The mean values of LDL-C and AI2 were significantly lower in women carrying the Leu/Leu genotype.

Discussion

Genetic polymorphisms of cytochromes P450s may affect the enzyme catalytic activity and have been reported among different populations to be associated with various diseases and adverse drug reactions (Elfaki et al. 2018a). Polymorphisms in *CYP1B1* were reported to be causes of disease phenotypes such as diabetes mellitus (Elfaki et al. 2018b), hypertension or coronary artery disease (CAD) (Mir et al. 2021). Park et al. (2015) reported that *CYP1B1* genetic variations in interaction with the 25-hydroxyvitamin D affect blood pressure, especially in individuals currently being treated for hypertension. Recently, we have revealed that *CYP1B1* rs1056836 was associated with hyperten-

sion in women, while Val allele was a risk factor for the increased hypertension incidence (Falbova et al. 2020).

Since estrogen has an antiatherogenic action along with lipid lowering abilities, and because the products of genes involved in estrogen metabolism markedly regulate estrogen concentrations, associations between the effect of these genes and lipid levels are also expected. Although, there are some studies indicating a significant association between DNA variants in genes related to estrogen biosynthesis and estrogen catabolism with serum lipid and lipoprotein levels, such as *CYP19A1* in Turkish non-obese females (Coban et al. 2015) or *CYP1A1* in Brazilian women of European descent (Almeida et al. 2005), there is a lack of studies tracking the relationship between *CYP1B1* and the lipid profile. In the present study, we have observed a significant association between *CYP1B1* rs1056836 and lipid profile in Slovak hypertensive women. To the best of our knowledge *CYP1B1* polymorphisms have not been investigated in relation to lipid profile in any east central European population study, with the exception of our two previous studies (Luptakova et al. 2012; Cernanova et al. 2018). The first study revealed that *CYP1B1* rs1056836 was responsible for higher values of atherogenic indices in apparently healthy pre-/perimenopausal women without any serious diagnosis; and, the second study observed the significant association between *CYP1B1* rs1800440 and plasma levels of HDL-cholesterol in postmenopausal women. There are, however, several studies that investigated the association of the *CYP1B1* polymorphisms with lipid profile in other countries/regions. In an Indian cohort with CAD the *CYP1B1* rs1056827 was strongly associated with an increased serum levels of cholesterol, HDL-C, and LDL-C (Mir et al. 2021). Hu, Lin and Chen (2008) observed significantly higher mean levels of HDL-C, LDL-C, and TC in workers from a municipal waste incineration plant in Taiwan carrying the *CYP1B1* rs1056836 Val allele than in those carrying the Leu/Leu genotype.

In this study, we found that women in premenopause with Val/Val genotype had significantly lower values of all investigated atherogenic indices than Leu allele premenopausal carriers. Several biological pathways might shed light on this finding. It has been shown that *CYP1B1* catalyzes the metabolism of 17 β-estradiol into reactive metabolites, such as 4-hydroxyestradiol (4-OH-E2) (Smerdova et al. 2014). Since the *CYP1B1* 432Val allele encodes an enzyme with higher activity to 17ß-estradiol than the 432Leu variants (Tang et al. 2000), women possessing the Val allele might have higher levels of 4-OH-E2. Wang and Zhu (2017) found that 4-OH-E2 had a markedly stronger effect in reducing the adipocyte size and serum cholesterol level in female rats compared to 17β-estradiol. Therefore, the *CYP1B1* Val variant, through a higher concentration of 4-OH-E2, may contribute to lower lipid levels in women before the 17 β-estradiol deficit causes the onset of menopause.

There is also a possible explanation for the observed association between *CYP1B1* and lipid profile in postmenopausal women. Accumulating evidence suggests that *CYP1B1* alters the expression of 560 genes in the liver, including PPARγ (Larsen et al. 2015). Duval, Müller and Kersten (2007) reported that PPARα modulates lipoprotein metabolism whereas activation of PPARα results in a reduction of plasma TG levels and in an increase of plasma HDL levels. However, estrogen inhibits the actions of PPARα on lipid metabolism through its

effects on PPARα-dependent regulation of target genes (Yoon 2009). Thus, this association between *CYP1B1* and lipid profile seems to be apparent in postmenopausal women, but not in premenopausal women with functioning ovaries. Moreover, the results of some studies demonstrate that Val/Val genotype is associated with lower *CYP1B1* mRNA expression than the *CYP1B1* Leu/Leu genotype after induction with environmental factors, such as benzo(a)pyrene or smoking (Helmig et al. 2009; Helmig et al. 2010; Helmig et al. 2014). Therefore, *CYP1B1* Val variant may have a lower impact on PPARγ activation than Leu variant, which may probably be reflected in negative changes in serum lipid levels. This evidence can at least partially explain the worse lipid profile in Val/Val genotype carriers in postmenopause detected in our study.

Despite the above studies and our seminal findings, there are also some limitations that need to be acknowledged. As our study was cross-sectional and may have had selection bias during case recruitment, this particular design can limit generalization of our results to all Slovak women. Our study was also limited by the sample size of study women (n = 255). Therefore, we would recommend that future studies enlarge the study sample for a more detailed analysis. Moreover, the role of *CYP1B1* polymorphism in lipid metabolism remains unexplained and the exact mechanism of its likely effect on the lipid profile in pre- and postmenopausal hypertensive women is unclear. Thus, future research into the mechanisms of *CYP1B1* is warranted.

Conclusion

In conclusion, our study results demonstrate that the Leu432Val polymorphism may be associated with the atherogenic indices and LDL-C in hypertensive women. Since the data presented here are the first attempt to associate *CYP1B1* polymorphism with lipid and lipoprotein parameters in hypertensive women, replications of the present findings in larger samples are warranted.

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The Authors' contribution

DF contributed to the conception, design, and performance of the study, and writing of the manuscript. LV participated in collection of data, analysis and interpretation of data, and writing of the manuscript. VCČ participated in collection of data. RB was responsible for the statistical analysis. DS was innovator for the project, participated in the conception, design, data collection andperformance of the study.

Conflict of interest

The authors declare that there is no conflict of interest.

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Sacral Spina Bifida Occulta: A Frequency Analysis of Secular Change

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Abstract: Substantial relaxation of natural selection beginning around 1900 changed the mutation/selection balance of modern genetic material, producing an increase in variable anatomical structures. While multiple structures have been affected, the temporal increase in variations of the sacrum, specifically, 'Sacral Spina Bifida Occulta,' have been reliably demonstrated on a localised scale. Calculation of largescale frequency has been hindered by the localised nature of these publications, the morphological variability of this variation, and potential pathological associations, which have produced divergent classifications, and conflicting reported rates of occurrence. A systematic review of the reported literature was conducted to provide an objective analysis of Sacral Spina Bifida Occulta frequency from 2500 BCE to the present. This review was designed to compensate for observed inconsistencies in reporting and to ascertain, for the first time, the temporal trajectory of this secular trend. A systematic review of Sacral Spina Bifida Occulta literature was conducted through the strict use of clinical meta-analysis criteria. Publications were retrieved from four databases: PubMed, Embase, the Adelaide University Library database, and Google Scholar. Data were separated into three historical groups, (1 = <1900, 2 = 1900 to 1980 and 3 = >1980), and frequency outcomes compared, to determine temporal rates of occurrence.

A total of 39/409 publications were included in the final analysis, representing data for 16,167 sacra, spanning a period of 4,500 years. Statistically significant results were obtained, with total open S1 frequency increasing from 2.34%, (79 to 1900CE), to 4.80%, (1900 to 1980CE) and to 5.43% (>1980CE). These increases were significant at $p<0.0001$, with Chi-squared analysis. A clear secular increase in the global frequency of Sacral Spina Bifida Occulta has been demonstrated from 1900 to the present. This research provides a novel and adaptable framework for the future assessment of variation distribution, with important implications for the fields of biological anthropology and bioarchaeology.

Key words: Sacral Spina Bifida Occulta (SSBO), frequency, classification, natural selection
Abbreviations: Sacral Spina Bifida Occulta (SSBO), Spina Bifida Cystica (SBC), Neural Tube Defect (NTD)

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Introduction

The relaxation of natural selection can be attributed to the decreased rate of infant mortality and the increased rate of adult survivability from 1900 onwards (Ulizzi et al. 1998). These changes were shaped by improved clinical understanding of disease, the invention of increasingly effective medication, and an improvement in prenatal and postnatal medical care (Ruhli and Henneberg 2013; Solomon et al. 2009). Consequently, survivorship to the age of reproduction (15 years) increased from <50% in 1850 to slightly >90% by 1900 (Greene 2001). Overall, the probability that an average person born into a population will be able to pass their genes to the next generation rose from 0.30 to 0.95 (Saniotis and Henneberg 2011). This reduction in the opportunity for selection, altered the mutation/selection balance which precipitated phenotypic variation (Cairnes and Gariepy 1990; Lee et al. 2011). Such an increase has been observed in a number of modern physiological, immunological, and morphological characteristics, the most well-known of which is the increase in lactose intolerance and the congenital absence of the third molar (Ingram et al. 2009; Swallow 2003). Occurring over a relatively short period of evolutionary history, (120 years), these modern secular changes have been observed in multiple correlating anatomical structures.

One such example includes increases observed in the retention of the embryonic variant, the median artery. This embryonic vessel typically regresses at 8 weeks gestation, but retention of this artery into adulthood has experienced an increase of 20%, over a period of the last 170 years (Lucas et al. 2020). The atypical fusion of one or more tarsal bones of the foot has also been subject to observed increases after 1900, with an increase of >12%, evidenced over a period of 50 years (Ruhli et al. 2003). The timing of these changes in anatomical structures, coincides with observed increases in sacral variations, most specifically the 'vertebral anomaly' Sacral Spina Bifida Occulta, (SSBO).

Sacral Spina Bifida Occulta (SSBO) is a condition difficult to define due to the variability with which it is described in the literature, and the broad spectrum of defects this condition can represent (Albrecht et al. 2007; Eubanks and Cheruvu 2009). SSBO is often considered the mildest manifestation of Neural Tube Defect (NTD), specifically of the highly debilitating Spina Bifida Cystica (SBC), which has been identified as the most common congenital anomaly of the 21st century (Kallen and Lofkvist 1984; Morrison et al. 1998). Characterised skeletally, by the absence or non-fusion of one or multiple posterior vertebral arches, SSBO variably includes deformation of the laminae, neural arch, or pedicles of vertebrae (Post 1966; Sutow and Pryde 1955). While this anomaly can occur at any level of the vertebral column, the malformation of the last lumbar vertebra and the first sacral vertebra is the most routinely observed, studied, and reported (Sairyo et al. 2006). Due to the severity of deformation caused by SSBO, (which typically presents as the exposure of the sacral canal, or absence of the dorsal wall), this condition is easily identifiable in dry human sacra, and can be reliably distinguished from post-depositional erosion or damage, (Figs 1, 2 and 3). Therefore, observations of this condition in dry human sacra are reliable, and publications which provide

frequency data in this context can be assumed to be accurate and objective.

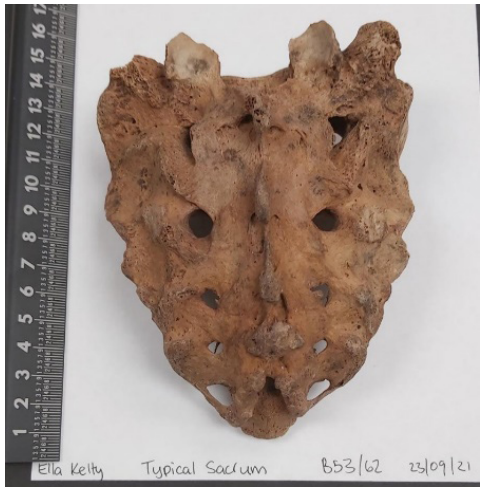

Fig. 1. Dry human sacrum with a typically formed dorsal wall – fully fused sacral vertebrae. (Photograph taken by lead author (Kelty 23/09/2021). Specimen B53 from St Marys archaeological collection, ethically held by The University of Adelaide).

Fig. 2. Dry human sacrum demonstrating 'Total SSBO' or non fusion of arches of all sacral segments. (Photograph taken by lead author (Kelty 23/09/2021). Specimen B79 from St Marys archaeological collection, ethically held by The University of Adelaide).

Fig. 3. Dry human sacrum demonstrating non-fusion of sacral segments, S1 and S4-S5. The most commonly observed configuration of S1 non-fusion. (Photograph taken by lead author (Kelty 23/09/2021). Specimen B61 from St Marys archaeological collection, ethically held by the University of Adelaide).

Deformation at all levels of the sacrum can occur with varying degrees of regularity, dependent on the sacral segment involved. The most common observation of non-fusion occurs at segments S4-S5, which can reach upwards of 90% of individuals with European ancestry (Fidas et al. 1987). Thus, this deformation pattern is clinically recognised as a natural morphological variation, termed the sacral hiatus (Abera et al. 2021; Henneberg and Henneberg 1999). Deformations of segments S2 and S3 have lower frequencies, (1% to 10%), but to date are also considered natural variations, due to their sometimes inclusion into the hiatus (Simriti et al. 2017). This inclusion of S2-S5 in the natural variation of the sacrum suggests that

these specific patterns of deformation have no pathological associations and are therefore of no clinical importance (Kumar and Tubbs 2011).

Non-fusion of the first sacral segment usually has a lower frequency, similar to that of S2 and S3, but is unreliably reported, with estimations ranging from 8% (Piontek 1971) to 23% (Sairyo et al. 2006). Unlike the segments S2-S5, S1 has clear pathological associations having been reliably correlated with enuresis, posterior disk herniation, and lower back pain (Eubanks and Cheruvu 2009; Sutow and Pryde 1955). Non-fusion of sacral segments inclusive of S1 can be considered morphologically and clinically important, irrespective of non-fusion or fusion of other segments. It is for this reason that Sacral Spina Bifida Occulta can be specifically defined as non-fusion inclusive of the first sacral segment (Henneberg and Henneberg 1999; Lee et al. 2011; Solomon et al. 2009).

The Pelvis (Os Coxae) is the most variable aspect of the human skeleton due to its high levels of sexual dimorphism, with the sacrum being considered the most variable aspect within that structure (Steyn and Iscan 2008). Variations to the structure of the sacral canal can also influence variation in the resulting morphology of the surrounding Os Coxae (Kurki 2013). The degree to which these variations can influence pregnancy, birth, overall health, and forensic sex identification, has resulted in a relative wealth of clinical, anthropological, and archaeological assessments of this variation over the last century (Henneberg and Henneberg 1999). The identification and classification of Sacral Spina Bifida Occulta was first described in the anthropological literature by Willis (1923). Willis popularised the characterisation of this condition as a 'vertebral anomaly' of no clinical significance, recording only a 1.2% frequency in 748 historical subjects (Willis 1923). Anthropological interest in SSBO was shaped by this definition but was characterised by inconsistency in reported frequencies. Ferembach (1963) famously reported a 76% frequency in a sample from 12,500 BCE, but this was hard to substantiate, due to the small sample size and the 8% to 23% occurrence which typified the literature of this period. Inconsistencies in reported SSBO frequencies were further exasperated by the clinical recognition of this condition, which aligned with investigations of neural tube defects in 1980 and introduced a new generation of conflicting classifications and frequency calculation methods (Molloy et al. 2017; Scatliff et al. 2013).

Investigations into the temporal increase of SSBO frequency in the modern era, and its correlation to the relaxation of natural selection around 1900, have ultimately been impeded by the number of academic debates, controversies and disagreements which characterise this research area (Shore 1930; Zemirline et al. 2013). A long-standing consensus within the medical community that anatomically modern humans are no longer evolving under the operation of natural selection, has prevented largescale research into these changing anatomical structures and their potential impact on the health of future populations (Kumar and Singh 2003; Rühli and Henneberg 2013). While small scale and localised studies have been conducted which reliably support this correlation between various changing modern anatomical structures and the relaxation of natural selection in the industrialised world from 1900 onwards, (Lucas et al. 2020; Rühli et al. 2003) large-scale assessments and

widespread acceptance of this phenomenon have yet to be established.

This observed lack of academic consensus has prevented reliable calculation of SSBO frequency over time, which is additionally impeded by the small number of publications which contain reliable data for this condition (Zemirline et al. 2013). These inherent limitations have been addressed by modern SSBO research, which provides more reliable assessments of frequency than historically observed (Kumar and Singh 2003). Interestingly, an 11% frequency of this condition was observed in Pompeii (79CE, Henneberg and Henneberg 1999), being about one half of modern European assessments of about 20% (Saluja 1988). This led Henneberg and Henneberg (1999) to suggest that a secular and microevolutionary trend could be observed in SSBO frequency. This increase was further substantiated by Solomon et al. (2009) and Lee et al. (2011), who demonstrated an increase in the frequency of SSBO at S1 in Australian and European birth cohorts, from 1940s to 1980s. This is interesting, as these localised studies demonstrate an increase that not only correlates with the observed relaxation of natural selection around 1900, but that also coincides with relative increases in similar anatomical variations during the same period. It is therefore hypothesised that the generation of a large-scale, geographical, and temporal assessment of SSBO frequency will produce evidence of a clear secular trend in the increase of this condition from 1900 onwards.

Materials and Methods

A literature review was performed to collect all available publications pertaining to SSBO frequency as previously defined. This review generated a total of 409 foundational or peer-reviewed publications. Predetermined exclusion criteria were used to determine the relevance of each publication and assess the quality of their reported segmentation data (Fig. 4). In total 39 of 409 (<10%) publications were included in the frequency analysis, producing a total sample size of 16,167 sacra, which spanned 25 international regions (Fig. 5) and a period of 4,500 years. Male and female sample sizes were also recorded where reported, with a total male sample size of 3,992 and female sample size of 3,818, with 8,357 (51.69% of 16,167) having undesignated sex.

In order to reliably evaluate the true frequency of SSBO it was necessary to design a method that could enable the review of all available and relevant literature, while overcoming observed inconsistencies in classification and frequency calculation. It was also imperative to demonstrate that modern human skeletal anatomy is subject to evolutionary change, and that increases in SSBO frequency directly correlate with the recent relaxation of selection shift. The literature was collected, assessed, and analysed according to strict clinical meta-analysis guidelines to ensure that data were reviewed systematically (Balduzzi et al. 2019; Page et al. 2021). As this research does not contain clinical trials or patients, some meta-analysis criteria could not be applied, and the decision was made to conduct a quantitative literature review/frequency analysis instead. To guarantee cohesion, validity and accuracy within the research design, all analyses were conducted according to the requirements of a meta-analysis where possible (Higgins et al. 2003; Page and Moher 2017).

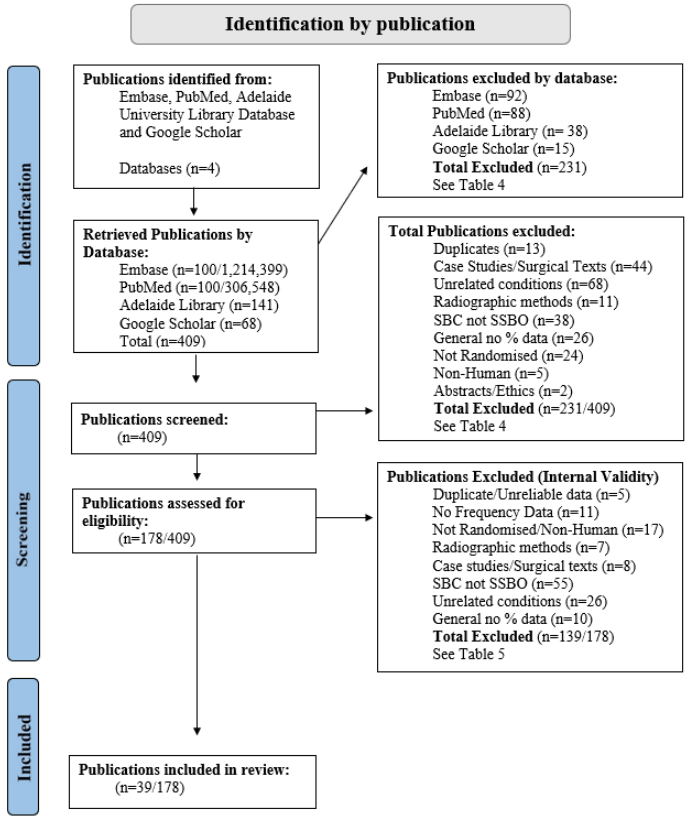

Fig. 4. PRISMA Flow chart of literature analysis method, with added inclusion/exclusion criteria.

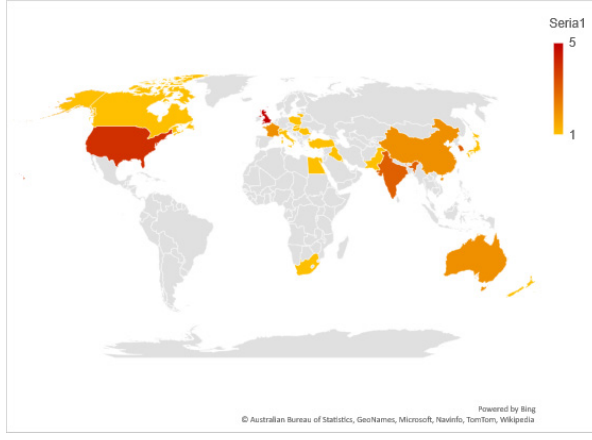

Fig. 5. World map showing distribution of included SSBO data for the literature analysis. Country of origin and number of publications per country included.

Literature Review Method

Publications for the literature review were collected through the use of Embase, PubMed, Google Scholar, and the University of Adelaide's library database. Embase and PubMed were used primarily for the collection of clinical literature relating to SSBO and did not include anthropological and archaeological data for this condition, particularly not from the early 20th century. Google Scholar was used as a means to find those publications not available in the medical literature databases, and the University of Adelaide Library was used to gain access to those publications found in Google Scholar that were restricted by paywalls. The Adelaide University Library database was the most practical resource to use for supplementary access to these publications, as both authors are members of The School of Biomedicine at this University.

Databases were searched for keywords; *Sacral Spina Bifida Occulta, Spina Bifida Occulta, Neural Tube Defect, Spina Bifida* and *Occult Spinal Dysraphism*. Keywords were supplemented by corresponding searches for, *incidence, prevalence, frequency,* and *rates*. Results from each database underwent two rounds of screening, the initial publication screening, (12/2/2021 – 03/05/2021) and the internal validity screening, (13/09/2021 – 2/10/2021), which included different criteria. The initial publication screening was conducted through a process of examining the abstract, results and conclusions of each publication, and including/excluding each publication based on a set of predetermined exclusion criteria (Fig. 4) (Balduzzi et al. 2019). Publications that were included through the initial publication screening were then analysed a second time with more stringent predetermined exclusion criteria, which focussed on the assessment of the internal validity of each study (Page et al. 2021).

Different retrieval strategies were used dependent on the database. PubMed and Embase are clinical databases that were used to source potential clinical data on the frequency of SSBO. Due to the volume of publications generated from such expansive databases, as a result of the search strategies outlined (Table 1), only the top 100 search results were included for screening. The University of Adelaide library and Google scholar databases were used primarily to retrieve anthropological and archaeological data on the frequency of this condition. Due to the nature of these databases, specific search strategies were not used, however, each afore mentioned key word was searched, and any relevant publications were retrieved. This was further complemented by the use of these databases to retrieve publications cited in already analysed works and to expand upon the key words to include, *sacral hiatus, paleoepidemiology, sacral anomaly, sacral deformity and osteoarchaeology*.

It should be noted that due to the nature of SSBO and its presence in historical and archaeological populations, this review of the literature is amalgamating already published data on the frequency of this condition in dry human sacra, from cadaver studies and through anonymised radiographic data. Therefore, this review is bio-anthropological and does not include patients, clinical trials, medical equipment, additional reviewers, ethics approval or funding grants. All publications were reviewed by the lead author manually, no automation process or equipment was used, and no additional reviewers or external parties were involved.

Table 1. Search strategies for publication retrieval from each database

Database	Keywords	Search String*	Retrieved/ Generated*
PubMed	Sacral Spina Bifida Occulta, Spina Bifida Occulta, Neural Tube Defect, Spina Bifida and Occult Spinal Dysraphism. + Incidence, Prevalence, Frequency, and Rates	(Sacral Spina Bifida Occulta) OR (Spina Bifida Occulta) OR (Neural Tube Defects) OR (Spina Bifida) OR (Occult Spinal Dysraphism) AND (Incidence) OR (Rates) OR (Frequency) OR (Prevalence)	100/ 306,548
Embase	Sacral Spina Bifida Occulta, Spina Bifida Occulta, Neural Tube Defect, Spina Bifida and Occult Spinal Dysraphism. + Incidence, Prevalence, Frequency, and Rates	Exp spinal dysraphism / or exp neural tube defects / or exp open spinal dysraphism / and exp incidence / or exp frequency / or exp prevalence /	100/ 1,214,399
The University of Adelaide	Sacral Spina Bifida Occulta, Spina Bifida Occulta, Neural Tube Defect, Spina Bifida and Occult Spinal Dysraphism. + Incidence, Prevalence, Frequency, and Rates + Sacral Hiatus, Paleoepidemiology, Sacral Anomaly, Sacral Deformity and Osteoarchaeology	N/A	141
Google Scholar	Sacral Spina Bifida Occulta, Spina Bifida Occulta, Neural Tube Defect, Spina Bifida and Occult Spinal Dysraphism. + Incidence, Prevalence, Frequency, and Rates + Sacral Hiatus, Paleoepidemiology, Sacral Anomaly, Sacral Deformity and Osteoarchaeology	N/A	68

*Search string and number of publications generated are not applicable to the University of Adelaide Library and Google scholar databases.

Initial publication screening parameters

Once all 409 publications had been retrieved, they underwent the initial screening process and were included/excluded based on stringent predetermined criteria, (Fig. 4). As the primary objective of this literature analysis was to obtain frequency data for SSBO, publications that did not include frequency data for this condition, data for SSBO specifically, or those that did not include random samples, were excluded. Case studies which discussed only single examples and therefore had no frequency data were excluded. Surgical texts were also excluded on the basis that they related to the diagnosis, management, treatment, and

surgical outcomes of spinal dysraphism. As such the frequency of occurrence was not reported, as all individuals observed had already been diagnosed with this condition. A singular list of abstracts for a conference on the neurosurgical management of spinal dysraphism was also excluded due to lack of detailed frequency data. Publications detailing novel radiographic methods for the identification of SSBO were also excluded, as prevalence data were not reported. One publication pertained solely to ethics, one was in reference to widescale arsenic poisoning, and a number were related to non-human clinical trials which were of no relevance to this research.

'General SSBO' included publications that were designed as informative documents on the identification, diagnosis, and treatment of SSBO from a clinical perspective. These publications did not include frequency data, and more than half were in reference to SBC not SSBO. This misidentification of SSBO as the neural tube defect SBC, was also an exclusion criterion. A number of publications retrieved from Embase, and PubMed also included publications on pathologies completely unrelated to SSBO. Issues in identifying SSBO data specifically, were further complicated by the number of associated pathologies researched clinically. Publications relating to these pathologies, were also assessed, and included only if the frequency data were wholly separated from those of the associated pathology, and if adequate and appropriate control groups were used (Page and Moher 2017; Page et al. 2021).

A disproportionate number of the retrieved publications were related to Spina Bifida Cystica (SBC) and Neural Tube Defects, and included no reference to, or data for SSBO. This was the consequence of an early proposal to compare frequency data for these two conditions to ascertain the importance of their relative patterns of occurrence. As this research progressed, it was determined that SBC frequency was already reliably established in the literature, and therefore these studies were not included in the final analysis (Fig. 4). Trusted data for SBC, however, were obtained from national and global birth registers, derived from these excluded publications, to compare the relative prevalence of this condition with that of SSBO for the same period (Atta et al. 2016). This was achieved through the calculation of mean values for reported births with SBC per 1000, in European populations. These are not included in the results but were generated for the purpose of aiding the discussion.

Internal Validity Screening Parameters

Once these publications had been screened for the more basic parameters, (inclusion of SSBO frequency data), the remaining 178 publications were subjected to an additional, more stringent, screening process, to access the internal validity and address the risk of bias in their results (Higgins et al. 2003). The quality of included segmentation data was assessed, and those that did not include data for deformation of S1 specifically, or which reported duplicate data, were excluded. Archaeological and anthropological texts from the early 20th century which included purely textual anecdotes, were thoroughly scrutinised to ensure that sample sizes and case numbers were accurate and did not contain any missing or unclear data. Any uncertainty as to the clarity, totality, or accuracy of the data

from these publications resulted in them being excluded to ensure the generation of meaningful and reliable results (Higgins et al. 2003). Publications that included duplicate data already screened in previous publications, or data that could not be reliably differentiated from other osteological assessments from similar or sometimes the same archaeological sites, were also excluded.

Those publications which assessed the association between SSBO, and a range of pathologies were also assessed to ensure that frequency data for SSBO were wholly separable from those of the associated pathology, and that adequate control groups had been used. Those which did not provide adequate control groups, or studies which included only patients with a pathology, or deformity, reliably associated with SSBO, (eg: Cutaneous stigmata), were excluded on the basis that they did not represent the true frequency of this condition. Radiological assessments of this condition, which focussed on novel methods for the identification of SSBO, were also excluded if they contained zero or duplicate frequency data for this condition, or if the level of deformity, (segment), was not reported.

Once completed, this review of the current literature produced 39/409 publications for inclusion into the frequency data analysis. The included publications, as outlined in detail in the appendix, ranged in publication date from 1932 to 2019, 30 of these were peer reviewed, with the remaining nine having been published before the introduction of the peer review system. All included publications reported SSBO frequency data that were analysed and deemed reliable, and no publication was assessed which examined the frequency of SSBO and did not produce at least one case of this condition.

Frequency Analysis Methods

A total of 39/409 publications were included in the final frequency analysis having conformed to the outlined inclusion criteria, (Fig. 4). Numbers were allocated to each publication and citation, location and dating details were recorded for each. Reported case numbers of identified SSBO were divided by reported sample sizes, and multiplied by 100, to produce percentage values. This was completed for each possible combination of reported deformation, across all sacral segments. This included deformation of segments inclusive of S1, (ie: L5-S1, S1andS5), and calculation of male and female frequencies (Henneberg and Henneberg 1999; Lee et al. 2011; Solomon et al. 2009).

Recalculations were made where reported prevalence was not clearly presented, with some cases and sample sizes being combined where necessary, (control/patient and multiple juvenile samples). Patient groups that were proven to be random (not commonly or primarily associated with SSBO) were combined with control group sample sizes, and case numbers, to determine frequency for the whole group. Publications that separated subadults (1–15 years) into smaller sub-divisions of age, (eg: 1–2 years, 3–4 years etc.) were also grouped together, and an identical method was used to determine the relative frequency (Page et al. 2021). Similar additions were also made with the male and female frequency calculations. This occurred where male and female cases were recorded for both the control and patient groups, which were then combined to determine the frequency, as per the method outlined above. Instances where sex was separated into age categories, of girl/boy, female/male structure, were also combined to determine the frequency by sex (Fidas et al. 1987).

Once this information had been collected for all 39 publications, the resulting data were separated into three distinct historical groups. This was done to consider the 4,500-year time span, to test the hypothesised increase of this condition after 1900 and 1980 and to ensure that each study would be accurately weighted. This separation was determined according to calculated date of birth of each group. Birth dates were either used as reported in more modern publications or estimated by subtracting average life expectancy figures from burial dates for historical collections (WHO 2012; WHO 2020). Historical Group 1 (HG1) included date range 2,500BCE to 1,900CE, the second Historical Group (HG2) encompassed all material dating from 1,900 to 1980CE while Historical Group 3 (HG3) included the remaining data for the period 1980CE to 2020CE. Male and female frequency data, where available, were also separated into historical groups, although an absence of reported sex data for HG3 did affect the results of this group.

Statistical methods

While data for SSBO were recorded for each sacral segment, only data for deformation inclusive of S1 were included into the statistical analysis. Total sacra observed, and total number of cases were determined for each historical group. Contingency tables were generated in the SSPS.25 software, (Tables 2 and 3) and Chi-squared calculation with Yates's correction, and corresponding *p*-values, were used to assess the direction of effect for these three groups (Henneberg and Henneberg 1999; Lee et al. 2011; Solomon et al. 2009). The available male and female data for all three historical groups were treated in the same way.

All statistical calculations were performed using the SSPS.25 software by the primary author with instruction and assistance from the secondary author. No external resources were used to complete this analysis and no additional reviewers were integrated into the assessment.

Table 2. Contingency table used to generate Chi-Squared statistic for the total frequency of SSBO

	Historical Group 1 <1900	Historical Group 2 1900-1980	Historical Group 3 >1980
Total Sacra Observed	6,901	8,074	1,192
Total SSBO Cases Identified	922	1,503	281

Total frequencies determined for comparison of Historical Groups 1 and 2, 2 and 3 and 1 and 3.

Table 3. Contingency table used to generate Chi-Squared statistic for the male/female frequencies of SSBO

	Historical Group 1 <1900	Historical Group 2 1900-1980	Historical Group 3 >1980
Total Male Sacra Observed	790	2,883	319
Total Male Cases of SSBO	98	738	46
Total Female Sacra Observed	720	2,830	268
Total Female Cases of SSBO	69	458	74

Male and Female frequencies determined through comparison of Historical Groups 1 and 2, 2 and 3 and 1 and 3.

Results

A total of 39 publications were included from the 409 retrieved and screened during the literature review process (Fig. 4). The results of both screening processes, with exclusion/inclusion criteria outlined for each publication, throughout each process, are presented in the appendix. Citation details and exact frequency data collected from each included study are also included in the appendix.

The frequency analysis results demonstrated a clear and statistically significant increase in the frequency of SSBO after 1900 (Table 4 and Fig. 6). The calculation of the total frequencies for historical groups one and two, evidenced a 5.25% increase in SSBO frequency, (<1900 to 1980), Chi-squared 54.503 (p<0.0001). The comparison of total frequencies between historical groups two and three also provided a very statistically significant result, with a 4.98% increase, Chi-squared 10.543 (p<and a p-value of <0.0012). Total frequency comparison was also completed between historical groups one and three, which demonstrated an increase of 10.23% from the period <1900, to the present, Chi-squared 57.843 (p<0.0001).

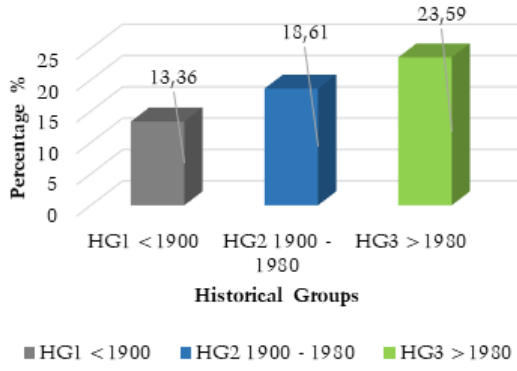

Fig. 6. Total SSBO frequency by historical group. Data derived from numerical analysis of included literature and frequency calculations outlined in Table 4.

Table 4. Total data included in each historical group

	Historical Group 1 <1900	Historical Group 2 1900-1980	Historical Group 3 >1980
Total Included Publications	18	17	7
Total Included Studies	28	40	7
Total Sacra Observed	6,901	8,074	1,192
Total SSBO Cases Identified	922	1,503	281
Total Frequency	13.36%	18.61%	23.59%
Total Increase	2.40%	5.25%	4.98%
Chi-Square Value	HG1-HG2 54.503	HG2-HG3 10.543	HG1-HG3 57.843
p-Value	<0.0001%	<0.0012%	<0.0001%

Detailed representation of the total data for each historical group includes significance calculations and data used to calculate frequency.

The male and female frequency calculations for the three historical groups also produced statistically significant results for an increase of SSBO after 1900 and 1980. A clear increase was demonstrated in males (13.19%) and females (6.60%) for historical groups one and two, generating Chi-squared values of 40.618 and 14.737 (for both $p<0.0001$). A decrease was observed in male frequency of -11.17%, (Chi-squared=12.209, $p<0.0005$) between historical groups two and three, but an increase was demonstrated for females (Chi-squared = 14.105, $p<0.0002$). (Table 5 and Fig. 7).

Table 5. Male and female data included by historical group

	Historical Group 1 <1900	Historical Group 2 1900-1980	Historical Group 3 >1980
Total Included Publications	6	10	4
Total Included Studies	10	23	4
Total Male Sacra Observed	790	2,883	319
Total Males Cases of SSBO Identified	98	738	46
Total Frequency	12.40%	25.59%	14.42%
Total Increase/Decrease	N/A	+13.19%	-11.17%
Chi-Squared Value	N/A	HG1-HG2 40.618	HG2-HG3 12.209
P-Value	N/A	<0.0001%	<0.0005%
Total Female Sacra Observed	720	2,830	268
Total Female Cases of SSBO Identified	69	458	74
Total Frequency	9.58%	16.18%	27.61%
Total Increase/Decrease	N/A	6.60%	+11.43%
Chi-Squared Value	N/A	HG1-HG2 14.737	HG2-HG3 14.105
p-Value	N/A	<0.0001	<0.0002

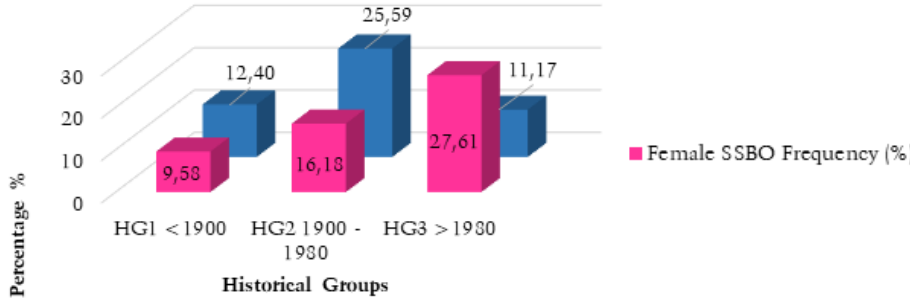

Fig. 7. Male and female frequency by historical group. Data derived from numerical analysis of included literature and frequency calculations outlined in Table 8.

Discussion

Almost all results pertaining to the overall frequency of SSBO demonstrated a substantial, statistically significant increase after 1900. These results provide compelling confirmation for the hypothesised increase in the frequency of SSBO and its correlation with the relaxation of natural selection around 1900. The importance of these results for the determination of current evolutionary change can be conceptualised by outlining the frequency for each historical group. HG1, (2,500BCE to 1900CE), had a total frequency of 13.36%, compared to HG2, (1900 to 1980) at 18.61%, which demonstrates a clear increase of 5.25% over a small 80-year period. HG3, (>1980) produced a frequency of 23.59%, and an increase of 4.98% from HG2, despite representing a smaller sample size and shorter period of history (40 years). When compared to HG1 (<1900), HG3 demonstrated an even more significant result, of a 10.23% increase in the frequency of SSBO between 1900CE and the present. When converted to increase per decade, these figures: 1.31% and 2.49%, demonstrate an accelerating non-linear increase in the frequency of SSBO in the modern era (Saniotis and Henneberg 2011).

The calculation of male and female prevalence for HG1 and HG2 also produced statistically significant results in support of an increase of SSBO after 1900. These results demonstrated higher percentages of increase than the total frequency calculation for these historical groups. Despite the sample sizes of each sex (3,992 males and 3,818 females) being similar, male prevalence was much higher and demonstrated a 6.59% greater increase than among females (13.19% male to 6.60% female) between HG1 and HG2. This could potentially represent a sex based evolutionary trend that has yet to be fully investigated, as male frequency was consistently higher with the exception of the decrease observed for males in HG3. This 11.17% decrease observed for males after 1980 is the only decrease noted across the entire analysis and is accompanied by a substantial 11.43% increase for females in this group. These calculations for >1980 are based on just four publications, with small numbers of sacra, and thus, may reflect regional differences rather than temporal trends. It is important to note that additional data are needed for HG3 to ensure that these relative increases/decreases can be substantiated.

While this research does represent the largest assessment of SSBO in the literature (16,167 sacra), the scarcity of relevant literature and the acknowledged limitations of these studies suggest that these results represent only a fraction of potentially recoverable data. The increase in the frequency of SSBO is relatively modest compared to the median artery, which shows an increase of 20% over the same 120-year period, about double that of the increase in SSBO (Lucas T et al. 2020). Similarly, tarsal coalitions experienced an increase of 12% over a 50-year period, double the reported frequency demonstrated for SSBO for this period (Ruhli et al. 2003). These modest increases in frequency recorded for SSBO may be the product of the lack of data and academic consensus. It may be possible that with the inclusion of additional, larger, and targeted datasets, rates of SSBO frequency may increase again, to parallel those observed in these other anatomical structures.

This research holds important implications for the general application of

both biological anthropology and bioarchaeology. The recognition of implications of natural selection on widescale secular change can improve the accuracy of differential diagnosis in skeletal remains. Awareness of this increase in skeletal variation, its frequency, and patterns of presentation, can improve future bioarchaeological interpretations of trauma, pathology, and health status. The potential for this phenomenon to produce previously unobserved skeletal changes must also be recognised and attempts to identify pathological associations with new forms of variation must be addressed. By acknowledging that the human skeleton is changing, collaboration with the medical community and the use of clinical methodology, can strengthen the capacity of bioarchaeology to provide insight into global future health outcomes as they relate to secular changes.

The incorporation of clinical parameters, statistical calculations, and bias assessments into this bioarchaeological assessment of SSBO frequency has provided a unique opportunity to design a systematic methodology which can be applied to a range of skeletal and anatomical variations. This framework has allowed for the traditionally small scale and localised anthropological datasets to be amalgamated into a broad temporal and geographic 'map' of SSBO frequency, emphasising overarching patterns not identifiable in smaller studies. This method allows for small datasets to be incorporated with a high degree of accuracy and can facilitate a continuous addition of new data. Potentially, this could produce an ever increasing 'map' of SSBO frequency, where the addition of datasets from a range of researchers would allow the eventual creation of a truly global representation of SSBO frequency and its secular trajectory. This method could then be expanded to include additional anatomical variations, from independent or future researchers, that would also lead to the creation of global 'maps' of diverse conditions frequencies.

Future bioarchaeological assessments of skeletal variation should be reconceptualised, with the traditional focus on individual and localised assessments of change replaced with wide reaching systematic evaluations of broad scale frequency. Clear patterns of secular change could be reliably assessed on a global scale, and these trends systematically compared. The potential for this style of analysis to identify trends that have explicit implications for public health and medicine, can be demonstrated through the comparison of SSBO and SBC frequency. The 4.98% increase in SSBO frequency observed after 1980 demonstrates a sustained increase of this condition and conforms with Solomon et al. (2009) and Lee et al. (2011) results on the confirmation of this microevolutionary increase and secular trend, despite the introduction of folate supplementation in 1980. This is in direct opposition to expected clinical outcomes for SSBO after folate supplementation introduction, which has resulted in a sharp decline of NTD related births worldwide after 1980, (Fig. 8) (Atta et al. 2016). This would suggest that SSBO potentially does not follow the same embryonic and etiological trajectory as SBC and has a separate cause altogether. While these results do not confirm or identify the underlying cause of SSBO, the large scale and systematic nature of this assessment, provides the foundation to test such hypotheses further.

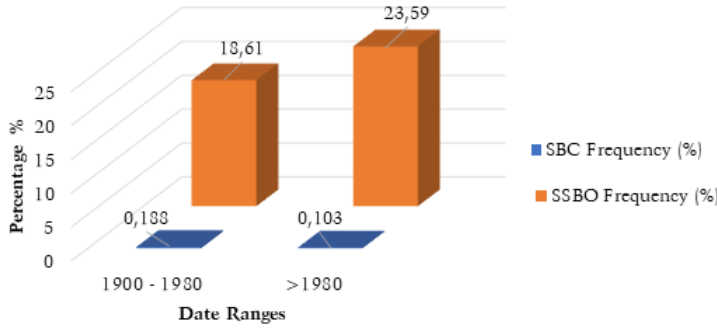

Fig. 8. Comparison between SBC and SSBO frequency by date. Resulting frequency data from literature analysis compared with reliably reported SBC prevalence.

While the results of this literature analysis were limited by the data available in the current literature, the primary objective of this analysis was achieved. The framework that this analysis has provided will facilitate the inclusion of additional SSBO data which will expand our understanding of this little-known condition and provide a uniform structure to ensure the replicability of all future research. In combination with the establishment of the most reliable frequency calculation to date, this framework will also enable the investigation of untested aspects of this condition, such as underlying etiology and additional pathological associations. Other anatomical variations, which have yet to be systematically evaluated, could also be incorporated into this framework, to establish a broader understanding of the trajectory and implications of secular evolutionary change in modern human populations.

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Authors' contribution

Both authors formulated the hypothesis. ERK collected data and drafted the text. MH helped with the analysis and edited text.

Conflict of interests

The Authors have no competing interests concerning this review.

Registration and Protocol

This review has not been registered as it is bioarchaeological.

The Protocol used for this review can be accessed in the appendix.

No amendments have been made due to registration or protocol.

Data Availability

PRISMA Flow Chart template 2020: http://www.prisma-statement.org/

Data for each individual study included in this analysis is available in the appendix.

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Supplementary Material

Table 6. Results of the initial screening process. Inclusion/exclusion criteria and number of publications included/excluded by database outlined

Exclusion reason	Number Excluded: Embase (n=)	Number Excluded: PubMed (n=)	Number Excluded: Adelaide Library (n=)	Number Excluded: Google Scholar (n=)	Total (n=) /409
Duplicates	9	2	1	1	13
Case Studies	11	5	1	1	17
Surgical Texts	12	13	2		27
Responses/Abstracts/Reviews	1				1
Radiographical Methods	6	3	2		11
Ethics		1			1
Non-Human Studies	1	3		1	5
General no % data	6	16	1	3	26
SBC not SSBO	10	13	10	5	38
Unrelated Clinical Conditions	26	25	16	2	68
Nonrandomised	11	7	4	2	24
Total Included	08/100	12/100	103/141	53/68	178
Total Excluded	92/100	88/100	38/141	15/68	231

Table 7. Results of the internal validity screening process. Inclusion/exclusion criteria and number of publications included/excluded by database outlined

Exclusion Criterion	Publications Excluded (n=)
Duplicate data	3
Unreliable data	2
Case Studies	5
Surgical Texts	3
SBC not SSBO	55
No segment data	5
No S1 data	6
Nonrandomised	15
Non-Human	2
Radiograph method	7
General no % data	10
Unrelated condition	26
Total Included	39/178
Total Excluded	139/178

Table 8. Included studies with sample size, date of samples as well as characteristics and any necessary assessments for bias in each publication

(n=)	Publication	Sample Size + Date	Characteristics	Risk of Bias Assessment*
1	Zemirline A et al. Lumbo-sacral malformations and spina bifida occulta in medieval skeletons from Brittany. Eur J Orthop Surg Traumatol. 2013;23: 149–153.	30 768 CE	Archaeological study of recovered skeletal human remains. (Dry human sacra)	Anecdotal data Clear, accurate and included segment data for SSBO, S1
2	Molto JE, et al. The paleoepidemiology of sacral spina bifida occulta in population samples from the Dakhleh Oasis, Egypt. Int J Palaeopathol. 2019;26: 93–103.	116 116 BCE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
3	Urrutia J, et al. Spondylolysis and spina bifida occulta in paediatric patients. Prevalence study using computed tomography as a screening method. Eur Spine J. 2016;25: 590–595.	228 2005 CE	Radiographic study of live patients with associated pathology.	CT and well-structured numerical results. SSBO and Spondylolysis data separate Clear, accurate and included segment data for SSBO, S1
4	Saluja PG. The incidence of spina bifida occulta in a historic and a modern London population. J Anat. 1988;158: 91–93.	112 1816 CE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
5	Lee YC et al. Confirmation of microevolutionary increase of spina bifida occulta among Swiss birth cohorts. Eur Spine J. 2011;20: 776–780.	384 1965 CE	Radiographic study of birth cohorts. Anonymised CT data.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
6	Ali S, et al. The prevalence of spina bifida occulta in a Pakistani population: a study of dry human sacra. Anaesth, Pain Intensive Care. 2014;18: 157–161.	200 1954 CE	Modern anatomical study of archived dry human sacra.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
7	Shin SH et al. Spina bifida occulta: not to be overlooked in children with nocturnal enuresis. Int J Urol. 2013;20: 831–835.	160 1999 CE	Radiographic study in live patients with associated pathology.	Well-structured numerical results. SSBO and enuresis data separated Clear, accurate and included segment data for SSBO, S1

(n=)	Publication	Sample Size + Date	Characteristics	Risk of Bias Assessment*
8	Wu L et al. Variable morphology of the sacrum in a Chinese population. Clin Anat. 2009;22: 619–626.	203 1961 CE	Modern anatomical study of archived dry human sacra.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
9	Cakiroglu B et al. The adverse influence of spina bifida occulta on the medical treatment outcome of primary monosymptomatic nocturnal enuresis. Archive Italian Urol. 2014;86: 270–273.	233 1999 CE	Radiographic study in live patients with associated pathology.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
10	Solomon LB et al. Secular trend in the opening of the sacral canal: An Australian study. Spine. 2009;34: 244–248.	200 1945 CE	Radiographic study of birth cohorts. Anonymised CT data.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
11	El-Din A et al. Congenital anomalies of the vertebral column: a case study on ancient and modern Egypt. Int J Osteoarchaeol. 2006;16: 200–207.	270 2424 BCE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
12	Maat GJ et al. Analysis of human skeletons from a Hellenistic period, buried at a ruined Bronze Age building on Failaka, Kuwait. Maison de l'Oreint. 1990;18: 85–102.	12 1770 BCE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
13	Kim DW et al. Morphological diversities of sacral canal in children: three-dimensional computed tomography study. Korean J Pain. 2014;27: 253–259.	143 1996 CE	Radiographic study in live patients with associated pathology.	Well-structured numerical results. SSBO data and other anomaly data separated Clear, accurate and included segment data for SSBO, S1
14	Wu JW et al. Prevalence of spina bifida occulta and its relationship with overactive bladder in middle-aged and elderly Chinese people. Int Neurouro J. 2016;20: 151–158.	1061 1954 CE	Radiographic study in live patients with associated pathology.	Well-structured numerical results. SSBO and bladder dysfunction data separated Clear, accurate and included segment data for SSBO, S1
15	Fidas A et al. Prevalence and patterns of spina bifida occulta in 2707 normal adults. Clin Rad. 1987;38: 537–542.	2707 1911 CE	Radiographic study in live patients with associated pathology.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1

Table 8 (cont.)

(n=)	Publication	Sample Size + Date	Characteristics	Risk of Bias Assessment*
16	Shore LR. Abnormalities of the vertebral column in a series of skeletons of Bantu natives of South Africa. J Anat. 1930;64: 206–238.	155 1945 CE	Archaeological study of recovered human remains. (Dry human sacra)	Anecdotal data Clear, accurate and included segment data for SSBO, S1
17	Masnicova S et al. Developmental anomalies in skeletal remains from the great Moravia and Middle Ages cemeteries at Devin, (Slovakia). Intl J Osteoarchaeol. 2003;13: 266–274.	150 1115 BCE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
18	Hussien FH et al. Spinal pathological findings in ancient Egyptians of the Greco-Roman period living in Bahriyah Oasis. Int J Osteoarchaeol. 2009;19: 613–627.	77 289 BCE	Archaeological study of recovered human remains. (Dry human sacra)	Anecdotal data Clear, accurate and included segment data for SSBO, S1
19	Mays S. Spondylolysis, spondylolisthesis, and lumbo-sacral morphology in a medieval English skeletal population. Am J Phys Anthropol. 2006;131: 352–362.	422 1465 CE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results. SSBO and Spondylolysis data separate Clear, accurate and included segment data for SSBO, S1
20	Kim Y et al. Lumbosacral defects in a 16th – 18th century Joseon Dynasty skeletal series from Korea. Biomed Res Int. 2018;28: 1–8.	198 1666 CE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
21	Schweitzer ME et al. Spina bifida occulta: incidence in parents of offspring with spina bifida cystica. Spine. 1992;18: 785–786.	177 1932 CE	Radiographic study in live patients with associated pathology.	Anecdotal data Clear, accurate and included segment data for SSBO, S1
22	McGrath M et al. Anatomical observations related to radiological findings in spina bifida -occulta of the lumbo-sacral spine. J Osteopath Med. 2004;7: 70–78.	40 1994 CE	Radiographic study specifically designed for SSBO.	Anecdotal data Clear, accurate and included segment data for SSBO, S1
23	Papp T et al. Changes of the lumbar spinal canal proximal to spina bifida occulta. An archaeologic study of clinical significance. Spine. 1994;19: 1508–1511.	104 367 CE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1

(n=)	Publication	Sample Size + Date	Characteristics	Risk of Bias Assessment*
24	Jankauskas R. Variations and anomalies of the vertebra column in Lithuanian palaeoosteological samples. Anthropol. 2001;39: 33–38.	633 1467 CE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
25	Merbs CF. Sagittal clefting of the body and other vertebral development errors in Canadian Inuit skeletons. Am J Phys Anthropol. 2004;123: 236–249.	218 1867 CE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results. SSBO and other anomaly data separated Clear, accurate and included segment data for SSBO, S1
26	Stewart TD. The vertebral column of the Eskimo. Am J of Anthropol. 1932;17: 123–136.	217 1990 CE	Archaeological study of recovered human remains. (Dry human sacra)	Anecdotal data Clear, accurate and included segment data for SSBO, S1
27	Eubanks J et al. Prevalence of sacral spina bifida occulta and its relationship to age, sex, race, and the sacral table angle. Spine. 2009;34: 1539–1543.	2866 1885 CE	Radiographic study specifically designed for SSBO.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
28	Sutow WW et al. Incidence of spina bifida occulta in relation to age. Am J Dis Child. 1955;90: 211–217.	540 1921 CE	Radiographic study specifically designed for SSBO.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
29	Albrecht TL et al. Radiographical method to access the prevalence of sacral spina bifida occulta. Clin Anat. 2007;20: 170–174.	53 1937 CE	Radiographic study specifically designed for SSBO.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
30	Karlin IW. Incidence of spina bifida occulta in children with and without enuresis. Am J Dis Child. 1935;3: 374–393.	75 1840 CE	Radiographic study in live patients with associated pathology.	Anecdotal data Clear, accurate and included segment data for SSBO, S1
31	Jozsa L et al. The occurrence of spina bifida occulta in medieval and contemporaneous Hungarian populations. Anthropol Hunarica. 1992;22: 51–60.	233 1328 CE	Archaeological study of recovered human remains. (Dry human sacra) + Radiographic study specifically for SSBO.	Anecdotal data Clear, accurate and included segment data for SSBO, S1
32	Avrahami E et al. Spina bifida occulta of S1 is not an innocent finding. Spine. 1994;19: 12–15.	1200 1949 CE	Radiographic study specifically designed for SSBO.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1

Table 8 (cont.)

(n=)	Publication	Sample Size + Date	Characteristics	Risk of Bias Assessment*
33	Piontek J. Variation in the level of closure in the sacral canal of man. Folia Microbiol. 1971;4: 459–464.	316 1911 CE	Modern anatomical study of archived dry human sacra.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
34	Kubauat DM et al. A study of non-fusion of laminae of the first sacral vertebrae in Western India. Int J Recent Trends Sci Tech. 2013;6: 122–124.	302 1953 CE	Archaeological study of recovered human remains. (Dry human sacra)	Anecdotal data Clear, accurate and included segment data for SSBO, S1
35	Groza VM et al. Frequency of spina bifida occulta and other occult spinal dysraphism's in the medieval population of Isas city: skeleton palaeopathology in the necropolis discovered in the eastern part of the Princely Court, 17th century. Biol Anim. 2012;58: 195–204.	28 1660 CE	Archaeological study of recovered human remains. (Dry human sacra)	Anecdotal data Clear, accurate and included segment data for SSBO, S1
36	Henneberg RJ et al. Variation in the closure of the sacral canal in the skeletal sample from Pompeii, Italy, 79AD. Perspect Hum Bio. 1999;4: 177–188.	124 79 CE	Archaeological study of recovered human remains. (Dry human sacra)	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
37	Singh R. Classification causes and clinical implications of sacral spina bifida occulta in Indians. Basic Sci Med. 2013;2: 14–20.	140 1953 CE	Archaeological study of recovered human remains. (Dry human sacra)	Anecdotal data Clear, accurate and included segment data for SSBO, S1
38	Al-Dahhan MH et al. Evaluation of spina bifida occulta in young patients presented with lower back pain. Eur J Mol Clin Med. 2020;10: 4416–4422.	180 2016 CE	Radiographic study in live patients with associated pathology.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1
39	Kumar P et al. Spina bifida occulta in functional enuresis. Indian J Paediatr. 2005;72: 223–225.	48 1997 CE	Radiographic study in live patients with associated pathology.	Well-structured numerical results Clear, accurate and included segment data for SSBO, S1

Table 9. Raw data from frequency analysis

Publication	Date	Total SS	Male SS	Female SS	S1-S5	S1	S1-S2	S1-S3
El-Din and El Banna 2006	-2424	270			0.74			
			135		0.74			
				135	0.74			
2. Maat et al. 1990	-1770	12			8.30			
3. Molto et al. 2019	-874	116			5.17	13.79	0.86	
			64		7.80	10.93	1.56	
				52	1.92	17.30		
	-860	77				2.59		
	-868	193			3.10	9.32	0.51	
			23			8.69		
	1766	144				11.11		
4. Hussein et al. 2009	-289	77			54.54			
			41		51.21			
	124	119		35	60.00			
	124	119			4.2	16.80	3.36	
			56		7.4	19.64	3.57	
	110	130		63	1.58	14.28	3.17	
	110	130			0.76	6.15	3.84	0.76
			47		2.12	6.37	8.51	
				83		6.02	1.20	1.20

Percentage values for each sacral segment recorded. Publications 1–4. SS=Sample Size.

Table 9 (cont.)

Publication	Date	Total SS	Male SS	Female SS	S1-S5	S1	S1-S2andS4-S5	S1andS3-S5
5. Mays 2006	1465	422			1.18	4.50	0.23	
	1515	115				82.60		
6. Zemirline et al. 2013	768	30			3.33	6.66		
7. Henneberg and Henneberg 1999	39	124				13.46		
8. Papp and Porter 1994	367	104				13.46		
	617	27				29.62		
	967	77				10.38		
9. Masnicova and Benus 2003	1115	150					2.00	
			61				3.27	
				65			1.47	
	865	76					3.94	
			38				5.26	
				28			3.57	
10. Jankauskas 2001	1467	633			2.21	12.95		
11. Jozsa et al. 1992	1328	233			1.71	0.42		
12. Groza et al. 2012	1660	28					3.47	
	1662	129						0.77
			62					1.61
				62				1.09
	1670	91			1.09			1.09
			66		1.51			
13. Kim et al. 2018	1666	198			0.50	4.04		
			81		1.23	7.40		
				68		1.47		

Percentage values for each sacral segment recorded in each publication. Publications 5–13.

Table 9 (cont.)

Publication	Date	Total SS	Male SS	Female SS	S1-S5	S1	S1-S2	S1andS4-S5
14. Saluja 1988	1816	112			1.78	11.60	1.78	
	1928	140			2.14	10.00	2.85	
15. Merbs 2004	1867	218				43.11		
16. Solomon et al. 2009	1945	200				10.50	8.00	
			100			17.00	13.00	
				100		4.00	3.00	
	1985	200				16.50	9.50	
			100			23.00	15.00	
				100		10.00	4.00	
17. Stewart 1932	1900	217			2.76			8.29
			107		4.67			8.41
				96	1.04			9.37
18. Avrahami et al. 1994	1968	273				24.24		
			137			28.46		
				136		20.58		
	1958	259				22.77		
			131			27.48		
				128		17.96		
	1948	248				19.35		
			128			25.00		
				120		13.33		
	1938	229				8.73		
			111			8.10		
				118		9.32		

Percentage values for each sacral segment recorded in each publication. Publications 14–18.

Table 9 (cont.)

Publication	Date	Total SS	Male SS	Female SS	S1-S5	S1	S1-S2	L5-S5
Avrahami et al. 1994 (cont)	1928	191				6.80		
			93			7.52		
				98		6.12		
19. Eubanks and Cheruvu 2009	1885	2866			1.22		11.13	
20. Lee et al. 2011	1965	384				2.34		
			194			2.57		
				190		2.10		
21. Karlin 1935	1913	75			12.00	62.00	16.00	12.00
22. Shore et al. 1932	1840	78				3.84		
23. Sutow and Pryde 1955	1945	155				51.61		9.03
			86			46.51		10.46
				69		57.97		7.24
	1941	95				44.21		6.31
			44			47.72		9.09
				51		41.17		3.92
	1936	108				39.81		4.62
			48			52.08		10.41
				60		30.00		
	1921	182				14.83		2.19
			79			54.43		3.79
				103		20.38		0.97
	1902	46				23.91		
	1902	87				16.57		
	1902	87				22.18		

Percentage values for each sacral segment recorded in each publication. Publications 19–23.

Table 9 (cont.)

Publication	Date	Total SS	Male SS	Female SS	S1-S5	S1	S1-S2	L5-S2
24. Singh 2013	1953	140			3.57			
25. Kubauat et al. 2013	1953	302				10.92		
26. Schweitzer 1992	1932	177						15.81
			32					15.62
				37				13.50
			53					16.98
				56				16.07
27. Cakiroglu et al. 2014	1999	233						1.28
			151			22.31	0.85	
				72		3.31	0.66	4.16
						65.27	5.55	
28. Mith and Tayles 2004	1994	40				22.50		
			20			22.22		
				20		27.77		
29. Piontek 1971	1911	316			1.26	3.48	3.48	1.26
			187		1.60	2.67	4.81	1.60
				129	0.77	4.65	1.55	0.77
30. 0 et al. 2004	1954	200			4.50		3.50	
31. Kumar et al. 2005	1997	48				16.66	4.16	10.41
32. Kim 2014	1996	143					15.40	
33. Wu et al. 2009	1961	203			2.95		18.20	
34. J.W. Wu et al. 2016	1954	1061				11.96	0.65	

Percentage values for each sacral segment recorded in each publication. Publications 24–34.

Table 9 (cont.)

Publication	Date	Total SS	Male SS	Female SS	S1	L5-S5
35. Fidas et al. 1987	1941	570			28.59	
			301		37.20	
				269	18.95	
	1931	877			20.98	
			411		29.68	
				460	13.47	
	1921	658			20.97	
			333		23.72	
				325	18.15	
	1911	380			20.58	
			208		25.00	
				172	15.11	
	1901	173			16.76	
			80		16.25	
				93	17.20	
	1891	52			19.23	
			24		20.83	
				28	17.85	
36. Shin et al. 2013	1999	160			16.25	
37. Urrutia et al. 2016	2005	228			35.08	
38. Al-Dahhan et al. 2020	2016	180			18.5	4.40
39. Albrecht et al. 2017	1937	53			1.25	

Percentage values for each sacral segment recorded in each publication. Publications 25–39.

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Klippel-Feil Syndrome: morphological findings in a 19th-century musealized skull from Viana del Bollo (Orense, Spain)

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Abstract: The aim of this study is to show the cranial alterations that Klippel-Feil syndrome produced in a case older than 200 years. Few paleopathological case studies diagnosed as Klippel-Feil Syndrome are focused on cranial abnormalities. A skull numbered 778, belonging to the Federico Olóriz Aguilera collection (Spain, 19th century AD), *Universidad Complutense de Madrid*, belonging to a young man born in a town in the North of Spain, was investigated. This cranium was visually inspected, hence macroscopically and paleoradiologically studied, using the images obtained through conventional radiology and CT scan imaging. In addition to the vertebral fusion between the atlas (C1) and the axis (C2), atlanto-occipital fusion, basilar impression, obliteration of the sagittal suture, enlarged parietal foramina and significant craniofacial asymmetry affecting maxillary bones, sphenoid, orbits, nasal bones and both palatines were observed. Morphological findings make it possible to diagnose a Klippel-Feil syndrome, possibly type-II, although the lack of the rest of the spinal column renders it impossible to verify other spinal anomalies. As a limitation, only the cranium and two cervical vertebrae were preserved, hence the possible involvement of the rest of the skeleton cannot be verified.

Key words: Olóriz collection, Klippel-Feil syndrome, atlanto-occipital fusion, basilar impression, facial asymmetry, enlarged parietal foramina

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Introduction

Klippel-Feil syndrome (KFS) is characterized by the congenital fusion of a variable number of cervical vertebrae (Vujasinovic Stupar et al. 2015), in the majority of cases accompanied by other abnormalities in different apparatuses, including otorhinolaryngological and craniofacial ones (Clarke et al. 1998). The classic clinical KFS triad comprises a short neck, restricted neck mobility, and a low dorsal hairline (Gunderson et al. 1967; Fietti and Fielding 1976; Taylor-Martínez et al. 2019). In the modern clinical setting, in cases in which the condition presents asymptomatically it can be discovered by chance, after a radiological examination in living patients. Although the etiology of KFS is not fully understood, some genetic involvement was postulated since mutations in the *GDF6*, *GDF* 3, and *MEOX1* genes were found in some families and an inheritance pattern was determined. For the first two, involved in bone formation and development, it is an autosomal dominant pattern, while for the third one, whose homeobox protein regulates vertebral separation, it is instead an autosomal recessive one (Manger et al. 2021). Before genetic correlations were found, families with KFS individuals within them were historically subjected to anthropological studies (Henneberg and Otocki 1974).

Several cases of KFS have been recorded in the paleopathological literature. Archaeological cases globally have been described since the Neolithic in Slovakia (site of Vráble-Veľké Lehemby) and Late-Final Neolithic in Greece (Alepotrypa Cave, Peloponnese, 5000-2300 BC) (Papathanasiou 2005; Hukeľová et al. 2021), the Chalcolithic Age in Peru (MacCurdy 1923), North America (Jarcho 1965), Central America (Urunuela and Alvarez 1994), Ancient Egypt (Aufderheide and Rodriguez-Martin 1998) or Europe (Barnes 1994; Gladykowska-Rzeczycka 1997; González-Reimers et al. 2001; Herrerín, 2004; 2011; Pany and Teschler-Nicola 2007; Fernandes and Costa 2007; Giuffra et al. 2009; Macías-López 2020). In Italy, four cases of KFS from the 1582–1583 AD plague phase of the San Michele cemetery in Alghero (Sardinia) were identified among 199 individuals making this the archaeological site with more such finds also dating to the same historical period (Varotto et al. 2020).

In this study the case of a skull from the Olóriz collection is presented: this specimen shows some of the typical features of KFS, despite the sole preservation of two cervical vertebrae and not of the entire skeleton. The Olóriz collection was formed for research purposes – as the history of anatomy teaches (Papa et al. 2020) – from corpses collected by Federico Olóriz Aguilera (1855–1912), Professor of Anatomy at the University of Madrid, reaching a total of 2,250 skulls, almost entirely from Spain and dating to the 19th century. At present the collection is distributed among various Departments of the *Universidad Complutense de Madrid*, and although the written documentation is now missing, all the skulls are marked with black ink indicating their basic data about sex, age at death and geographic provenance. In addition, it must be underlined that the collection is dispersed and many specimens have disappeared from the register. However, it has been possible to verify that there is no similar case among those preserved, after a thorough review of all the preserved skulls.

Material and Methods

The analyzed skull (inventory number 778 – Olóriz collection) comes from Viana del Bollo, in the Spanish province of Orense. Data about sex and age at death are labelled on the skull itself, but sex determination and age estimation were anyhow performed utilizing Ferembach et al.'s (1979), Masset's (1982) and Buikstra and Ubelaker's (1994) sets of methods. The skull was completely preserved, although the mandible is missing, and, as a result on the fusion, the atlas and axis are still present. Its state of preservation can be grossly assessed as good although no preservation indexes, more commonly used for bioarchaeological material originating from excavation sites – and, as yet, not for scientifically musealized anatomical specimens – were calculated. Together with the visual inspection and macroscopic examination of the skull, image analysis was carried out using conventional radiology and CT scan imaging (Equipment: HP 15.0; Parameters: 120kV/150mAs; 0.5/3.0mm).

Results

The information about sex and age at death was written on the right parietal bone by Dr. Olóriz himself (Fig. 1): the skull belongs to a 22-year-old male. Morphologically and anthropologically, the skull confirmed the above-stated information: male features and 20–25 years age range.

The following pathological findings were observed in skull 778.

Neurocranium:

1. Absence of sagittal suture;
2. Enlarged parietal foramina;
3. Basilar impression;
4. Atlanto-occipital fusion with a significant spinal canal synostosis.

Splanchnocranium:

5. Asymmetry of orbits and nasal region;
6. Asymmetry of nasal and palatal bones;
7. Displacement of maxillary massif.

Spinal column:

8. C1-C2 fusion.

Fig. 1. Lateral view of skull number 778 with information on sex, age at death and provenance labelled on the ectocranial surface of the right parietal bone.

In the superior view (Fig. 2a), the sagittal suture was totally missing and the coronal one showed no obliterated areas. In the frontal view, a significant lateral asymmetry was observed, with deviation of the nasal bones and nasal septum to the right (Fig. 2b). This asymmetry also affected the orbits, the left being more cranially located, taller and narrower than the right. In the posterior view (Fig. 2c), the asymmetry was also evident, with significant lateral displacement of the sagittal suture and the lambda craniometric point, while the lambdoid suture appeared visible and unobliterated. Additionally, two parietal enlarged foramina are present (Fig. 2c).

In the inferior view the skull showed asymmetry of the entire facial massif

(Fig. 3a, b), with a very significant displacement of the median sagittal plane (red and white lines). This asymmetry was very evident in the maxillary palatal processes and palatal bones, with smaller dimensions in the left hemicranium (Fig. 3c).

The palatal suture was asymmetrical in its course and length. Four teeth remained *in situ* (16, 17, 25 and 26) as well as two roots (18, 27), while the rest were lost *post-mortem*. The postero-inferior view (Fig. 4a) showed an atlanto-occipital fusion, with fusion of the lateral massif and a normal appearing posterior arch of the atlas. The axis was found to be fused with the atlas at its articular facets. The odontoid process was fused with the anterior arch and the right lateral mass of the atlas. Part of the odontoid process had already been lost historically, torn off as a result of past manipulations performed to separate the skull from the spine at the time of its musealization (Fig. 4a+). On the other hand, the atlas was fused to the occipital but not symmetrically, leaving a space between the left postero-lateral edge of the foramen magnum and the left postero-lateral arch of the atlas (Fig. 4a*). The lower view (Fig. 4b) also showed the reduction in the antero-posterior diameter of the vertebral canal.

Conventional radiographic analysis showed a 4 mm basilar impression on McGregor's line (hard palate-occipital scale; red line; Fig. 5a). At the same time the CT study made it possible to

Fig. 2. Macroscopical examination. a) Superior view; b) Frontal view; c) Posterior view.

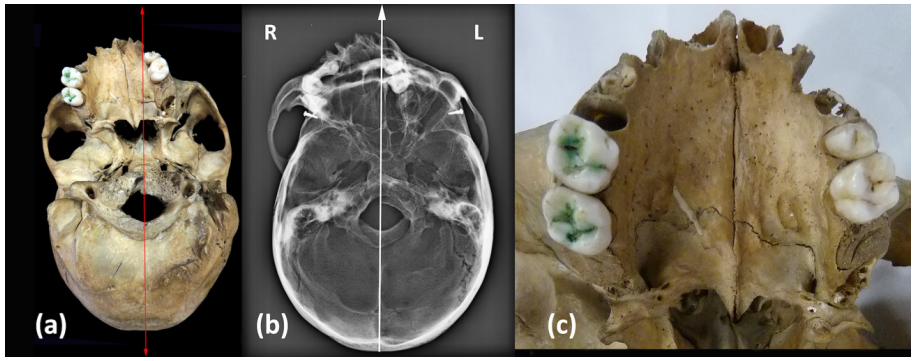

Fig. 3. a) Inferior view (red line, sagittal plane); b) Inferior view, X-Ray image (white line, sagittal plane); c) Maxillar and palatal bones, inferior view.

verify how the asymmetry also affected the sphenoid bone, with a marked shift towards the right side of its pterygoid processes (Fig. 5b), and likewise the maxillary bone and the nasal cavity (Fig. 5c). Finally, the tomography verified the stenosis of the vertebral canal at the level of the foramen magnum (Fig. 5d, e), due to the fusion of the atlas in an asymmetric, rotated position.

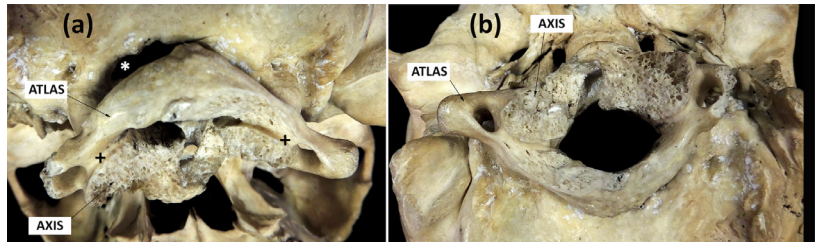

Fig. 4. Postero-inferior view. a) atlanto-occipital fusion + axis-atlas fusion. * Space between the left postero-lateral edge of the *foramen magnum* and the left postero-lateral arch of the atlas. b) Stenosis of the vertebral canal.

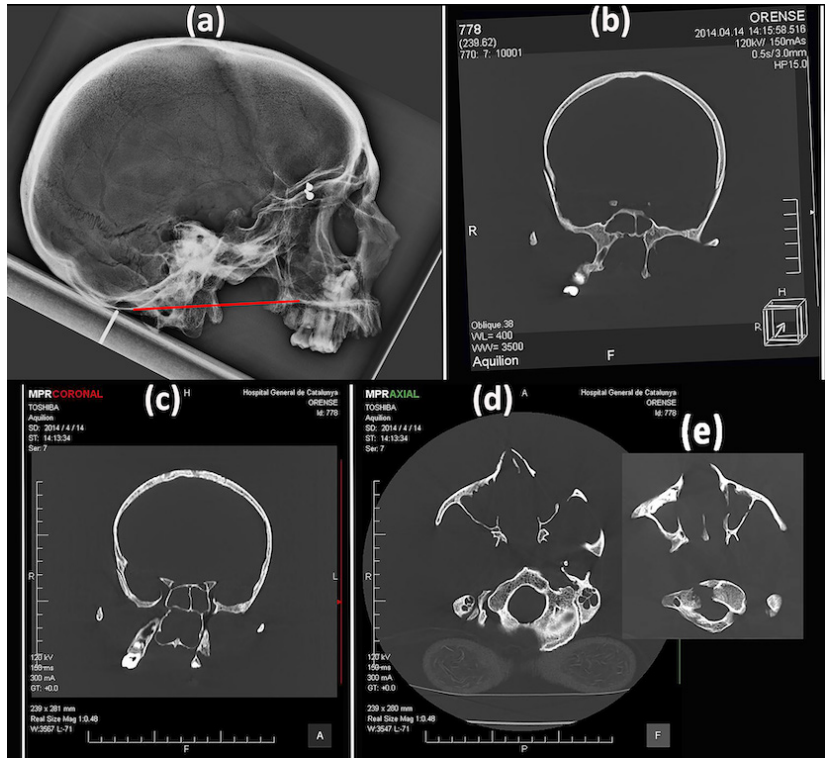

Fig. 5. a) Conventional radiographic image. McGregor's line (red line). b) CT image. Frontal view. Deviation of the pterygoid processes. c) CT image. Frontal view. Deviation of the maxillary bone and the nasal cavity d) and e) CT image. Inferior view. Stenosis of the vertebral canal at the level of the *foramen magnum*.

Discussion

KFS was first described in 1912 by Maurice Klippel and André Feil (1912) in a 46-year-old patient with a massive fusion of the cervical vertebrae, although the first clinical descriptions are attributed in 1745 to Haller and in 1746 to Morgagni (Gunderson et al. 1967).

Three different forms of KFS are defined (Barnes 1994; Pany and Teschler-Nicola 2007; Toker et al. 2009):

- Type I: with formation of a bony block of several cervical and upper thoracic vertebrae (three of more levels) often associated with more severe defects (Barnes 1994; Pany and Teschler-Nicola 2007).
- Type II: fusion of two or three vertebral segments. The second and third cervical vertebrae are the most frequently affected, followed by the fifth and sixth. When the thoracic vertebrae are affected, it is mainly between T2 and T5 (Barnes 1994). It can appear with an atlanto-occipital fusion, hemivertebrae and other anomalies. It is the most common form of KFS, although with minimal clinical symptoms (Barnes 1994; de Rubens-Figueroa et al. 2005; Pany and Teschler-Nicola 2007).
- Type III: fusion of the cervical block together with other anomalies in the lumbar and thoracic regions. Scoliosis is present in 60% of cases (Barnes 1994; Pany and Teschler-Nicola 2007; Toker et al. 2009).

Therefore, the cervical vertebrae are the most commonly affected, leading to the description, especially in old clinical textbooks, of a syndrome in which patients are characterized by a shortened or absent neck as the result of a complete cervical block.

The prevalence of KFS is estimated at 0.71% (Brown et al. 1964; Papagrigorakis et al. 2003; Nouri et al. 2017) and the incidence of one case per 30,000–42,000 (de Rubens-Figueroa et al. 2005), with a female predilection (Jones 1997; Aufderheide and Rodriguez-Martin 1998; Martínez-Quintana and Rodríguez-González 2015; Sirico et al. 2015; Gruber et al. 2018).

The final cause of this vertebral fusion is found in a failure in spinal segmentation between the third and eighth weeks of embryogenesis (Fietti and Fielding 1976; Mahirogullari et al. 2006; Fernandes and Costa 2007). There is no unanimity of criteria on its genetic origin (Daum and Jones 1988; de Rubens-Figueroa et al. 2005; Pany and Teschler-Nicola 2007; Toker et al. 2009), with a genetic inheritance being proposed, dominant in Type II and recessive in Types I and III (Gunderson et al. 1967; Juberg and Gershanik 1976; Lowry et al. 2001).

Cervical fusions in KFS can remain asymptomatic, it being discovered accidentally after radiological analysis (Copley and Dormans 1998), although limitation of neck movement and its relationship with other abnormalities occurs in a high percentage of cases (Dietz 2001). On the other hand, atlanto-occipital fusion can lead to severe neurological symptoms after even minor trauma (Gray et al. 1964) due to the excessive mobility of the vertebral segments adjacent to the fused area (Strax TE, Baran E. 1975; Adeleye and Akinyemi 2010).

Multiple disorders associated with KFS have been described, such as kyphosis, spina bifida, cleft palate, atlanto-occipital fusion, basilar impression, scoliosis, supernumerary cervical vertebra, Sprengel's deformity, dwarfism, hypodontia, meningocele, renal and cardiac anomalies (Gunderson et al. 1967; Hensinger et al. 1974; Hensinger and MacEwen 1986; Daum and Jones 1988;

Tachdijian 1990; Barnes 1994; Copley and Dormans 1998; Herman and Pizzutillo 1999; Warner 1998; Dietz 2001; Papagrigorakis et al. 2003; Narang and Goyal 2006; Pany and Teschler-Nicola 2007; Toker et al. 2009). Specifically, atlanto-occipital fusion is produced by a failure in the segmentation between the skull and the first cervical vertebra. It can be partial or complete, and it usually produces associated basilar impression (Boleaga-Durán et al. 2006). It can appear as an isolated sign or be part of different syndromes, among them (very frequently), type-II Klippel-Feil syndrome. With particular reference to the basilar impression or basilar invagination, it consists of an elevation of the floor of the posterior fossa, with displacement of the odontoid process towards the interior of the foramen magnum (Chamberlain 1939). The primary or malformative basilar impression is almost always associated with atlanto-occipital fusion and narrowing of the foramen magnum (List 1941). Among the many causes that can produce a basilar impression (osteomalacia, Paget's disease, Chiari malformation, syringomyelia, hydrocephalus, etc.), the Klippel-Feil syndrome is counted (Matson 1969). In skull 778, the radiographic analysis showed a light but obvious basilar impression (4 mm) on McGregor's line (hard palate-occipital scale; red line; Fig. 5a). Moreover, two enlarged parietal foramina (EPF) detected in the parietal bones are present. EPF are developmental defects characterized by variable intramembranous ossification, normally located on each side of the dorsal portion of the sagittal suture. They differ from normal parietal foramina, which are smaller (less than 1 mm in diameter) and considered anatomical variants. EPF can be associated with syndromic condition such as KFS or Saethre-Chotzen syndrome (Thompson et al. 1984) or can be found isolated (Piagkou et al. 2013).

Differential diagnosis

A typical paleopathological diagnosis of KFS involves a morphological examination of the column and any other areas of the skeleton to determine if other anomalies may be present, a paleopathological and clinical comparison with the available scientific literature, and an imaging study (conventional X-ray and/or CT scan) in order to confirm the suspected skeletal condition ruling out other pathologies such as fusion caused by traumatic conditions like fractures. Occasionally, but not routinely (especially with historic remains in which invasive sampling is not always justified), a genetic test can be made to corroborate phenotypic observations with the discovery of a matching genotypic background. A sample may be taken and examined to see if there are mutations in genes like GDF3, GDF6, or MEOX1, which are known to be commonly affected in KFS (Mohamed et al. 2013). However, it must be stressed that, both clinically and paleopathologically, radiological analyses can be considered sufficient to make a substantiated diagnosis of KFS.

Differential diagnoses include Paget's disease, fibrous dysplasia, cleidocranial dysostosis, osteogenesis imperfecta, osteoporosis, rickets.

Paget's disease: Paget's disease of bone is a chronic bone disorder of unknown cause. It was first described by Sir James Paget (1814–1899) in 1877. There is an increase in osteoclast activity, resulting in increased bone resorption, the clinical expression of which is the lytic bone lesions observed in conventional radiography

(Resnik and Niwayama 1988; Bolland and Cundy 2013). In response, accelerated and chaotic bone formation occurs, resulting in sclerotic bone that is functionally weaker than normal bone without the characteristic laminar pattern (Menéndez-Bueyes and Soler-Fernández 2017). The clinical manifestations are usually expressed after years of evolution when bone deformity appears that leads to pain, osteoarthritis, and pathological fractures (Roodman and Windle 2005; Bolland and Cundy 2013; Corral-Gudino et al. 2013; Galson and Roodman 2014). The disease usually presents at an older age than 55 and its frequency increases with age (Resnik and Niwayama 1988; Bolland and Cundy 2013), with a slight predominance in males. The bones most frequently involved are the pelvis, femur, spine, skull, and tibia (Bolland and Cundy 2013). In the postcranial skeleton, thickening of the diaphyses of the limb bones are observed, which tend to deform laterally and fracture (in extreme affectations), with the femur and tibia being the most affected bones (Ortner 2003). The alteration in the cranium can cause a symmetrical or asymmetric growth of the parietal or frontal bones causing a greater size of the cephalic portion (Favus and Vokes 2005). This cranial expansion can narrow the diameter of the cranial foramina and cause neurological complications including hearing loss due to cochlear nerve damage caused by involvement of the temporal bone, cranial nerve palsy, and softening of the skull base with risk of compression of the brainstem. In the facial bones, a deformity and/or loss of teeth is caused (Aufderheide and Rodríguez-Martín 1998; Ortner 2003; Favus and Vokes 2005).

Of all the listed features, only platybasia is present in skull 778. But some features of skull 778 may allow this disease to be ruled out, even though the vertebral column and the rest of the postcranial skeleton have not been preserved. First of all, age makes it possible to exclude this possibility. It would be extremely rare for a 22-year-old young adult to show signs of Paget's disease as advanced as the pathological changes shown in this case. Secondly, the widening of the diploe (hyperostosis of the cranial vault), the typical "cottony" radiological image of Paget's disease and circumscribed osteoporosis, do not appear in this case (Resnick and Kransdorf 2006; Herrerín et al. 2009). Third, CT images do not show involvement of the temporal bones, or stenosis of the auditory canal. And, lastly, teeth are not affected.

Fibrous dysplasia (FD): it is a bone development disorder in which the lesions form fibrous tissue and spicules of bone tissue (Ortner 2003). The spongy medullary bone is replaced by fibrous tissue. It can be monostotic (a single affected bone) or polyostotic (multiple lesions). The monostotic form is the most common (Herrerín et al. 2009). The monostotic forms mainly affect the long bones, ribs and radius. The polyostotic forms usually involve the proximal femur and the base of the skull (Parekh et al. 2004; Alonso and Muñoz-Torres 2009). The most frequently affected bone in fibrous dysplasia is the femur (44%) followed by the skull (38%), the pelvis (23%), the ribs (16%) and the spine (9%) (Benhamou et al., 2006). When the skull is affected, there is an expansion of the diploe associated with a reduction in the thickness of the internal and external tables of the cranial vault (Herrerín et al. 2009). Radiographic images are very similar to those obtained in Paget cases (*cottony* images). But computed tomography is more im-

portant in diagnosis, because it shows expansive focal areas with a homogeneous "*ground glass*" appearance (Herrerín et al. 2009; Raus and Coroiu 2016). Despite not having recovered the postcranial skeleton, we ruled out fibrous dysplasia because it does not show a widening of the diploe or the typical CT image in "thin glass" (Herrerín et al. 2009).

Cleidocranial dysostosis (CCD). The pathogenesis of CCD is currently unknown: it is probably caused by an ectodermal and mesodermal tissue disorder during the bone growth phase (Hernández et al., 1980). The difficulty in making a diagnosis of CCD lies in the variability of the alterations. The skull has the anthropometric characteristics of brachycephaly, protruding fronto-parietal fontanelle with large sutures and numerous small supernumerary (Wormian) bones (Herrerín 2011). Affected individuals show a slight hypertelorism and exophthalmos (Hernández et al., 1980; Ortner, 2003; Roberts et al., 2013; Russell, 2015; Lewis, 2019). In the dentition, skull 778 does not show supernumerary teeth or agenesis of premolars, which are also frequently seen signs in patients with CCD (Herrerín 2004; 2011).

Osteogenesis imperfecta (OI): OI are a group of inherited genetic pathologies of the connective tissue characterized by bone fragility and fractures (Jones 2006). They result from constitutional bone fragility (cortical bone thinning, trabecular bone rarefaction) but also from acquired bone fragility due to muscle wasting and immobilization. Wide fontanelles are a known symptom of OI. Typical radiographic signs are thinning of the cortical bone and excessive transparency of the trabecular bone. The main radiographic features are osteopenia, bone fractures and bone deformities (Renaud et al. 2013). These signs do not appear on this skull.

Osteoporosis: there are no radiological osteopenic signs suggesting some form of osteoporosis (Resnick and Kransdorf 2006). The age of the individual (22 years) also makes this disease very unlikely.

Rickets: rickets is ruled out in the absence of cranial thickening and cranial porosity, frequent in the frontal and parietal bones of individuals affected by this disease (Resnick and Kransdorf 2006).

Among the various craniofacial anomalies associated with KFS, facial asymmetry occurs in 13% to 20% of cases (Martínez-Quintana and Rodríguez-González 2015; Naikmasur et al. 2011; Kerai and Saxena 2014; Jovankovičová et al. 2012). This facial asymmetry is very significant in skull 778, including the nasal area, where the part of piriform aperture and the nasal bones are asymmetrical, with the nasal septum laterally deviated from the sagittal plane. Both the displacement of the maxillary mass and the asymmetry of the orbits and nasal region may have their origin in genetic factors (as occurs in the case of hemifacial microsomia, multiple syndromes, craniosynostosis of the coronal suture or labio-palatal fissures) or in environmental and/or functional factors (such as intrauterine pressure, especially in multiple births, *ante-mortem* trauma with deficient fusion or even infections in the area during growth). In this skull we have not found any sign that would allow us to relate it to synostosis of the coronal suture, as this is the only synostosis that can present asymmetrical compensation of the cranial and facial bones. Neither have we detected, both in the conventional radiological study and in the CT scan, the presence of signs of any *ante-mortem* fracture nor any signs of infection.

These nasal features have also been described in other patients with KFS (Fragoso et al. 1982). The asymmetry of the palatine processes of the maxilla and palatine bones observed are also not unusual findings in KFS cases with facial asymmetry (Martínez-Quintana and Rodríguez-González 2015). Giuffra et al. (2009) describe the case of Cardinal Carlo de' Medici (1595–1666), who had a clear asymmetry of the nasal and maxillary bones, larger on the left side, together with a marked hypoplasia of the right hemimandible. All of these characters produced an easily recognizable facial asymmetry in the portrait that the artist Justus Sustermans (Galleria Palatina, Rome) made of the Cardinal.

Anomalies associated with KFS, such as atlanto-occipital fusion or basilar impression are also rarely mentioned in the paleopathological literature. The fusion of the atlas has been described in an individual of medieval chronology, in Portugal (13th–15th centuries), presenting partial atlanto-occipital fusion together in the skull with very slight differences in the outline of the nasal cavity (Fernandes and Costa 2007). Regarding the atlanto-occipital fusion, we have not found signs of infection, fracture or rheumatoid arthritis (subluxation, erosion, sclerosis, basilar impaction, etc.), which could indicate a different origin than congenital. In a juvenile individual from Gnadendorf (Hungary, 10th century AD), in addition to congenital fusion of several cervical and thoracic vertebrae, symmetric hypoplasia of the occipital bone, marked curvature of the occipital scale, basilar impression, and asymmetry of the occipital condyles have been described (Pany and Teschler-Nicola 2007). Such malformations are rarely described in the anthropological literature. In the presented specimen, none of these signs are present. The age of the individual (22 years), together with the data provided by the analysis of the radiological images, has made it possible to rule out other cranial malformations with similar signs, such as Paget's disease, fibrous dysplasia, cleidocranial dysostosis, *osteogenesis imperfecta*, osteoporosis or rickets. Therefore, in skull 778, all the described findings are compatible with Type-II KFS, although, as only two cervical vertebrae are available, a completely accurate diagnostic assessment cannot be made. Type-II KFS corresponds to about 26% of all cases of this syndrome (McGaughran 2004), and usually includes atlanto-occipital fusion.

Conclusions

The pathological findings observed in skull 778 include the absence of sagittal suture, enlarged parietal foramina, basilar impression, atlanto-occipital fusion, asymmetry of orbits and nasal region, asymmetry of nasal and palatal bones, displacement of maxillary massif and C1-C2 fusion. This leads to the diagnosis of Type-II Klippel-Feil syndrome. X-rays and CT scans corroborate the morphological findings. Due to the absence of most of the vertebral column, in this article we have also paid a great of attention to other important skeletal alterations at the cranial level which are known to accompany this syndrome and, in this particular, have its detection more straightforward. This description also adds to the list, still under scrutiny, of previously reported KFS cases in the Spanish bioarchaeological record, covering a chronological span from the Bronze Age to the 19th century AD. As a final general note to this historical dissertation, it must be stressed how a careful analysis of cranial asymmetries and

splanchnocranial modifications should always constitute an important part of retrospective assessments of this condition.

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Authors contribution

J.H., E.D., R.D.S. = conceptualization, first draft, writing, data analysis and synthesis, diagnosis, palaeoradiological analysis;

F.M.G, E.V = writing, data analysis and synthesis, diagnosis, palaeoradiological analysis, literature review

Conflict of interest

The authors declare they have no conflict of interest.

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Moral foundations tracked over 200 years of lexicographic data, and their predictors

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Abstract: The prediction that reduction of negative selection decreases group-level competitiveness, as reflected in increased individual-focused and diminished group-focused moral foundations, is tested. To measure this hypothesized shift in moral foundations, we conduct a culturomic analysis of the utilization frequencies of items sourced from the moral foundations item pool, tracked among Britannic populations from 1800 to 1999 using Google Ngram Viewer. The resultant higher-order factor, which tracks increasing individualizing values and decreasing binding values, is termed *Asabiyyah* (capturing social cohesion and collective purpose). Two predictors of this factor are examined: change in the strength of intergroup competition and change in levels of indicators of developmental instability. Both the strength of intergroup competition and levels of developmental instability associate with *Asabiyyah*. Rising developmental instability mediates the impact of inter-group competition, indicating that reduced between-group competition might have relaxed negative selection against mutations, which might reduce *Asabiyyah* via their effects on inter-genomic transactions. These results must be interpreted carefully, given the clear real-world evidence that explicit commitment to group-oriented values often features in harmful and maladaptive social and political ideologies of an extreme character.

Key words: Asabiyyah, Lexicographic data, Moral foundations, Multi-level selection

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Introduction

Social epistasis refers to intergenomic transactions that occur between at least two organisms, and which modify the gene expression of at least one of the involved organisms. Such social-epistatic changes of gene expression evidently can have phenotypic effects. For instance, in the work of Linksvayer (2007), an early user of the term "social epistasis," evidence is reported that in "three species of closely related *Temnothorax* ants … adult worker size was determined by an interaction between the genotypes of developing brood and care-giving workers, i.e., intergenomic epistasis. Such intergenomic social epistasis provides a strong signature of coevolution between social partners" (p. 1). Evidence of social-epistatic effects has also been found in mice (see Bachmann et al. 2018; Cross 2019; Kalbassi et al. 2017). Among the most impressive findings concerns a mutation, specifically a gene deletion, in mice that is related to autistic-like behavior; it has been found that social association of mice who carry this mutation with mice who do not can lead to the latter exhibiting the autistic-like behavior of the former (Kalbassi et al. 2017).

Investigating the mechanism through which these behavioral changes in mice occur, Cross (2019) found evidence that social contact of mice that were carriers of this mutation with non-carriers changed RNA expression in the latter's brain cells, which is consistent with a social-epistatic effect.

A relatively new line of primarily theoretical research into social epistasis in humans has concerned development of the social epistasis amplification model or SEAM (Woodley of Menie et al. 2017a). The SEAM was devised to offer a unified explanation of the apparent falling fitness and declining physical and mental health of Western populations from roughly the twentieth to twenty-first centuries (Woodley of Menie et al. 2017a). This model posits, first, that deleterious mutations have been accumulating in Western populations since the substantial relaxation of negative-selective pressure (i.e., selection that removes deleterious mutations) brought on by industrialization and many of its effects on social, technological, and economic development, especially improved sanitation and increases in wealth, which reduced burdens of infectious disease and general environmental harshness (for research on relaxed negative selection, see Kondrashov 2017; Lynch 2016; Rühli and Henneberg 2017). Second, it posits that the fitness costs of these accumulating deleterious mutations may be amplified via social epistasis, with certain deleterious mutations causing harmful changes in patterns of gene expression even in those who do not carry these variants, potentially reducing physical and mental health as well as reproductive success. A simulation indicates that this social-epistatic amplification of the fitness costs of a certain class of deleterious mutations, known as *spiteful mutations* because of their ability to externalize their fitness costs onto others via (in this instance) their social epistatic effect on the gene expression of other organisms in the same population[1], can theoretically cause very rapid decline in

1 The term *spiteful mutation* was coined by Hamilton (1971), who speculated that mutations that were spiteful in their action in terms of their fitness costs to both carriers and others would have difficulty evolving because of their inability to bring into being complex, individual-level genetic adaptations that would theoretically be needed for them to produce such spiteful effects (he notes specifically that "it seems

the fitness of entire populations (Woodley of Menie et al. 2017a).

One possibility is that differential expressions of moral foundations and

unlikely that a multigenic spiteful adaptation could evolve"). Were they to arise in "[a] population which is small enough, and sufficiently bunched together, to make possible the distribution of such extensive harm," he notes that "any strongly spiteful mutation is very likely to cause its extinction" and "must act like a final infection that kills failing twigs of the evolutionary tree." Woodley of Menie et al. (2017a) presented a solution to the problems raised by Hamilton in noting, first, that the carriers of such mutations do not need to be executing individual-level adaptative strategies that are specifically tailored toward spite; instead, such mutations can capitalize on preexisting individual-level adaptations (such as those that subserve social cognition) and group-level adaptations (such as religious and social ritual). The impacts of spiteful mutations on the integrity of the latter can negatively affect the fitness of others, who depend on optimal cultural expressions of these adaptations, via social epistasis. Second, based on simulations, it has been found that mutations that are spiteful in their action can only accumulate if the rate at which such mutations are arising due to the relaxation of negative selection in a growing population exceeds their individual and (critically) their group-level fitness cost. Such mutations can be expected to have cumulative and harmful effects on patterns of social epistasis, reducing the fitness of a growing population of socially associated genotypes, with such a process eventually leading to the rapid collapse of the population. It is necessary to note that the term "spiteful mutant" has found currency among some commentators on both the political left and right, who use the term to denigrate political opponents whom these individuals regard as being in some way socially dysfunctional and/or undesirable. But it is inappropriate to use the term in such partisan and abusive ways. In the first place, there is no reliable method by which to comprehensively take account of the relative genetic and environmental contributions to the particular traits or behaviors of any given individual. So, to attribute with certainty a specific trait or behavior in a specific individual simply or even primarily to spiteful mutations is quite ridiculous. Spiteful mutations might partly contribute to a large number of extreme and maladaptive behaviors and beliefs found on both sides of the political spectrum and in other contexts. Therefore, politically partisan use of the term is, to repeat, inappropriate. It is worth noting that in the contemporary political context, individuals on the far left *and* the far right, such as those associated with the "alt-right" and neo-Nazi movements, have exhibited profoundly maladaptive behaviors that disturb and threaten the wellbeing and integrity of the groups in which they are embedded; the behavioral profiles of such persons on the whole are at substantial remove from the normative "centers of gravity" of their societies, both in those societies' modern and in many respects historical forms, and it is possible that unusual genetic factors could have a role in the tendency of certain individuals to gravitate to extreme, non-normative ideologies on both sides of the political spectrum. (While it is true that there is evidence that greater rightism is associated with higher fertility (Fieder and Huber 2018), which would obviously relate positively to fitness, it is unclear if this association persists into the truly extreme right-wing end of the political spectrum, since studies measuring political orientation often fail to adequately capture the strikingly non-normative parts of the left-right continuum; moreover, the association could be driven entirely by the positive association between rightism and religiosity, with the typically pro-natal aspect of at least the Abrahamic faiths being the true causal basis of higher fertility in those on the political right. Although prior research by some of the current authors, in addition to this paper, emphasizes the potential role for harmful mutations in the adoption of individualizing [both in Haidt's more narrow sense of the term and in the broader sense of promoting atomization or individualization] ideologies, past research has failed to emphasize the astonishing frequency with which politically extreme views, both on the left and right, promote destructive behaviors at the group and individual levels that belie many of those ideologies' explicit commitment to pro-group orientations). This case of politicized misrepresentation of a biological concept is not dissimilar to that faced by Richard Dawkins in the 1970s after coining the term selfish gene. The concept, and some associated ideas of which Dawkins availed himself, was widely misunderstood by the lay public and misused by politically motivated actors (in particular those on the far right) as a basis for advancing their goals. Just as Dawkins (1981) took the opportunity to oppose in print this misuse of his concept, we, too, take this opportunity to state our opposition to the casual (mis)labeling of people as "spiteful mutants" for abusive and other objectionable purposes. All uses here of the term spiteful mutations (and associated terms and concepts), as in related scientific literature, are meant to be purely descriptive of biological and psychological phenomena in humans and nonhuman animals (Woodley of Menie et al. 2017a; Woodley of Menie et al. 2020).

associated systems[2], which Haidt (2007, see also Haidt 2012) defines broadly as "[i]nterlocking sets of values, practices, institutions, and evolved psychological mechanisms that work together to suppress or regulate selfishness and make social life possible", may correspond to the relative strengths with which group- and individual-level selection have acted on populations over time, as well as their patterns of social epistasis. According to this hypothesis, greater expression of group oriented (or what Haidt (2012) calls *binding*) moral foundations, which are those prioritizing loyalty, sanctity, and respect for authority, reflects higher relative strength of group-level selection than greater expression of more individual-oriented (or what Haidt (2012) calls *individualizing*) moral foundations, which are those prioritizing fairness and avoidance of harm[3].

Hertler, Figueredo and Peñaherrera-Aguirre (2020), proposed that the interpretation of these chronometric factors as reflecting a culture's orientation toward group- versus individual-level selection, resonates quite strongly with the ideas of medieval historian and sociologist Ibn Khaldun (1377), who believed that empires flourish when they are high in *Asabiyyah*. This concept describes a type of cohesive tribalism, and shares similarity with the concepts of *esprit de corps* and *vigor*, which denote a group's legacy of toughness, grit, and resilience. Taken together, these traits contribute to exceptionally well-integrated groups which are thought to be more organized and capable in the face of conflict with rival groups. Ethnographic and historical evidence suggests that complex sociopolitical systems featuring ultrasocial mechanisms promoting within-group cooperation and proscribing social defection tend to outcompete simpler sociopolitical systems (Hertler et al. 2020). *Asabiyyah* as a prospective measure of groupishness conceptually overlaps with several subsets of terms that are characteristic of highly group-selected populations, including but not limited to *élan*, *panache*, and *dash*, as indicators of martial enthusiasm, *comradery*, *loyalty*, and *compatriotism,* as indicators of fraternal solidarity, and *jingoism*, *nationalism*, and *patriotism,* as indicators of national commitment and cohesiveness. The leixical basis of Haidt's *Binding* higher-order moral foundation clearly also conceptually overlaps with *Asabiyyah,* with the production of words connoting loyalty, sanctity, and deference toward authority, serving as verbal-behavioral markers of orientation toward *Asabiyyah.* Conversely, when cultural emphasis is placed on concepts such as fairness and harm avoidance, reflecting an *individualizing* morality, this can be said to reflect an orientation away from *Asabiyyah*, as such values tend to be associated with personal flourish-

2 Woodley of Menie et al. (2020) found that advanced paternal age (a strong proxy for de novo mutation load in offspring), net of covariates, is a negative predictor of church attendance in U.S. cohorts born in the 1970s and 1980s, but not among those born in the 1930s and 1940s. One interpretation of this finding is that the accumulation of prospectively spiteful mutations has undermined group-oriented cultural adaptations (such as religious ritual) possibly by promoting attrition. Thus, historically, when cultural pressures to conform to religious norms were strong, we see no effect of paternal age on church attendance; however, among younger cohorts, where these pressures are much weaker, and in some regions virtually absent, we see the expected effect of paternal age on avoidance of engagement with religious ritual.

3 It should be noted that there is some controversy concerning the diachronic stability of the association between moral foundations and ideological dispositions (Smith et al. 2016); however, these objections have been addressed (Haidt 2016).

ing rather than groupishness (Hertler et al. 2020). Ultimately, therefore, the term *Asabiyyah* simply denotes the distinctive observable quality of group-selected populations at the cultural level, and (based on arguments advanced in Hertler et al. 2020) this, in turn, might be reflected in a culture's verbal behavior (e.g., in the generation of texts utilizing certain terms connoting a high *binding* and low *individualising* moral psychology).

Although complex polities initially benefit from the spoils of war, the influx of wealth and ease of living reduces a group's level of *Asabiyyah* (Hertler et al. 2020; Khaldun 1377). Hertler and colleagues (2020) identified a stark macrohistorical decline in a lexicographic *Asabiyyah* factor across two centuries. According to the authors, GDP per Capita significantly reduced the level of *Asabiyyah* above and beyond any temporal autoregressive effects. This negative effect is expected since polities featuring greater macroeconomic growth and stability may allocate their available resources toward reducing morbidity and mortality rates. It follows then that such epidemiological transition should also be associated with relaxation of negative selective pressures facilitating the accumulation of deleterious mutations. Although consistent with SEAM, the authors of the latter study did not explore whether a reduction in between-group competition, an indicator of selective pressures, could positively influence (potential proxies for) mutation accumulation, which in turn may reduce *Asabiyyah* over time.

A prediction deriving from the SEAM is that *Asabiyyah* is likely to decline with time as a consequence of this relaxed negative selection leading to the accumulation of (in particular) spiteful mutations reducing the group-level cohesion of populations, and that this trend might be captured and measured lexicographically, as in Hertler et al. (2020), using changing cultural expressions associated with decreased *binding* morality, coupled with increased *individualizing* morality, marking the shift away from the sorts of values that are essential to the internal cohesiveness of groups (this being a key component of *Asabiyyah*).

There are also other factors that might influence a population's level of *Asabiyyah*. Heightened expression of individualizing moral sentiment is very likely adaptive under low intergroup competition (peace), as reflected in the conditions that characterize Western late modernity (Hertler et al. 2020). This is likely because being more focused on the mitigation of harms and the promotion of fairness and personal flourishing, and a reduced emphasis on matters promoting group-level fitness, would be more beneficial to individuals under conditions of intergroup peace. This hypothesis is consistent with the *evoked culture* model of Tooby and Cosmides (1992), as flexibility in the development of moral foundations may constitute a kind of evolved plasticity which facilitates the adaptive calibration of behavior in response to various evolutionarily familiar environmental cues. Having gone through periods of both intergroup conflict and peace, human populations may have acquired behavioral and innate moral repertoires that adapt their members to both conditions, with such populations having become evolutionarily prepared for the expression of a range of moral sentiments that adaptively match the situation. Selection may also act via gene-culture coevolution to differentially promote the fitness of various moral genotypes under different regimes of group- and individual-level selection.

To compare these two models (the SEAM versus the evoked culture plus gene-culture coevolution model), in the current study Haidt's *moral foundations theory* will be used to derive a lexicographic diachronic measure of *Asabiyyah*, as indicated by the decline in a latent common factor among the levels of both *binding* and (reverse scored) *individualizing* morality, measured via the utilization frequencies of words corresponding to Haidt's moral foundations in the textual outputs of Britannic populations. It will then be determined whether a diachronic trend in measures of the strength of group-selection have direct effects on the level of this factor, or whether this is mediated by measures of increasing developmental instability (as a proxy for increasing mutation load). It is expected that the developmental instability factor should largely mediate the impact of the intergroup competition measure on the moral foundations factor. This is because decreased intergroup competitiveness likely relaxes negative selection via reductions in social conflict.

Methods

Populations

Data were collected for the following Britannic nations ranging from AD 1800 to 1999, essentially comprising the former British Empire and its various successor states: UK, USA, Canada, New Zealand, and Australia (Figueredo et al. 2019a).

Lexicographic Measures

The historical utilization of these specific classes of words was quantified via their relative frequencies of usage in English language texts across the 200 years spanning AD 1800–1999 through Google Ngram Viewer, an interactive textual corpus encompassing more than 5.9 million texts and 500 billion written words from AD 1500 to 2019 (Michel et al. 2011). The forward extent of our analysis is restricted to 1999, as the post-2000 corpus is known to be highly incomplete. This is consistent with other studies using this database (see: Greenfield 2013; Hills and Adelman 2015; Younes and Ulf-Dietrich 2019). Google Ngram Viewer has been used to track temporal trends in public sentiment (Figueredo et al. 2019a, 2019b; Greenfield 2013; Ladle et al. 2016; Michel et al. 2011), changes in expressions of religiosity (Younes and Ulf-Dietrich 2019), changes in population-level cognitive characteristics (as ascertained by the utilization frequencies of words with known item-level psychometric difficulties; see Roivainen 2014; Woodley of Menie et al. 2015 and historical estimates of word learnability; see Hills and Adelman 2015), shifts in lexicographically estimated life history characteristics (Woodley of Menie et al. 2019), and the temporal stability of cultural stereotypes (Del Giudice 2012). These applications of Ngram to the quantification and study of cultural trends are referred to as *culturomics* (Michel et al. 2011).

The lexical items connected with the moral foundations were retrieved from Graham, Haidt, and Nosek's (2009) moral foundations dictionary: (1) harm, (2) fairness, (3) loyalty, (4) authority, and (5) purity (an abridged list of the words collected is presented in Table S1). The lexical items used in constructing each of these scales were psychometrically selected based on their possessing satisfactory part-whole correlations for each word to the corresponding aggregate scale score for each lexicographic scale. The best words were thus empirically selected from the initially larger item pool. This psychometric procedure for selecting

items has the benefit of being the most straightforward approach to creating robust and internally consistent chronometric constructs, with the items exhibiting differential validity based on their degree of convergence with each of the five moral foundations.

Unit-weighted common factor scales (Gorsuch 1983) were estimated as the means of the standardized scores for the lexicographic items on each scale (Figueredo et al. 2000). As per moral foundations theory, the five scales were aggregated into two lower-order factors: (1) binding and (2) individualizing. By reverse-scoring the individualizing factor, these two lower-order factors were further aggregated into a single higher-order factor—the *Asabiyyah* factor discussed in Hertler et al. (2020). The resultant chronometric factors, along with their Cronbach's alpha values and unit-weighted factor loadings are presented in Table 1. Binding and individualizing exhibited a strong tendency toward negative correlation across time, indicating that these two trends were diverging from each other systematically. Therefore, there is a clear tendency for the rise in cultural expressions of morality emphasizing personal flourishing and self-actualization to occur at the expense of groupishness, deference to authority, and sanctity, which would be consistent with a decrease in *Asabiyyah* over time. This trend is graphed in Figure 1. It is important to note that the division of moral foundations into the categories of individualizing and binding does not reflect the explicit intent of the original authors to attribute these categories to individual versus group fitness; rather, this division more accurately describes alternate loci of moral values that both function to inhibit selfishness (Graham et al. 2011; Haidt 2008). The attribution of these diachronic changes to individual versus group selection was made by some of the current authors based on the application of multilevel selection theory (e.g., Hertler et al. 2020). Nevertheless, as discussed in the introduction, Haidt (2007, 2012) does speculatively attribute the evolution of binding values to cultural group selection, foreshadowing the present application. The logic expounded by Graham, Haidt, and Nosek (2009) is as follows: a society that takes a predominantly individualizing approach to suppressing selfishness will honor the rights and well-being of other individuals (care and fairness); in contrast, a society that takes a binding approach to suppressing selfishness will emphasize the imperative for individuals to conform to the needs of the group (loyalty, authority, sanctity/purity). If this reasoning is correct, then the implications for multilevel selection are quite manifest.

Table 1 displays the psychometric results of these analyses. *p*-values here and later are based on two-tailed tests unless otherwise noted.

Following Woodley of Menie et al., (2017b), we also used the ten altruism words employed by Charles Darwin in *The Descent of Man* (1871) to describe changing levels of within-group altruism and between-groups competition in humans[4]. Diachronic part-whole

4 In the *Descent of Man* Darwin uses a set of ten terms (including *self-sacrifice*, *obedience*, and *heroism*) in describing broadly altruistic virtues that would lead to group-level benefits in competition. In previous research (e.g., Woodley of Menie et al. 2017b) it has been found that a diachronic factor comprised of the utilization frequencies of these "Darwin altruism" terms sampled from the Ngram viewer, exhibits high levels of internal consistency, in addition to external validity with respect to other prospectively more direct measures of inter-group competition (such as per capita war fatalities). This can be taken

Table 1. Cronbach's alphas and part-whole correlations (unit-weighted factor loadings) for the lexicographic scales and lower-order Moral Foundations factors of the higher-order Asabiyyah factor from AD1800–1999

	Cronbach's Alpha	Unit-Weighted Factor Loading
	INDIVUALIZING	
Harm Scale	0.974	0.988*
Fairness Scale	0.980	0.988*
	BINDING	
Loyalty Scale	0.974	0.985*
Authority Scale	0.991	0.995*
Purity Scale	0.994	0.990*
	ASABIYYAH	
INDIVUALIZING	0.988	-0.987*
BINDING	0.997	0.978*

* $p<.05$

correlations of the Darwin *Descent of Man* Altruism Words ranged from .29 to .92 ($p<.05$), with the overall factor scale accounting for 55% of the chronometric factor variance.

Biodemographic Measures

Population sizes were obtained from the *Maddison Project* database (Bolt at al. 2018), a repository curated by the Groningen Growth and Development Center. These were used to construct a corporate or group-level fitness measure by dividing the share of the Britannic populations by the rest of the world's population at different points in time, yielding a relative measure of the success of their biocultural-group relative to the rest of the world's population. The proportion of the world population was estimated based on the various demographic database compiled by Roser, Ritchie, and Ortiz-Ospina (2013, see also references therein).

Warfare mortality estimates (a fairly uncontroversial measure of inter-group competitiveness) were obtained from Sarkees and Wayman's (2020) *Correlates of War* database, we excluded civil (within-state) conflicts, retaining only between-state conflicts. Any conflict involving one or more Britannic nation was retained. Mortality rates (expressed per capita, per 100,000) were estimated, controlling for population size. This was necessary, as population size could confound warfare intensity owing to the observation that larger populations will exhibit greater absolute death numbers.

Following Woodley of Menie et al. (2017b), a latent Intergroup Competition (*IGC*) Factor was constructed using the three convergent group-selec-

as evidence that "everyday language" contains information about the sorts of selective pressures that shape the attitudes and behaviors undergirding its use. Thus, Darwin's choice of these terms in relation to describing altruistic attitudes that promote group competitiveness makes them potentially good stand-alone indicators of societal altruistic sentiment of a sort that is specifically directed toward a particular biocultural group.

tion indicators: (1) Darwin's Descent of Man Altruism Words; (2) Britannic corporate fitness; and (3) War Mortality per 100,000. The single lexicographic indicator used in the *IGC* factor model, the Darwin *Descent of Man* Altruism Words, converged well with the two biodemographic ones, the proportion of the world's population and war mortality; part-whole temporal correlations of the *IGC* factor ranged from .42 to .66 ($p < .05$), with the overall factor scale cumulatively accounting for 27.5% of the chronometric factor variance.

Developmental Instability Measures

This measure includes three convergent phenotypic measures that are believed to be associated (in part) with individual differences in burdens of deleterious mutations. The measures are percentage sinistrals (meaning those who are left-handed, sourced from McManus et al. 2010), craniofacial fluctuating asymmetry (sourced from Kimmerle and Jantz, 2006, with supplementary data from Woodley of Menie and Fernandes 2016), and body mass index (BMI; sourced from Komlos and Brabec 2010). The data were recovered from graphs in their respective publications using *WebPlot Digitzer* (Rohatgi 2017). Both sinistrality and fluctuating asymmetry have long been theorized to be indicators of developmental instability, and possibly also elevated mutational load (e.g., Markow 1992; van Valen 1962). The association between both the level and variance in BMI and deleterious mutations has only recently been evidenced however, with national-level indicators of relaxed negative selection functioning as substantial predictors of national differences in levels and variance of BMI (Budnik and Henneberg 2017), even when lifestyle covariates (e.g., calories consumed and levels of exercise) are controlled. Budnik and Henneberg (2017) have hypothesized that variation in BMI might be partly reflective of the action of deleterious variants that reduce the efficiency of metabolic processes, leading to either excessive body mass, or (in some cases) an inability to accumulate body mass.

In total, these variables are available for the years spanning 1825 to 1985. Sinistrality was sampled between the years 1835 to 1976, for a total of 99 measurement occasions. BMI was sampled between the years 1885 to 1985, for a total of 21 measurement occasions. Craniofacial fluctuating (specifically size) asymmetry was sampled between the years 1825 to 1985 for a total of 16 measurement occasions.

All variables were sourced from the population of the USA. The reason for focusing on the European-American samples in this instance is because the majority of the USA population for the majority of the set of years sampled here were of European descent. The incorporation of data on non-European-origin populations might therefore bias the sample characteristics in ways that are unrepresentative of the true time trends.

The use of phenotypes as proxies for tracking the underlying burden of deleterious mutations has been promoted in the absence of sufficiently high-resolution genomic sequencing and variant-calling protocols to detect the hypothesized increase in mutation accumulation that may have accompanied the reduction in opportunity for negative selection through mortality since industrialization (for discussion of this topic see Kondrashov 2017). It should

furthermore be noted that factors independent of mutation accumulation may partly, and in some cases, mostly account for the time trends associated with these variables. For these variables to serve as useful proxies for mutation accumulation, it is only necessary that some of the temporal trends among them stem from relaxed negative selection however.

The results of the unit-weighted factor model (estimated using multivariate imputation; see Figueredo at al. 2000; McKnight at al. 2000) on the developmental instability factor are presented in Table 2. All factor loadings are statistically significant and high magnitude ranging in value from .68 to .99 ($p < .05$). The latent factor accounted for 75.5% of the chronometric variance.

Table 2. Part-whole correlations (unit-weighted factor loadings) for the Developmental Instability factor from AD1825-1985

	Unit-Weighted Factor Loading
Developmental Instability	
Craniofacial Fluctuating Size Asymmetry	0.68*
Body Mass Index	0.91*
Sinistrality	0.99*

* $p<.05$

Hypotheses

The following set of hypotheses are examined with reference to the data employed in the present study.

H1: Year will negatively predict the *Intergroup Competition Factor*.

H2: The *Intergroup Competition Factor* will negatively predict the *Developmental Instability Factor*.

H3: Year will positively predict the *Developmental Instability Factor*.

H4: The *Developmental Instability Factor* will negatively predict the *Asabiyyah Factor*.

H5: The *Intergroup Competition Factor* will positively predict the *Asabiyyah Factor*.

H6: Year will negatively predict the *Asabiyyah Factor*.

Statistical analyses

All univariate and multivariate analyses were performed using SAS 9.4 (SAS Institute Inc., 2015) and Unimult 2 (Gorsuch 2016). Using SAS PROC STANDARD and DATA, unit-weighted common factor scales (Gorsuch 1984) were estimated as the means of the standardized scores for all non-missing subscales on each factor (Figueredo et al. 2000). Using SAS PROC CORR, Cronbach's alphas and the part-whole correlations of the subscales with the unit-weighted factor scales were also computed.

Results

Multilevel Models

The lexicographic scales function as manifest variables for the purposes of longitudinally estimating multilevel models (MLMs). We estimated four nested MLMs in total, so as to determine the need for increasing parameterization as a function of testing alternative hypotheses. The four models are as follows: *MLM1* was an unconditional *Asabiyyah* model, in which a single logarithmic slope and intercept were estimated for all lexicographic factors, scales, and items (words) over time. *MLM2* involved the estimation of a separate intercept and logarithmic slope over time for each lexicographic factor. However, the same intercepts and

logarithmic slopes were estimated for all within-factor scales and words over time. *MLM3* involved the estimation of separate, lexicographic-scale-specific, logarithmic slopes and intercepts over time, but with each within-factor scale word having the same logarithmic slope and intercept over time. *MLM4* involved the estimation of separate word-specific logarithmic slopes and intercepts over time. All MLMs were statistically controlled for the effects of the year of *FirstUse* recorded for each word in the analyses; this is an important control, as it has been found that older words tend to be better known to users of texts as a result of the lag between changes in spoken and written texts (Curzan 2009; Woodley of Menie et al. 2015); LNT is the natural logarithmic function of time.

All nested model comparisons are displayed in Table 3. Systematic -2RLL and AIC comparisons were performed by comparison among nested models. AIC and AIC weights were computed with the statistical package *qpcR* (Ritz and Spiess 2008) in R version 4.1.0. Each level of the aggregative hierarchy contains and accounts for specific variance components. Their estimation revealed that the majority of incremental model fit improvements were relatively trivial in magnitude, but nevertheless statistically significant ($p<.05$). When the four nested MLMs were compared in terms of the squared multiple correlations among them, it was found that they yielded basically the same results. Although statistically significant, the magnitudes of specific variances associated with each level of aggregation (ΔR^2), were negligibly small, which contrasts sharply with the finding that the common factor variance associated with the highest-level of aggregation (unconditional *Asabiyyah*) was quite large (69%). It is worth noting that the model comparison identified *MLM4*, with Word and the Word*LNT interaction, as the best model based on its AIC weight (1.000).

Table 3. Fit Indices for Nested Multilevel Models (MLMs) for Haidt Moral Foundation Dictionary Factors, Scales, and Words from AD1800–1999

Multilevel Model	**MLM1**: FirstUse + LNT	**MLM2**: + Factor + Factor*LNT	**MLM3**: + Scale + Scale*LNT	**MLM4**: + Word + Word*LNT
AIC	42323.8	42313.8	42307.1	41700.1
AIC	623.70	613.70	607.00	0.00
AIC weight	0.000	0.000	0.000	1.000
-2RLL	42313.8	42299.8	42281.1	41186.1
	$\Delta\chi^2$ =	**14.0***	**18.7***	**1095.0***
R^2	0.68869	0.68886	0.68909	0.70221
	ΔR^2=	**0.00017***	**0.00023***	**0.01312***
NDF	2	4	10	253
	ΔNDF=	2	6	243

* $p<.05$

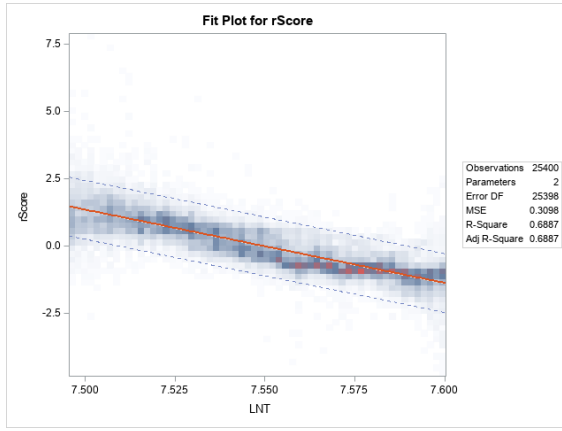

Fig. 1. Heat Map for Bivariate Linear Regression of Asabiyyah as a Natural Logarithmic Function of Time (AD 1800–1999).

Only the model parameters associated with the unconditional *Asabiyyah* level (*MLM1*) were retained, as the extra model parameters added by all the lower levels of aggregation (*MLM2, MLM3,* and *MLM4*) in the moral foundations dictionary factors, scales, and words only increased the proportion of variance explained from 69% to 70%. The logarithmic slope of this unitary higher-order *Asabiyyah* factor over time was negative and statistically significant: r = -.83, $F(1,25398)$ = 56190.9, $p<.0001$. No significant heterogeneous serially autoregressive effects were identified (*ARH1* = 0), and the effect of year of *FirstUse* (of each word) was statistically nonsignificant ($p>.05$).

Cascade Model: Hierarchical Multiple Regressions

We constructed a sequential canonical cascade model from the following system of three ordered hierarchical multiple regressions:

1. *IGC* = *YR*
2. *DI* = *IGC* + *YR*
3. *ASABIYYAH* = *DI* + *IGC* + *YR*

The purpose of a sequential canonical cascade model is to test for mediation by using each prior criterion variable as the first predictor in each successive hierarchical regression to control for any indirect effects transmitted through it, thus estimating only the residual direct effects of each subsequent predictor variable (Figueredo and Gorsuch 2007). As fewer historical data were available for the developmental instability (*DI*) variable, the sequential canonical cascade model was estimated exclusively on data spanning the years from AD 1825–1985. The protective omnibus Pillai-Bartlett trace test for the entire sequential canonical analysis model was statistically significant: V = 0.984, E = 0.57, *90%CI* = (0.56, 0.58), $F(3,104)$ = 2068.47, p <.0001.

Table 4 displays Cascade Equation 1, with time (*YR*) having a statistically significantly and negative direct effect on intergroup competition (*IGC*; supporting *H1*). The semipartial correlation coefficient is indicated by the symbol *sR*; Figure 2 shows this relation graphically, using the standardized (z) scores of both predictor and criterion variables. These results indicate that intergroup competition has been decreasing across time since AD 1825.

Table 4. Cascade Equation 1: Hierarchical Regression for IGC with YR from AD1825–1994

Predictor	*sR*	**C.I. (90%)**	*F*	*df1, df2*	*p*
YR	-0.19*	-0.36,0.01	3.77	1, 106	0.05

*$p < .05$

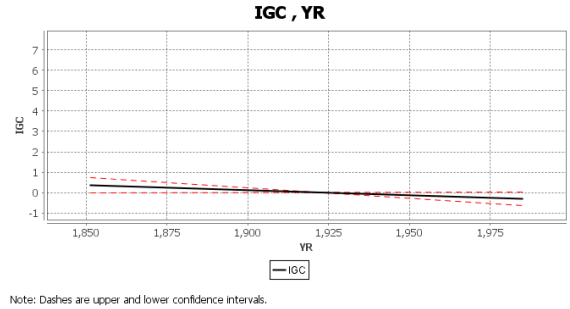

Fig. 2. Hierarchical Regression of the direct effect (semipartial correlation) of YR on IGC (AD 1825–1985).

Table 5 displays Cascade Equation 2, in which *IGC* had a statistically significant and negative direct effect on developmental instability (*DI*; supporting *H2*), while *YR* had a statistically significant and positive residual direct effect on *DI* (supporting *H3*). The semipartial correlation coefficient is indicated by the symbol *sR*; Figures 3 and 4 illustrate these relations graphically, using the standardized (*z*) scores of both predictor and criterion variables. These all indicate that *DI* is reduced by higher levels of intergroup competition, which Cascade Equation 1 shows to be declining, but that *DI* has otherwise been increasing through time since AD 1825.

Table 6 displays Cascade Equation 3, wherein *DI* had a statistically significantly and negative direct effect on *Asabiyyah* (supporting *H4*), while *IGC* had a statistically significantly and positive residual direct effect on *Asabiyyah* (supporting *H5*), and *YR* had a statistically significant and negative residual direct effect on *Asabiyyah* (supporting *H6*). The semipartial correlation coefficient is indicated by the symbol *sR*; Figures 5, 6, and 7 show these relations graphically, using the standardized (*z*) scores of both predictor and criterion variables. These all indicate that *Asabiyyah* is reduced by higher levels of developmental instability, which Cascade Equation 2 has shown to be rising, but that *Asabiyyah* increases with higher levels of intergroup competition, which Cascade Equation 1 shows to be declining, and *Asabiyyah* has otherwise been decreasing over time since AD 1825.

Table 5. Cascade Equation 2: Hierarchical Regression for DI with IGC and YR from AD1825–1985

Predictor	*sR*	C.I. (90%)	*F*	*df1, df2*	*p*
IGC	-0.33*	-0.49, -0.14	22.89	1, 105	<0.0001
YR	0.63*	0.50, 0.74	86.23	1, 105	<0.0001

*$p < .05$

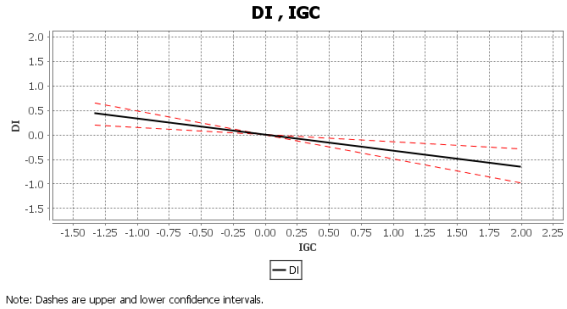

Fig. 3. Hierarchical Regression of the residual direct effect (semipartial correlation) of IGC on DI (AD 1825–1985).

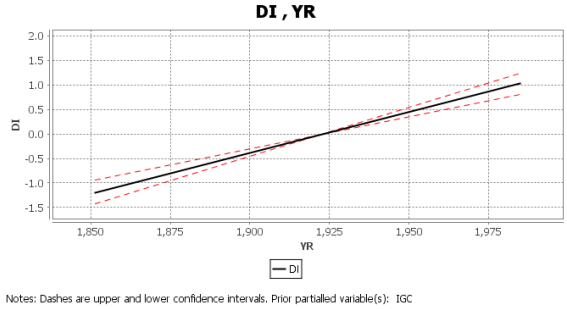

Fig. 4. Hierarchical Regression of the residual direct effect (semipartial correlation) of YR on DI, statistically controlled for the effect of IGC (AD 1825–1985).

Table 6. Cascade Equation 3: Hierarchical Regression for ASABIYYAH with DI, IGC, and YR from AD1825–1985

Predictor	*sR*	C.I. (90%)	*F*	*df1, df2*	*p*
DI	-0.61*	-0.72,-0.48	543.44	1, 104	<0.0001
IGC	0.06*	-0.25,0.14	4.75	1, 104	0.03
YR	-0.74*	-0.82,-0.64	785.70	1, 104	<0.0001

*$p < .05$

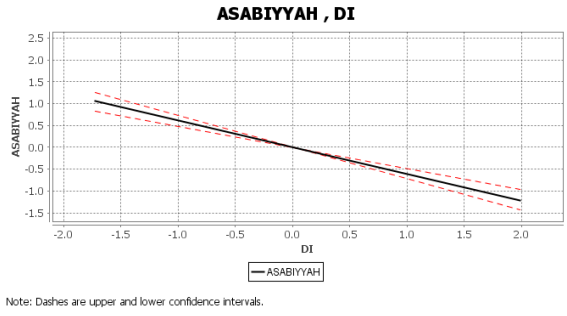

Fig. 5. Hierarchical Regression of the direct effect (semipartial correlation) of DI on ASABIYYAH (AD 1825–1985).

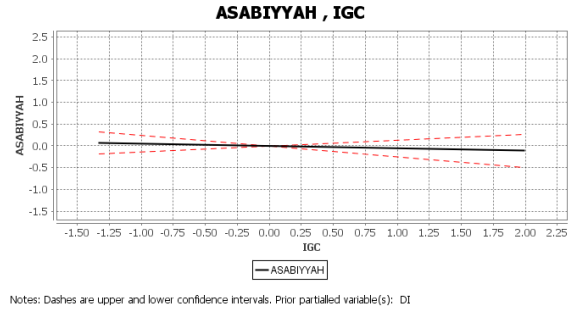

Fig. 6. Hierarchical Regression of the residual direct effect (semipartial correlation) of IGC on ASABIYYAH, statistically controlled for the effect of DI (AD 1825–1985).

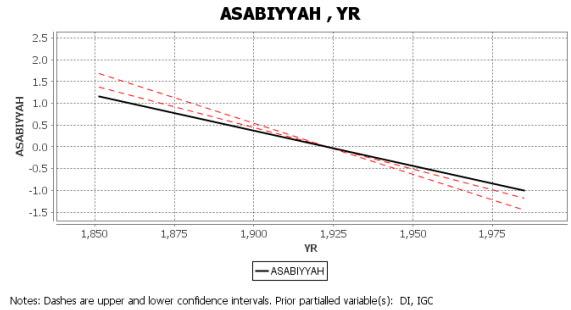

Fig. 7. Hierarchical Regression of the residual direct effect (semipartial correlation) of YR on ASABIYYAH, statistically controlled for the effect of DI and IGC (AD 1825–1985).

Discussion

Consistent with predictions from both the SEAM and the evoked culture model, the temporal decline in the *Asabiyyah* factor is independently predicted by both the (declining) intergroup competition and (rising) developmental instability factors. Critically, the developmental instability measure seems to substantially mediate the impact of declining intergroup competition on the *Asabiyyah* factor, consistent with the hypothesized impact of diminished intergroup competition on mutation accumulation, as predicted by SEAM. Furthermore, these results demonstrate a novel application of Haidt's moral foundations theory to the elucidation of culturomic shifts and their determinants. We also note that the decline in Asabiyyah noted here is consistent with the work of Younes and Ulf-Dietrich (2019), who, in employing Google Ngram Viewer, found indications of a general decrease in collectivistic religious expression across multiple languages between 1900 and 2000, with (temporary) reversals to this trend having occurred during times of conflict (e.g., World War II).

The small and positive residual direct effect of intergroup competition on the *Asabiyyah* factor, after statistically controlling for that of developmental

instability, was minimally consistent with the predictions of the evoked culture model. This model would hypothesise that declining intergroup competition might serve as a direct driver of changes in preferences from group-oriented to individual-oriented textual expressions of moral psychology. As discussed in the introduction, the process by which this path-dependency arises might relate to diminished levels of intergroup competition evoking pre-existing evolved psychological mechanisms that adaptively upregulate preferences for individualizing morality, with more fairness- and harm-avoidance-oriented moral expressions being more adaptive under conditions of intergroup peace. This process might also establish a selective context in which, via gene-culture coevolution, rapid selection can take place favoring the fitness of genotypes that predispose toward the development of these individualizing moral foundations. Such selection can conceivably even act over relatively short periods of time[5].

Further, the measurement model for the latent structure of *Asabiyyah* was not meaningfully confounded by temporal autocorrelations, which were found to be of negligible magnitudes, nor were the lexicographically convergent results confounded with the age of the words sampled, which is significant as age has been found in previous work on Ngram viewer to be a significant predictor of temporal changes in the utilization frequencies of words (Woodley of Menie et al. 2015). The developmental instability factor may therefore capture changes in the strength of negative selection on indicators that may serve as proxy measures of disturbed patterns of social epistasis. Moreover, evidence for this mediational pathway strengthens the case for lexicographic moral foundation measures serving as diachronic indicators of either positive or negative social epistasis.

These findings also have relevance for ongoing debates in evolutionary psychology concerning the possibility that certain levels of trait expression might be maladaptive – meaning that they stem from some process that is leading to long-term reductions of fitness. A related debate concerns the possibility that the extremely low fertility rates characteristic of post-demographic-transition Western populations, in particular, might be maladaptive in so far as their fertility is at sub-replacement levels. Arguments have been made to the effect that this consequence of the demographic transition merely reflects changes in patterns of bioenergetic investments stemming from adaptive, developmentally mediated transitions into slower life history, and specifically a regime of significantly diminished child, infant, and general mortality. Based on this alternative argument, lower individual-level fertility might therefore be "paid for" in other ways by changes in such patterns of investments resulting in for example, greater somatic persistence (longevity) and greater allocations of effort into certain communitarian domains (e.g., Colleran 2016).

These results should be interpreted with caution, insofar as some may

5 Consistent with the idea of rapidly shifting selection on moral foundations is the finding of Huber and Fieder (2018) who have observed that selection, as proxied by relative fertility, has increasingly come to favor those whose political values are closer to the extremes, with significant shifts in the focal point of this selection having occurred over just a handful of decades. But as noted in an earlier footnote, how far this tendency goes is unclear.

be inclined to read them as suggesting that group-oriented ideologies are simply "better," in terms of their effects on group fitness, than their individual-oriented counterparts. It is becoming increasingly apparent, from real-world evidence, that this is flatly incorrect, however. In the contemporary Western context at least, the adoption of extreme ideologies, including and perhaps especially group-oriented ones, for instance Communism on the left and neo-Nazism on the right, seems to be uniquely attractive to those whom criminologists would term "socially deviant" individuals. Such individuals are at elevated risk of a number of undesirable outcomes, such as engagement in crime (including violent crime and terrorism) and low social status. The organized actions of these individuals often seriously harm, particularly through violence, the welfare of the broader populations in which they are embedded (Institute for Economics and Peace 2020) and occasion the rapid dissolution of the movements with which they are associated. Even if it is true, as these results suggest, that genetic change in Western populations has been favoring the rise of excessively individualistic (from a group-fitness perspective) values, it does not follow from this that all groupish alternatives are functional or in some way desirable. Indeed, it could be that genetic and social-epistatic dynamics have taken long-standing individualistic tendencies in Western populations to problematic levels, while also giving rise to damaging group-oriented efforts to "correct" these developments. Pathological manifestations of extreme and groupish political and social ideologies obviously are nothing new. The especially catastrophic results, in moral and biological terms, of Communism and Nazism in the twentieth century speak for themselves – and it is hardly surprising that the intellectual heirs of these movements clearly share psychological traits widely considered to be highly socially undesirable (Costello et al. 2022; Moss and O'Connor 2020 a,b). Although the groupish social and political arrangements of premodern societies in the more distant past across the world were hardly liberal, and involved various moral evils, the disastrous totalitarianisms of recent history, and their contemporary ideological progeny, suggest the appearance of uniquely perverse extremisms. It must be stressed that all authors on the current paper unequivocally oppose extremist political views.

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Availability of data and material

Google Ngram Viewer data can be obtained from the following url: https://books.google.com/ngrams. The lexical items used as the basis for constructing diachronic measures of moral foundations were obtained from Graham, Haidt, and Nosek's (2009) moral foundations dictionary. The diachronic data used here have been made publicly available in Hertler et al. (2020), Woodley of Menie et al. (2017b), and from and sources contained therein.

Code availability

All code will be made available upon request.

Ethics approval

Not applicable.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Consent to participate

Not applicable.

Authors' contributions

MWOM, MAS, and MPA drafted the manuscript. AJF, MPA, and MJ prepared the data analyses. All authors approved the final version.

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Abandon	Brutality	Cruelty	Disgust	Exploiting	Hierarchy	Insider	Loyalty	Position	Respect	Solidarity	Unchaste
Abstemiousness	Cadre	Crusher	Dishonest	Exploits	Holiness	Insubordination	Maiden	Preference	Respected	Spurn	Uncleanliness
Abstention	Care	Damage	Disloyalty	Fair	Holy	Insurgent	Member	Prejudice	Respectfulness	Spy	Unequal
Abstinence	Caring	Debasement	Disobedience	Fair play	Homeland	Integrity	Miscreant	Preserve	Respects	Stain	Unfairness
Abuse	Caste	Debauchery	Disproportion	Fairly	Homologous	Intemperate	Modesty	Pristine	Rights	Status	Unfaithful
Adultery	Celibacy	Deceiver	Disrespect	Fairmindedness	Honesty	Jilter	Mother	Profanity	Riot	Sterility	Unison
Agitation	Chastity	Decency	Dissent	Fairness	Honorable	Joint	Mothering	Profligate	Ruin	Stomp	Uniter
Alienate	Class	Defector	Dissident	Familial	Humble	Justice	Motherland	Promiscuity	Sacredness	Subversion	Unjustness
Ally	Cleanliness	Defense	Dissociate	Families	Hurt	Justification	Mothers	Prostitute	Safety	Suffering	Unprejudicedness
Amity	Clique	Defiance	Egalitarianism	Family	Illegality	Justness	Mutinous	Protection	Saint	Supremacy	Unscrupulous
Annihilation	Cohort	Defiler	Empathy	Father	Immaculate	Kill	Nation	Protest	Security	Taint	Upright
Apostasy	Collective	Denounce	Endangerment	Favoritism	Immigrant	Killed	Nonconformist	Pureness	Sedition	Tarnish	Violence
Apostate	Command	Depravity	Enemy	Fellowship	Impair	Killer	Obeyance	Purity	Segregation	Terrorism	Virgin
Attack	Communal	Desecration	Equable	Fight	Impartiality	Killing	Obscenity	Rank	Sequester	Together	Virginal
Austerity	Commune	Deserted	Equality	Filth	Impiety	Kills	Obstruct	Ravage	Shelter	Tolerant	Virginity
Authority	Communism	Deserter	Equity	Foreigner	Impious	Law	Oppose	Reasonable	Shield	Tradition	Virgins

Balance	Community	Deserting	Equivalent	Gross	Imposter	Lawfulness	Order	Rebel	Sickness	Traitor	Virtuous
Benefit	Compassion	Destroy	Evenness	Group	Indecency	Lawlessness	Peace	Reciprocity	Sin	Tramp	Wanton
Betrayal	Compliance	Detriment	Excluder	Guard	Individual	Lax	Permission	Refined	Sinfulness	Trashy	War
Bias	Comrade	Devotee	Exclusion	Guild	Inequitable	Leader	Permit	Refuse	Sinned	Treachery	Warlord
Bigotry	Constant	Dirt	Exploit	Harm	Ingroup	Legality	Pervert	Remonstrate	Sinner	Treason	Warring
Blemish	Contagion	Discrimination	Exploitation	Harm	Injustice	Lewdness	Piety	Renegade	Sinning	Unadulterated	Wars
Bourgeoisie	Control	Disease	Exploited	Heretic	Innocent	Limpid	Pious	Repulsion	Sins	Unbiasedness	Wholesomeness

S1. Abridged list of Moral Foundation words collected from Haidt (2012) and the online version of the Moral Foundations Questionnaire.

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Post-medieval stelae cemetery in Nowy Dwór: preliminary results of an anthropological and archaeological study

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Abstract: The paper presents preliminary results of an anthropological analysis of a previously unknown post-medieval stelae cemetery in the village of Nowy Dwór in Podlaskie Voivodeship, Poland. The main aim of the study was to identify the site itself, and to create the probable biological profile of the local population. The research confirmed the existence of a post-medieval necropolis in which remains of at least 181 individuals were unearthed, with 111 individuals discovered in 88 intact graves and their closest proximity. Few individuals were equipped with what can be interpreted as "obol of the dead", and at least three burials could be classified as deviant. Biological analysis showed that 33% of analysed individuals regardless of age bore infection-related lesions and post inflammatory pathologies. Constructed mortality tables also correspond more with tables for medieval rather than post-medieval populations. As a conclusion, collected evidence and results of analysis seem to verify the historical accounts mentioning several plague outbreaks in the region, occurring from the 16th to 18th centuries. Individual findings such as "obol of the dead", as well as the "deviant grave", likely belonging to a whisperer (witch), can also provide useful to further research on local traditions and beliefs.

Key words: epidemic, Podlasie, Christian Orthodoxy, Uniates, grave markers, excavation, necropolis

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Introduction

Being characterised by large, upright stones on graves, stelae cemeteries are a cultural phenomenon native to north-eastern Poland and Belarusian Grodno region (Fig. 1). Until recently, these necropolises have been associated with local Yotvingian communities, or – alternatively – interpreted as epidemic cemeteries. During the European late medieval and post-medieval period, it was a common, although not universal practice to establish epidemic cemeteries far from human settlements or sacred grounds. It should be noted, however, that in such cases the deceased were usually buried in mass graves, and the marking of their resting place was rarely permanent (Duma 2015: 142—143). Since in terms of funerary rite stelae cemeteries were often a necropolis with singular graves, thus, stelae cannot be interpreted as indicators of plague victims' burials. On the other hand, the tradition of marking graves with stones or boulders is present in many local cultures – not only Yotvingian, but also Christian Orthodox, Uniate, Jewish and Muslim Tatar.

Since 2018, stelae necropoleis have been a subject of a research identification program led by the Podlasie Museum in Białystok, Poland. As part of the project, in 2020 the Podlasie Museum conducted rescue excavations in Nowy Dwór, where a previously unknown stelae cemetery was discovered during construction works. The study had two points: archaeological research and prospection, and anthropological analysis of unearthed remains. The following paper presents and discusses preliminary results of this study.

The exact date of the Nowy Dwór foundation is unknown and is, therefore, a subject of discussion. The earliest possible period was in the years 1440 and 1492 (Wiśniewski 2006: 149; Ryżewski 2006; 2019) or in 1505 (Ryżewski 2019: 124). During the time it was a royal property, Nowy Dwór became the centre of colonization and floatation of forest goods. In 1578 it was granted town privileges based on Magdeburg rights – an event that only sealed its status (Ryżewski 2006: 308—310).

Fig. 1. Map with the location of Nowy Dwór. Created by Hubert Lepionka.

The town population and its closest vicinity consisted mainly of colonizers coming from the east, from territories of the Grand Duchy of Lithuania (Wiśniewski 1978, Ryżewski 2006; 2019). In the first half of the 16th century, at the same time the Catholic Church of John the Baptist and an Orthodox church were founded in the town (Ryżewski 2019). With the Union of Brest, at the turn of the 16th century the Orthodox parish became Uniate.

Excavations in Nowy Dwór were conducted after the discovery of human remains and stones during an archaeological supervision of the reconstruction of a local road near the Orthodox

church, and covered a 4-meter wide and 60-meter-long trench located on the North-Western border of the cemetery (Fig. 2). As a result, 88 graves typical of a Christian burial rite were discovered.

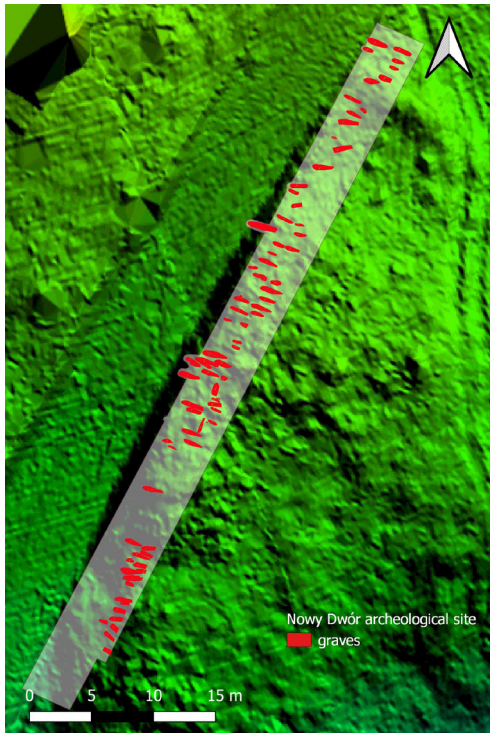

Fig. 2. Map of the site with graves. Created by Hubert Lepionka, drawn by Olga Dec.

Material and methods

On the excavated part of the cemetery 88 singular skeletal flat graves (occasionally with additional loose bones) were discovered, with the dead resting in extended supine position with either both upper limbs folded on the pelvis, or with the left hand over the heart area. In several graves remains of wooden caskets were found, although, they were not present with most burials, thus, leading to the conclusion that the deceased were buried primarily with shrouds. Similarly, barely any grave goods were discovered, and only in a few graves coins were present. Graves were mainly oriented from the North-West to South-East, with skulls pointing towards North-West. However, there were few deviations to this rule (Fig. 3). Only in two graves there was a completely different orientation: in the grave no. 75 the deceased was buried with their head to the South, and in the grave no. 73 to the South-West. Some of the graves were marked with a stone stelae with no visible traces of carvings or engravings. Preserved stelae were located in parts of the cemetery where the topsoil has not been previously disturbed (Fig. 4).

In the surveyed section of the cemetery at least 181 people were buried. These graves had been intact by the time of discovery. The rest of the remains belonged to two different types of burials. First, there were features that can be described as consisting of small pits with a layer of loose bones of 2 to 3 individuals, who were buried in what resembled a pile. These were probably secondary burials of loose bones found on the surface of the cemetery (Fig. 5). The rest of bone material consisted of at least 60 individuals found in a mixed layer of various bones devoid of any anatomical arrangement upon discovery. It can be presumed that this may be related to the long-term intensive use of the cemetery, during which many graves were at least partially destroyed (Fig.6). Regardless of the condition of the in situ burials, no significant differences between people buried in regular graves and people from the mixed layer were observed. It is highly probable that original burials from this layer were identical to that of graves from other layers. Therefore, we treated all collected and analysed bones as one set.

Fig. 3. Selected grave examples. (1) Grave no. 3, (2) Grave no. 63, (3) Grave no. 60, (4) Additional skull from the grave no. 60. Photographed by Hubert Lepionka.

Fig. 4. Grave-marking stone stelae in the trench with an undisturbed top soil. Photographed by Hubert Lepionka.

Fig. 5. Loose bone feature no. 2 (upper) and no. 3 (lower). Photographed by Hubert Lepionka.

Fig. 6. Mixed layer during exploration. Photographed by Hubert Lepionka.

The sex of the adult individuals, whose remains were preserved in anatomical arrangement, was assessed based on the dimorphic features of the skull (Acsádi and Nemeskéri 1970) and pelvis (Buikstra and Ubelaker 1994). The pelvic bone was used for sex assessment for adult individuals belonging to the loose bones category. To sum up, the examined material included the remains of 65 children up to 15 years of age, 28 females, 36 males and 52 individuals of unspecified sex (tab. 1). The highest percentage of the remains of unknown sex derived from the loose bone material, by which the assessment of this parameter was insubstantial due to a limited number of pelvic bones. A second negative factor influencing sex assessment was the different state of bone preservation. This factor also contributed to the determination of the parameter of biological age at time of death. This characteristic in adult individuals was estimated based on morphological features of the surface of ribs' sternal ends (Işcan et al. 1984), pubic symphysis (Todd 1921, Brooks and Suchey 1990), pelvic auricular surface (Lovejoy et al. 1985, Buckberry and Chamberlain 2002), and the phase of teeth wear (Lovejoy 1985).

In non-adult individuals, biological age at death was assessed by the stage of skeletal ossification and degree of development of primary and permanent teeth (Ubelaker 2018). Based on this data, examined individuals were assigned to one of the seven standard anthropological categories: fetus; infans I – up to 7 y.o.; infans II – up to 14 y.o.; juvenis – up to 22 y.o.; adultus – up to 35 y.o.; maturus – up to 55 y.o.; senilis – over 55 y.o. In the case of incomplete or badly preserved material, where the assessment of biological age at death was impossible, individuals were assigned to the child/adult category based on the development of preserved bones. This data, for the need of group and population analysis, was statistically evaluated between the anthropological age at death categories. To establish the demographic profile of the population, 14 age categories with 5-year ranges were created. Individuals were assigned to a specific category based on the features of biological age at death. Number of cases in certain categories is shown in table 1. Body height was calculated by measuring the length of the long bones (Trotter and Gleser 1952).

Results

Among the analysed remains we recorded both sex variants, with a slight prevalence of male individuals (Table 1). However, this disproportion was probably due to the overall low percentage of adults with defined sex parameters. Remains in the surveyed area also represented all categories of age at time of death – from prenatal period to individuals over 60 years of age (Table 1). Based on the mortality tables constructed for the sample (Table 2) it is apparent that the highest number of deaths and one of the highest probabilities of death rates was recorded in the 0-4 years of age category. The second most numerous was 30-34 years of age category. The lowest number of deaths was observed in the 55-59 years of age category. Continued adult life expectancy for 20-year-old individuals – which is a measure of population quality – was approximately 22 years; meaning the average adult life expectancy of the population was 42 years. Similarly, the approximate percentage of life expectancy of 23 years was recorded for newborns.

Variation in body height was linked with determined sex. Average height was estimated at 159 centimetres for the females and 170 centimetres for males (Table 3).

Table 1. Age and sex estimation of individuals from post-medieval population of Nowy Dwór

Age (years)	Sex	N of indiv. in reg. graves	% of indiv. in reg. graves	N of indiv. in objects	% of indiv. in objects	N of indiv. in coming-led layer	% of indiv. in coming-led layer
<0	Undefined	2	1.11	0	0.00	1	0.60
0-4	Undefined	23	12.71	2	1.11	20	11.05
5-9	Undefined	8	4.42	1	0.60	2	1.11
10-14	Undefined	4	2.21	0	0.00	2	1.11
15-19	F	0	0.00	0	0.00	2	1.11
	M	3	1.66	0	0.00	0	0.00
	NN	6	3.32	1	0.60	0	0.00
20-24	F	2	1.11	0	0.00	0	0.00
	M	0	0.00	0	0.00	0	0.00
	NN	0	0.00	0	0.00	0	0.00
25-29	F	1	0.60	0	0.00	0	0.00
	M	2	1.11	0	0.00	0	0.00
	NN	0	0.00	0	0.00	0	0.00
30-34	F	4	2.21	0	0.00	2	1.11
	M	4	2.21	0	0.00	3	1.66
	NN	0	0.00	0	0.00	0	0.00
35-39	F	0	0.00	0	0.00	0	0.00
	M	3	1.66	0	0.00	2	1.11
	NN	0	0.00	0	0.00	0	0.00
40-44	F	4	2.21	0	0.00	0	0.00
	M	2	1.11	0	0.00	1	0.60
	NN	0	0.00	0	0.00	0	0.00
45-49	F	1	0.60	0	0.00	0	0.00
	M	4	2.21	0	0.00	0	0.00
	NN	0	0.00	0	0.00	0	0.00
50-54	F	3	1.66	0	0.00	0	0.00
	M	1	0.60	0	0.00	0	0.00
	NN	0	0.00	0	0.00	0	0.00
55-59	F	0	0.00	0	0.00	0	0.00
	M	0	0.00	0	0.00	1	0.60
	NN	0	0.00	0	0.00	0	0.00
60+	F	2	1.11	0	0.00	0	0.00
	M	4	2.21	0	0.00	0	0.00
	NN	1	0.60	0	0.00	0	0.00
NN	F	6	3.32	1	0.60	0	0.00
	M	5	2.76	1	0.60	0	0.00
	NN	16	8.84	2	1.11	26	9.39
Total	–	111	61.56	8	9.04	62	29.45
Total number							181
Total %							100

Table 2. Mortality table for the post-medieval population of Nowy Dwór

Age (in years)	D_x	d_x	l_x	q_x	L_x	T_x	e_x
0-4	61	34.27	100.00	0.34	414.33	2 328.43	23.28
5-9	12	6.74	65.73	0.10	311.80	1 914.10	29.12
10-14	6	3.37	58.99	0.06	286.53	1 602.30	27.16
15-19	15	8.43	55.62	0.15	257.03	1 315.77	23.66
20-24	3	1.69	47.19	0.04	231.73	1058.74	22.44
25-29	5	2.81	45.50	0.06	220.48	827.01	18.18
30-34	23	12.92	42.69	0.30	181.15	606.53	14.21
35-39	9	5.06	29.77	0.17	136.20	425.38	14.29
40-44	13	7.30	24.71	0.30	105.30	289.18	11.70
45-49	9	5.06	17.41	0.29	74.40	183.88	10.56
50-54	7	3.93	12.35	0.32	51.93	109.48	8.87
55-59	2	1.12	8.42	0.13	39.30	57.55	6.84
60+	13	7.30	7.30	1.00	18.25	18.25	2.50
Total	178*	100.00	–	–	–	–	–

* Remains of 37 adult individuals and 18 subadult individuals of undetermined precise age at time of death, were statistically evaluated. Remains of three individuals who died at prenatal age were not included in the mortality table.

Abbreviations:
D_x – number of individuals in x age (at time of death) category.
d_x – percentage of individuals in x age (at time of death) category.
l_x – percentage of individuals living to x years of age.
q_x – probability of death at x years of age.
L_x – number of years lived overall by all individuals in the x age category.
T_x – number of years left to live for all individuals in the x age category.
e_x – average life continuity for individuals in x age category.

Table 3. Height estimation of adult post-medieval population of Nowy Dwór

Sex	Lowest height value (in cm)	Highest height value (in cm)	Average height (in cm)	SD	N
Female	150	169	159	5,13	16
Male	160	180	170	4,66	23

Recorded markers of physiological stress on child remains, linear enamel hypoplasia from a mild to a moderate degree (acc. to Garcin 2010) was the most common and was present in approximately 33% of analysed subadults with preserved teeth. The second most numerous was cribra orbitalia, being from weak to moderate severity (acc. Steckel at al. 2006), which was observed in 25% of skeletons with preserved upper orbital edges. Lesions on the greater wings of sphenoid bones, posterior surfaces of maxillae, hard palate, in the form of porosity or periostitis occurrence were observed in approximately 12.5% of cases.

A similar pattern of physiological stress markers was observed within adult population. Additionally, dental caries of varying degrees of severity were recorded in approximately 66% of adult individuals, whereas with subadults these occurred only in a few cases. Apart from cribra orbitalia (12.5%), singular cases of cribra cranii were also reported. Porosities and periostitis around the base of skull and on the mandible, (probably due to vitamin C deficiency-related lesions in adults), were observed in 12.5% of cases. Furthermore, significant ante-mortem tooth loss and bone inflammations were common.

Lesions of an overload-degenerative nature, often associated with an active lifestyle, were among the most common in the analysed sample. Individuals aged 16-18 years of age at time of death were the youngest in whom musculoskeletal overuse was observed – based on severe ligamentum flavium ossification, Schmorl's nodes on the vertebral bodies, and traces of osteochondritis dissecans in joints. However, it should be noted that only few juveniles were affected, and lesions of this type were common with older age groups, starting with young adults aged 20-29 years of age at the time of death. Enthesophytes on limb bones, vertebral osteophytes of varying severity, Schmorl's nodes, ligamentum flavium ossification, and numerous cases of osteochondritis dissecansin were also observed in all individuals aged 20-29 years of age. All degenerative-related lesions – particularly in limb joints – in Nowy Dwór inhabitants increased both in severity and frequency in people over 45 years of age.

The examined material showed many cases of infection diseases as well. Based only on skeletons from intact graves, infection-related lesions were observed in 24% of cases. This percentage increased, however, if loose bone material was also included. Thus, it can be estimated that infection-related changes occurred in at least 33% of the population. One of the most frequently recorded indicators of possible infection was long bone periostitis in varying forms and severity (Fig. 7:1) – it was often present within a pair of limb bones (e.g. right and left tibia), but cases of single and multifocal periostitis involving different bones have also been reported. In several instances, these coexisted with lytic lesions, e.g. on the ribs or vertebrae. Periostitis was recorded twice as often in remains of children. Several examples of inflammatory reaction resulting in osteomyelitis of varying severity were also present, occurring in both singular bones, and larger parts of the entire skeleton (Fig. 7:2). Among both subadults and adults, inflammatory reaction of periosteum of varying severity and spread was also present, occurring on the inner surfaces of the cranial bones (Fig. 8:1).

Traces of infection were also been observed in the ribs, and can be divided into three categories. The first category, included cases of bone tissue superstructure on the inner surface of the rib shafts with uninterrupted tissue continuity (Fig. 8:2); the second category refered to local bone loss causing lytic lesions (Fig. 9:1); the third category included lytic lesions observed on the external surface of the ribs at the junction with thoracic vertebrae. Regardless of the type, these markers were present in both adult and subadult remains, and often co-occured with lytic lesions on vertebrae or with infection indicators on long bones. Lytic lesions on vertebrae could also be categorised according to the incidence location. The first category included local bone loss within vertebral bodies – these refer to singular incidences recorded only in adult remains. The second group consisted of lytic lesions within vertebral arch structure (Fig. 9:2), present in subadults.

Fig. 7. Selected pathological condition examples. (1) Left tibial shaft with periosteal reaction, grave no. 70, (2) Distal fragment of left radius with advanced stage of *Osteomyelitis*, grave no. 22. Photographed by Angelika Słodka.

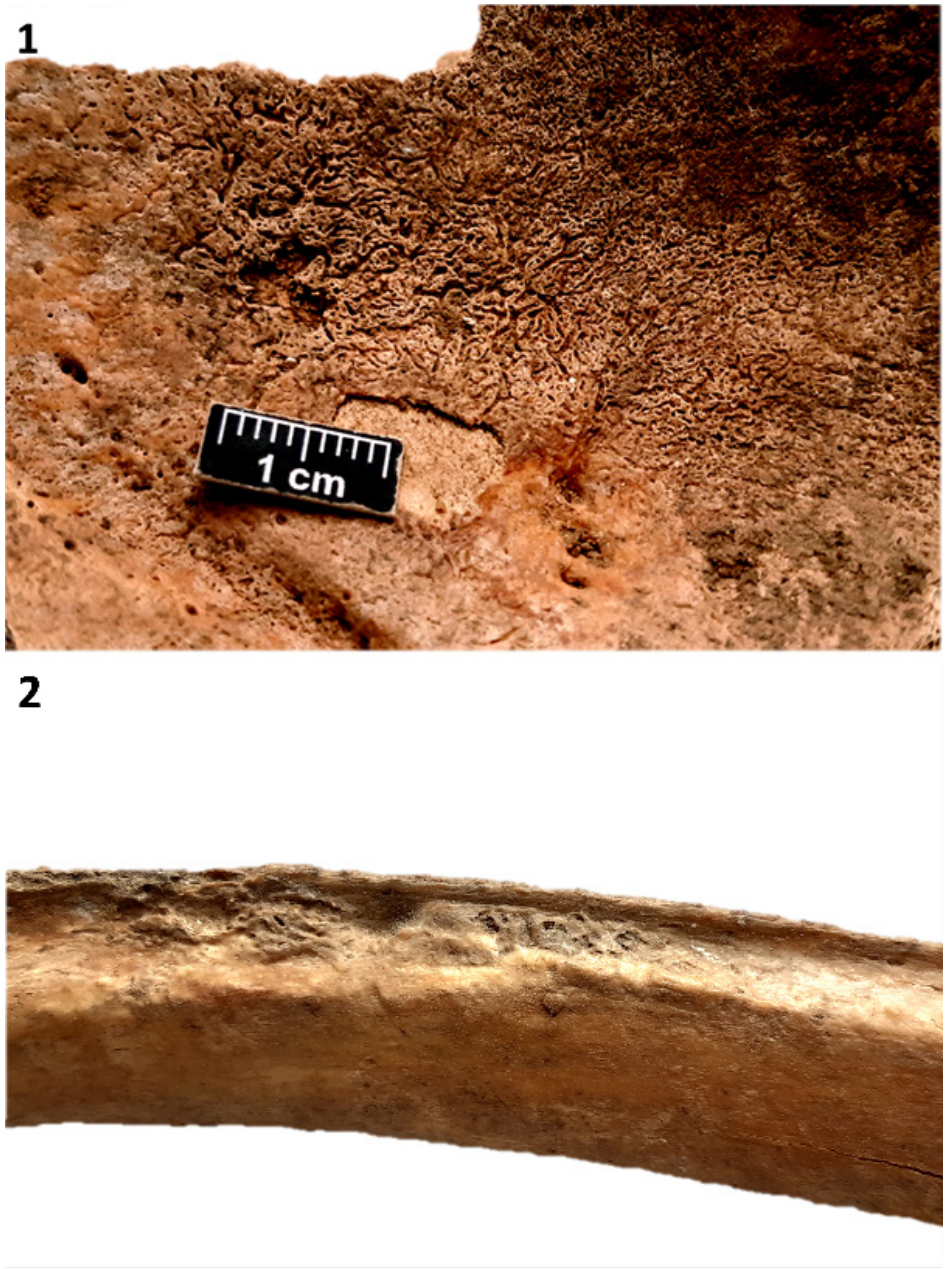

Fig. 8. Selected pathological condition examples. (1) Internal surface of occipital bone with periosteal reaction, loose bones, (2) Internal surface of rib with periosteal reaction, grave no. 53. Photographed by Angelika Słodka.

Fig. 9. Selected pathological condition examples. (1) Internal surface of rib with lytic lesions, grave no. 24, (2) Vertebral arch with lytic lesions, grave no. 57. Photographed by Angelika Słodka.

Furthermore, the repeatable occurrence of non-metric, epigenetic features in multiple individuals was also noted, among them numerous instances of cervical enamel projection in majority of examined teeth, repetitive patterns of Wormian bones, and several examples of non-obliterated metopic sutures. This in turn suggests probable high homogeneity and low gene flow within analysed population.

Discussion

This study has showed that people were buried regardless of their biological profile within the surveyed part of the cemetary. What is puzzling, is the abundance of remains of children under 10 years of age at time of death, which accounts for 41% (after statistical evaluation) of all individuals. It is generally accepted that in most historical periods there was a high mortality rate in children, estimated at approximately 33% (Lewis 2007). In the Nowy Dwór case, this figure exceeds the generally accepted child mortaily rate. However, it should be noted that excavations covered only parts of the cemetery, and therefore, the examined sample represents only a fragment of entire population. It is possible that the observed high percentage of subadults may be related to the occurrence of additional environmental factors increasing the mortality, e.g. an epidemic, or location of child burials located near the end of the cemetery. For this reason, the estimated values correspond more to medieval than post-medieval populations (Kozłowski 2012; Pudło 2016; Budnik and Pudło 2017). Despite the significant underestimation of the parameters of the extinction table for a post-medieval population, the noted pattern does not characterise catastrophic extinction. What seems more likely was the occurrence of several events in short intervals, or a single long-term event. Confronting these values with historical data, it is possible that during the 18th century – and perhaps even earlier – events in Nowy Dwór significantly affected the town's inhabitants, and thus, their life expectancy. In terms of body length, the analysed sample can be described as of middle height (Piontek 1992) for both females and males regardless of their age.

Several types of physiological stress were recorded. Disturbances in the enamel layer formation are usually associated with a general deficiency of minerals, proteins, or vitamins due to malnutrition. However, it cannot be excluded that linear enamel hypoplasia could form because of infection or various childhood diseases – therefore, it is considered a non-specific indicator (Irish et al. 2015). Cribra orbitalia formation is also not clear. In most cases it is associated with iron deficiency due to anaemia, yet among other possible causes are infectious diseases, parasites, or anaemias of genetic origin (Grauer 2011). Occurrences of local porosity and periostitits on the skull bones are lesions quite clearly identified as effects of scurvy and vitamin C deficiency; it should be taken into account that such deficiency may be due to both insufficient diet, as well as bodily need for vitamin C in case of infectious diseases (Halcrow et al. 2014). Lesions of overload-degenerative nature and their frequency are indicative of severe, long-time musculoskeletal overload most likely associated with physical labour from early adulthood.

An important task in material analysis is the verification of possible indicators of an epidemic mentioned in historical sources. Observations concerning the

high mortality of children and mortality tables indicated the presence of an undefined factor that increased population mortality. Assessment of health and life conditions showed significant nutritional deficiencies among both subadults and adults, which impacted general immunity and in turn could have resulted in an epidemic increase. These elements, however, are not unequivocal indicators of an infectious disease in the population. For further verification, bone markers of infection – which were noted in up to 33% individuals and twice as often among children – were analysed. Periostitis is commonly regarded as an inflammatory lesion associated with infection, however, its nature does not point to any single pathogen that could be its cause. In addition, the issue of non-infectious factors (such as genetic, metabolic, and other diseases) have been raised (Weston 2012, Ortner 2003). Unlike periostitis, however, a relevantexample of an infection-related lesion is osteomyelitis. The most common aetiological agent associated with it is *Staphylococcus aureus* and *Streptococcus* causing purulent inflammation (Pinhasi and Mays 2008). Another possible pathogen is *Mycobacterium tuberculosis*. Several examples of inflammatory lesions of infectious origin occurred on the inner surfaces of the cranial bones. They were most likely results of a reaction of the periosteum – varying in severity and spread and noted in remains of both children and adults. Usually, they did not co-occur with other bone pathologies of infectious origin. It is probable that the placement of inflammatory lesions indicates its secondary nature and points towards infections of other systems, especially the respiratory system. Infections on the inner surfaces of the skull are often present in remains infected with *Mycobacterium tuberculosis*. Other pathogens (causing pneumonia) that are mentioned were equally as likely (Hershkovitz et al. 2002).

Traces of infection observed in the ribs can be divided into three categories. The most common cause of bone superstructure on ribs with uninterrupted tissue continuity are diseases associated with inflammation of the lower respiratory tract. On the other hand, cases of lytic lesions present at the rib-vertebral junctions are mostly attributed to tuberculosis (Davies-Barrett et al. 2019). Lytic lesions observed on the vertebrae are also categorised according to the place of their occurrence. These lesions are of varying degree of severity, progression and spread, and their occurrence – as with other infection markers – cannot be linked with one specific pathogen. However, changes of this type are most frequently present in cases of tuberculosis. Therefore, it may be possible, to some extent, to confirm historical reports on probable epidemics decimating a local population. Based on recorded and analysed pathologies, it is difficult to confirm their aetiological factor, since markers are most often non-specific. Nevertheless, it is worth noting that in the analysed material the most frequently recorded cases were those attributed to pulmonary infections and tuberculosis.

Based on available historical records, observed infection-related lesions can be associated with several time periods. The earliest information is one of the supposed demises of two post-Yotvingian villages near Nowy Dwór – Kopno and Jatwieź. The populations of both villages were believed to have died from plague at the end of 16th century (acc. to Żuk and Bujnowski 2009). Another period can be dated to the first half of 17th century, ac-

cording to information included in letters of the servants of Radziwiłł family who had eyewitnessed the plague. The most reliable account derives from information that in 1706, 1707 and 1712 Nowy Dwór was first ravaged during the Northern War, and then (around 1760) affected by a plague which devastated almost the entire population, with only five families remaining (Ryżewski 2006: 316). It is possible that after the plague the population of the town was supplanted by the inhabitants of neighbouring villages.

Based on chronological findings, it was possible to trace the period of cemetery usage from 16th to half of the 18th century (Fig 10, 11). This closely corresponds with historical data from 1804, when inventory documentation of local Uniate parish was made, describing the church as being in bad condition at the time, as well as mentioning an old cemetery located next to the temple which was still in use. A new cemetery located on the peripheries of the town was also mentioned, however, it had not been in use during that time by the inhabitants. This suggests that the excavated area can be linked with the existence and functioning of the Orthodox and Uniate churches: beginning in the year 1530, and finally being abandoned in the first

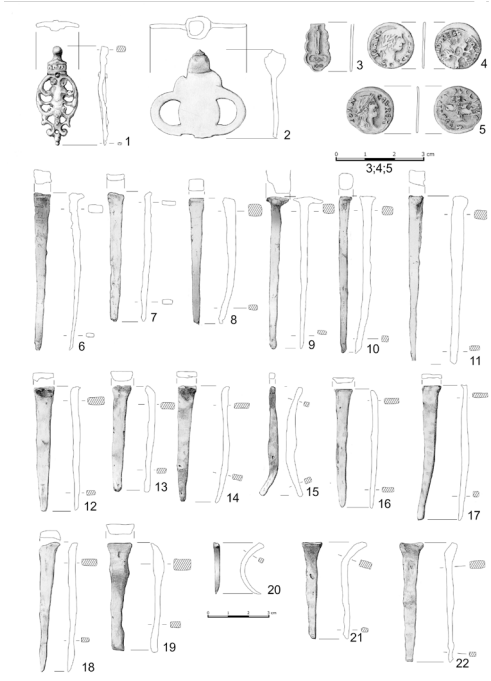

Fig. 10. Book binding piece (1), metal object (2), silver fragment of crucifix (3), copper coins of John II Casimir Vasa (4, 5) – loose finds from the layer between graves, coffin nails from graves (6–17). Drawn by Olga Dec.

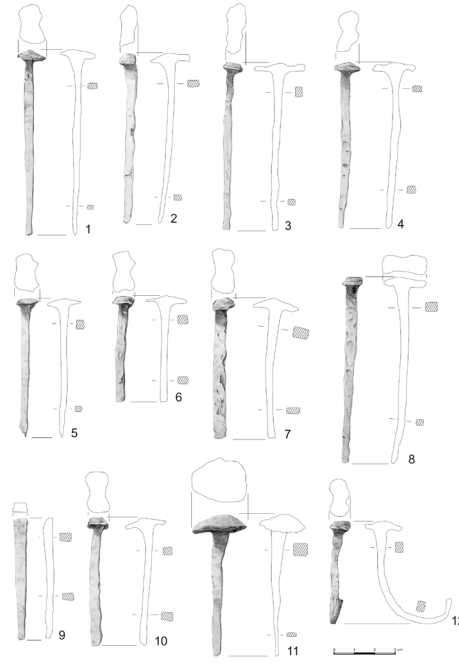

Fig. 11. Coffin nails from graves. Drawn by Olga Dec.

half of the 19th century. The abandonment of the cemetery also corresponds with the delegalisation of the Uniate religion by the decree of Tsar Nicholas I of Russia, and the foundation of a new Orthodox church next to where the previous temple was located (Ryżewski 2019).

Among the findings, were a few numismatics which can be interpreted as "obols of the dead" – these are silver denars and two denars of Alexander and Sigismund II Jagiellon, minted in the period from early 16th to mid-16th century. Another set consists of small copper coins of John II Casimir Vasa, dated to the years 1659–1668, and used until the half of 18th century (Fig. 12). Several coins were found in the layers outside grave cavities, and most likely came from destroyed graves. The practice of the obol tradition can be divided into roughly two periods. First, it was from around the Early Middle Ages, then disappeared, and then emerged once again in the 14th century – only to reach its peak in the following centuries. This tradition was practiced in the regions the territory of modern Poland, Ukraine, Belarus, and Russia. In the case of the Polish-Ruthenian borderlands, it occurred mostly in rural areas. The cultural meaning and significance of the "obol of the dead" unclear. Some researchers raise the possibility of Arabic, Christian or even ancient influences, while others suggest probable fear for the fate of the deceased in the afterlife, or – alternatively – the fear of their return as malicious revenants (Miechowicz 2019).

Among the discovered graves some can additionally be described as "atypical" or "deviant" – i.e., as differing from the majority of graves in some respects. These included graves no. 75 and 73 – which were oriented differently from the rest, facing respectively South and South-West – as well as the grave marked with number 60 (Fig. 3:3,4). The latter is of particular interest, as it could have been subjected to unusual practices, exceptional in the scale of the examined parts of the necropolis.

Fig. 12. "Obols of the dead". Upper: (1) Coin of Alexander Jagiellon, 1501-1506, grave no. 56. (2) Coin of Sigismund II Augustus Jagiellon, 1559, grave no. 1. (3) Coin of Sigismund II August Jagiellon, 1560, grave no. 1. (4) Coin of Sigismund II Augustus Jagiellon 1566, grave no. 3. (5) Copper coin of John II Casimir Vasa, grave no. 43, (6–7) Copper coins of John II Casimir Vasa. Low: grave no. 30 with "obols" *in situ*. Photographed by Hubert Lepionka, drawn by Olga Dec.

Grave no. 60 can theoretically be considered a double grave, as it consisted of one mostly complete skeleton and an additional skull deposited near the skel-

eton's legs. Upon discovery, the skeleton rested in an anatomical arrangement in extended supine position; despite its medium degree of completeness and imperfect state of preservation, it was possible to determine that the remains belonged to a person presenting female characteristics, who had died at the age of approximately 40–44 years of age. Further analysis did not reveal any peri-, ante- or postmortem injuries, however, pathologies in the form of loss of three molars, inflammation of the jawbone, and degenerative-strain lesions of the spinal bones and ribs were noted. The skull of the second individual – which was poorly preserved, also presented female characteristics and indicated adult age at time of death – was located between the legs of the skeleton.

The deviant forms of the burial in question is further emphasised by the fact that post-depositional processes and overlapping of two independent burials – an older and younger one – can be excluded with a fair amount of certainty. Both the skeleton and the lone additional skull were buried not only within the same burial cavity, but also in one coffin that bore no signs of post-depositional disturbances or partial destruction. No other graves, grave remains, bones, or other types of features were recorded in the immediate vicinity of the burial, ruling out the possibility of skull being moved unintentionally.

Furthermore, the position of the skull raises questions as well. As one of the determinants of burials of the revenants, it is generally associated with the belief in the undead rising from the grave to harm the living community. Alternatively, mostly in regard to Late Middle Ages, these burials can be interpreted as those of criminals and convicts. It should be noted, however, that in burials of the alleged revenants what the issue here is that the decapitated skeleton is from one person, and not – as it is in the case of grave no. 60 – an additional skull. It is possible that in this case we are in fact faced with a material trace of some local, unknown belief or resulting behaviours, but it is difficult to determine exactly what kind it would be: whether the grave is that of a revenant, or perhaps a whisperer (witch). There is the possibility of the grave was an ordinary double grave of two people buried together – one who had died earlier, and who later had their skull removed and placed in the coffin of the other. However, this does not explain the placement of the skull, a position clearly associated with the burials of those suspected of revenantism or of other forms of a supernatural nature. Yet, associating grave 60 with revenantism itself is unlikely. It may, however, be a burial of a szeptucha – a whisperer (whispess) or witch, as witchcraft activities are to this day practiced in the region. Information obtained from various sources from the local community indicate that in the past whisperers used bones collected from graves in rituals and for healing purposes. Thus, the additional skull could be a specific designation of the deceased, marking them as a witch.

Conclusion

The Nowy Dwór necropolis bears a number of features which characterised the health and socio-cultural elements of the community which used it. Firstly, based on population studies it is possible to explain the relative homogeneity and genetically undiversified character of local society. Pathological lesions, such as periostitis, osteomyelitis or lytic lesions, which were

present in 33% of all examined individuals, seem to provide at least some argument based on historical accounts for several epidemic events which decimated the town's population, and ultimately caused the entire settlement to regress into a village. The cemetery itself, however, is with all certainty not a specifically epidemic cemetery. The necropolis stands out from the rest of similar stelae burial sites due to its location within the settlement, and not along its peripheries. Based on numismatic finds and historical sources, it is possible to set the usage period of the cemetery between the 16th to mid-19th centuries. Moreover, it most probably functioned in connection with the now non-existent Uniate church built in 1530, the location of which was initially estimated based on geomagnetic and geo-radar surveys. However, further and more extensive research is required to verify the hypothesis concerning the church itself.

In general, results obtained from Nowy Dwór make a note-worthy contribution to the process of identification and characterisation of the whole phenomenon of stelae cemeteries and post-medieval period culture in North-Eastern Poland – providing a wider perspective on local traditions and memory, as well as cultural and religious diversity of the region.

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Conflict of interests

No conflict of interests was declared

Authors' contributions

HL wrote introduction, used photographs, and fragments covering archaeological record and history of Nowy Dwór; AS did an anthropological analysis, statistical analysis, interpretation of results and wrote part of Results section; OD id an author of used drawings, Abstract, part of Results, Conclusions, and was responsible for translation entire manuscript to English.

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The assessment of the biological age of children`s characters created in the convention of Japanese animation in forensic practice

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Abstract: Introduction: According to criminal codes of most Western countries, possessing, producing and disseminating of fictional paedopornography is a crime. In light of these laws, the shotacon/lolicon (popular and widely available Japanese animations or comic books showing minors in a sexual context) seems to deserve special mention. There have been several convictions for violations of these laws, however, the methodology of a depicted person's age estimation is still unestablished.

The aim of this study was to assess the suitability of anthropometrical prediction of age to the analysis of characters animated in the Japanese style.

Material and methods: The metric (distance between facial landmarks) and non-metric (type of chin shape) features of 173 animated characters' faces were obtained. Material was collected from 90 most popular Japanese anime series. Measurements were conducted in ImageJ software. The correlations of age and standardized measurements: *en-ex, en-en,* eye height, *pu-prn, pu-sto, pu-gn* were examined. The chin shape was described by three independent 'judges'.

Results and conclusions: Correlations for *pu-prn, pu-sto, pu-gn* and eye height in females and in all males were statistically significant. Age prediction was made using linear regression equations. Good prediction (± 1 year) was obtained for 44% males and 17% females. Prediction within the acceptable range (± 2 years) was achieved for 23% of males and 18% of females. In total, the prediction with an error of no more than ± 2 years was obtained for 67% of males and 35% from females, which is comparable to the results obtained in the study of real children. Moreover, triangular or rounded chin shape was significantly more frequent in boys aged 10–12 years, and square in older boys 16–18 years. Current research provides a basis for developing a methodology for assessing the age of animated characters. There is a need for further research in this area.

Key words: anime, anthropology, child pornography, face, lolicon, measurement

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Introduction

Child pornography is a significant ethical, social and medico-legal issue (Dayal et al. 2018). The term includes sexually marked materials picturing an individual(s) below the legalage of consent (for sexual activities), which can range from 12 years of age (e.g. China, Mexico, Paraguay) to 18 years of age (e.g. Egypt, Guatemala, Haiti) (Franklin et al. 2015). Currently, the major transmission channel of child pornography is the Internet (a major medium in disseminating child pornographical material (Vitorino et al. 2018)). However, concomitant with modern technological development in the sphere of creating virtual reality, the cultural perception of child pornography is also being transformed. This is reflected in global penal codes. Legal systems pertaining to many Western countries impose legal sanctions for possessing, producing and disseminating not only real images of minors participating in sexual activities, but also graphic representations of fictional characters (Hinton 2014). *Shotacon* and *lolicon* are relevant examples of such representations.

Shotacon and *lolicon* are the genres of *manga* (Japanese style comics) and *anime* (animations), which frequently depict males and females under the age of sexual maturity (usually 8–13 years old) in a sexualised context (Savage 2015). For example, showing intimate relations between children (including siblings) or between a child and an adult (Burdzik 2014). Therefore, these comic genre are pertinent in relation to criminal law (Holloway 2020).

The international legal status of *lolicon* and *shotacon* productions remains unclear. It is worth noting that Japan, where the majority of this sexual genre is produced, is reluctant to prohibit their construction, citing freedom of artistic expression (Holloway 2020). Arguments raised against the criminalization of this type of art genre include the fact that the characters depicted in them are completely fictitious, and no real person participates in the process of their creation. Consequently, they should not be compared with real pornographic materials presenting children (Savage 2015). It is worth noting that Japanese-made *shotacon* and *lolicon* comics that depict children in an erotic or even pornographic way are not part of the illegal black market. They are available through legal mass distribution channels. Examples of such distribution channels are the 'Boku no Pico' franchise (Natural High studio) or 'Enzai' franchise (Adonis / Japan Home Video studio). They are also available outside of Japan (especially on websites that provide fan translations of anime). Due to the nature of the content presented, these comic genre may be considered illegal in most Western countries. Due to easy access to these comic genre, children are especially at risk of exposure to unattended inappropriate content.

In many countries (e.g. Canada, Australia, United Kingdom) there have been cases of convictions issued in connection with the possession or dissemination of fictional child porn (The Sydney Morning Herald 2008; Thompson 2011; Romano 2014). However, in order to make possession of such materials punishable, it is necessary to prove that the presented character is a child under the legal age (Cattaneo et al. 2009). Although, there are a few established methods of living persons' age prediction, there is no methodology suitable for fictional images.

In cases concerning children pornography the age estimation of an individual depicted in photographic, video or

other media plays a crucial role (Cunha et al. 2019; Mayer et al. 2014). Currently used methods are based on morphology, dentition or secondary sexual characteristics development. However, all of these are limited by differentiation of growth rate (which may be observed e.g. in endocrine disorders), technical restrictions and costs or inapplicability for every stage of human ontogenesis (Schmerling et al. 2016). In cases involving 2D or 3D materials, the restrictions concern: i) lack of reference point suitable for measuring, ii) poor quality of materials, iii) photo manipulations, iv) characterization and v) widespread depilation of anogenital area (Bednarek 2006; Łabęcka et al. 2011). Currently, the most commonly applied methods for assessing the age of people depicted in images (2D or 3D) use the variability with age of the features of the construction of individual body parts such as a hand or a face. Face proportions are particularly suitable. Anthropometric methods of age estimation are based on distances between facial landmarks, and their dependence on age. Current methods involve both 'manual' and 'automatic' approaches. In the 'manual' approach facial landmarks are positioned by an expert (e.g. Koruga et al. 2011, Borges et al. 2018, Deitos et al. 2020), and their values are further processed in order to obtain the models of craniofacial growth (Ramanathan and Chellappa 2006) that allow to estimate the individual's age from its known facial measurements value. In the 'automatic' approach advanced computer methods are used, like machine learning (Liu et al. 2020, Porto et al. 2020).

The aim of the research was to examine the 'age-related variability' of the anthropometric features of animated characters' faces in order to assess whether it would be possible to predict in this way the age (assigned by author) of character depicted in Japanese comic books or animations.

Materials and methods

Ninety of Japanese anime series were selected by popularity (according to MyAnimeList.net) and occurrence of young characters (according to Animecharactersdatabase.com) (access: December 2018 – March 2019). One hundred and seventy-three pictures of *en face* shots of characters (86 males and 87 females) in ages 10 years of age to 18 years of age, were obtained respectively according to following inclusion criteria: i) metrical age of an individual was well-known (from anime plot, author's complementary information, character's biographic note), ii) character was a human (without any animal-like features, e.g. cat ears), iii) the sex of each individual was clearly determined, iv) face of the individual was shown as clearly as possible, v) in the cases of significant timelapses the images of an individual from early episodes were selected, vi) the face was as motionless as possible. They were all included in the metric analysis.

Moreover, 16 additional images that did not meet one of the requirements of the metric analysis were included in the non-metric analysis of the chin shape (189 individuals: 96 male and 96 female).

Pictures were examined in ImageJ, a free software suitable for photometry. They were treated as if they were human faces. Anthropometric landmarks (according to Martin and Saller 1957) were positioned at designated points (Fig. 1). The chosen landmarks were: 1) *pu* (*pupillare*) – the center of the pupil; 2) *en* (*entokanthion*) – internal eyelid angle; 3) *ex* (*ektokanthion*) – external eyelid angle; 4) *prn* (*pronasale*) – tip of the nose; 5) *sto*

(*stomion*) – oral fissure (in the midline); 6) *gn* (*gnathion*) – lower edge of the mandible (in the midline). In cases where the eyes were drawn without closing the internal and external angles, the *en* and *ex* landmarks were localized in the points that were most inward (*en*) or lateral (*ex*).

The selected landmarks were chosen because they were easiest to identify on the simplified facial model of an anime-style 2D drawing. For practical reasons, the points closely related to the craniofacial bone elements (such as *glabella, zygion, frontotemporale, gonion*) were excluded because there was a higher risk of their incorrect placement. Moreover, the landmarks localized in the points that could change position due to facial expression (such as corners of the lips or landmarks localized on the eyebrows) were also excluded. Additionally, for the purposes of the study, points were defined that mark the height of the eye, defined in the middle of the width of the upper and lower lash lines.

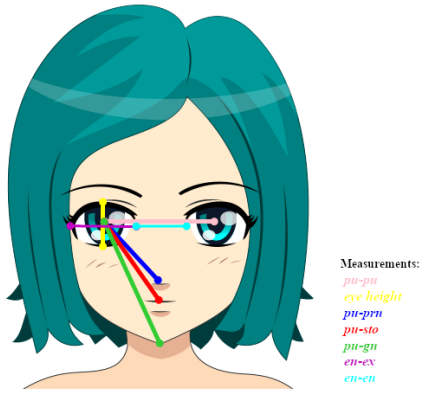

Fig. 1. Measurements between anthropological landmarks. Source: Rafael Javier on Pixabay; in own modification.

To standardize data, the measurements were divided by reference measurement *pu-pu*. This measurement was selected due to the references in the literature regarding the developmental stability of the pupil, the diameter of which does not increase after 2 years of age (Borges et al. 2018). However, the measurement between the centers of two irises was chosen instead of mean value of iris diameter, in order to avoid possible errors caused by differences in the iris shape, that could be elongated (vertically) in some drawing styles.

The proportion ratios obtained in this method were statistically analyzed using the STATISTICA 13.5 software. The distribution of all features was normal.

Pearson's *r* coefficient values were calculated for the correlation between age and proportion ratios. The regression equations for age estimation were proposed for each feature, which was statistically significant. The final result (estimated age) averaged the partial results for individual features. Prediction accuracy (Ac) was determined on the basis of the absolute value of the difference between real age (Ar) and estimated age (Ae):

$$Ac = |Ar–Ae|$$

The coordinates of the points obtained in the program were substituted into the Pythagorean formula (Koruga et al. 2011) for the length of the segment, obtaining following measurements: *pu-pu, en-en, en-ex, pu-prn, pu-sto, pu-gn,* eye height.

For the value of Ac ≤ 1, the estimation was considered good, and for Ac > 1 and ≤ 2 years – acceptable. Statistically significant results were assumed for $p \leq 0.05$.

Three forms of chin shape were observed: triangular, round and square. The differences in their occurrence in three categories of age (10–12, 13–15, 16–18) were examined using the χ^2 test. Statistically significant results were assumed for $p \leq 0.05$.

Results

Pearson's correlation coefficients (*r*) for all of the examined features in males and for 4 of 6 (*pu-sto, pu-prn, pu-gn* and eye height) in females were statistically significant (Fig. 2, 3). In both sexes there was a tendency to decrease with age the values of proportion indicators related to eye morphology (*en-ex,* eye height) with a simultaneous tendency to increase the indicators of the lower face (*pu-sto, pu-prn, pu-gn*). Features with *r* equal to 0.4 or more were further analyzed by calculating the regression equations (the searched variable was age; table 1).

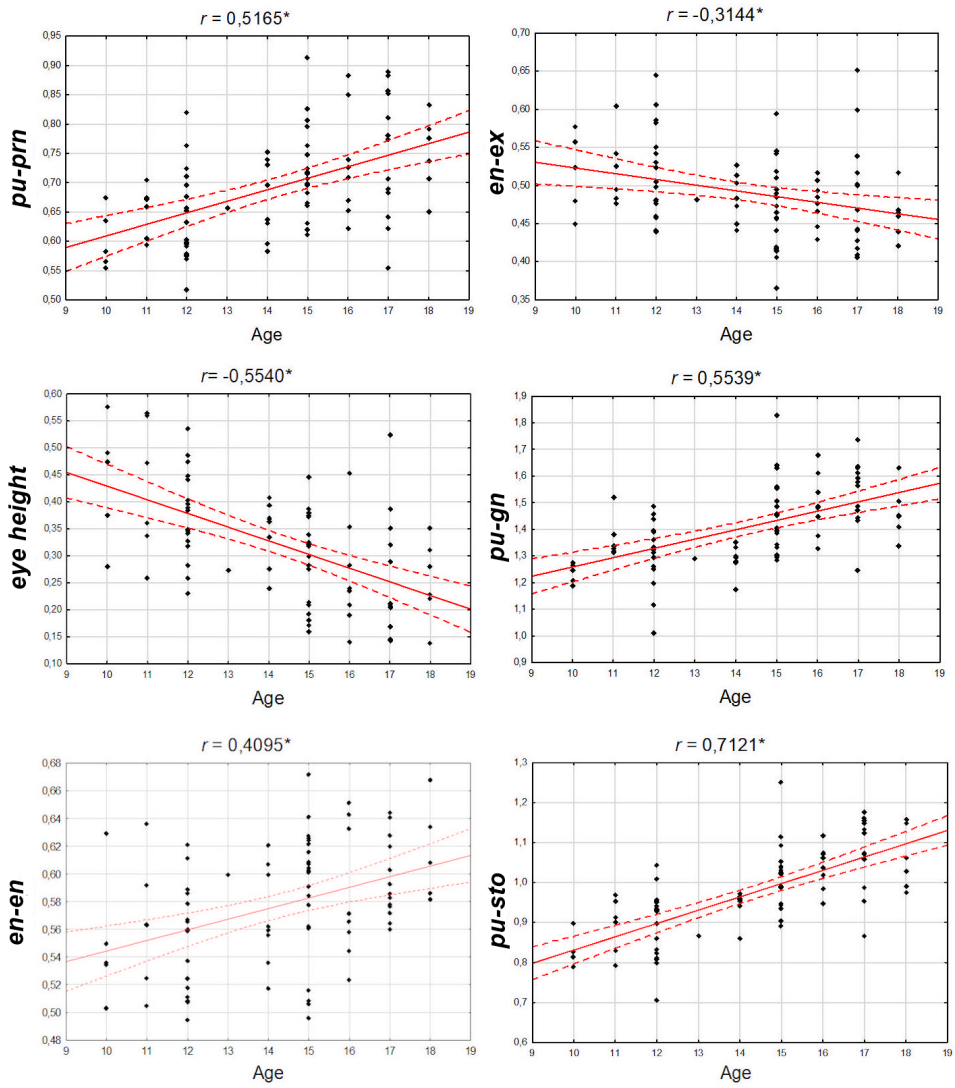

Fig. 2. Correlation diagrams of analyzed metrical features – males (* – statistically significant, $p<0.01$).

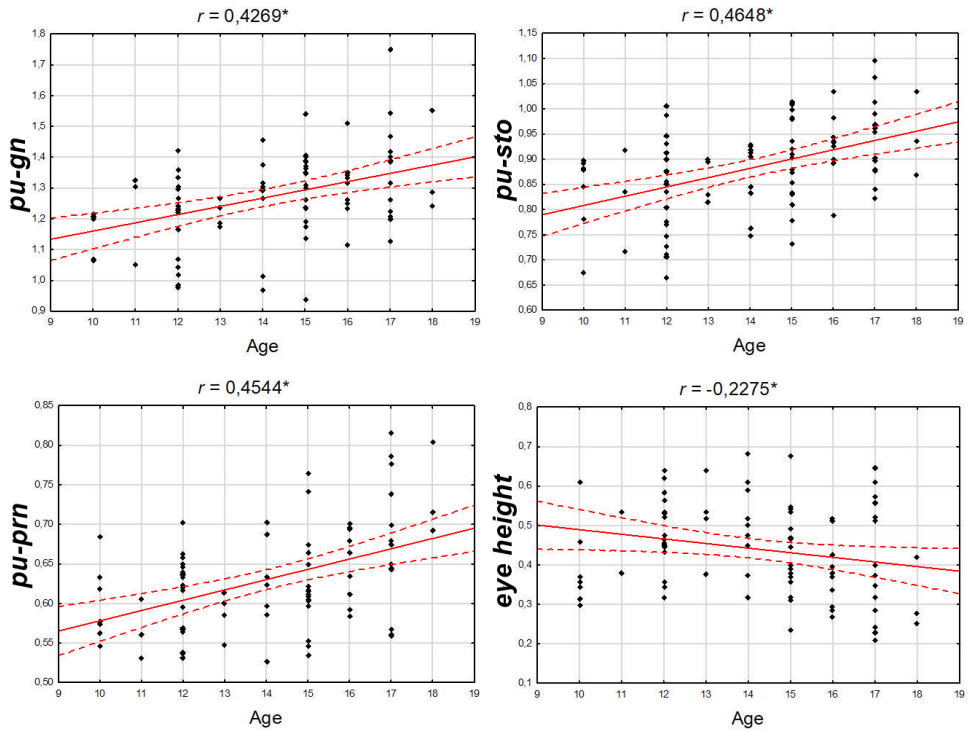

Fig. 3. Correlation diagrams of analyzed metrical features – females (* – statistically significant, $p<0.01$).

The final result was achieved by determining the arithmetic mean of the age obtained in each prediction based on the following equations:

For males:

$$\frac{\sum(Ae_{eye\ height} + Ae_{pu-sto})}{5} + \frac{\sum(Ae_{pu-prn} + Ae_{pu-gn} + Ae_{en-en})}{5}$$

For females:

$$\frac{\sum(\mathrm{Ae}_{pu-sto} + \mathrm{Ae}_{pu-prn} + \mathrm{Ae}_{pu-prn})}{3}$$

The estimated age of the individuals, the accuracy of which was then assessed, was obtained by applying combined methods: i) with the use of five indicators – for male, ii) with the use of three indicators – for female, in accordance with the above-mentioned equations.

To assess the validity of the method, the difference between the age assigned to the character and the age estimated anthropometrically was assessed in each case. Since the age assigned to the characters was a natural number (number of years), and it is not possible to refine this value (e.g. by calculating the number of months lived or indicating the exact date of birth that can be related to the date of measurement), the cases of difference that did not exceed 1 year (absolute value) were considered a good estimation accuracy. Cases where the difference did not exceed 2 years (absolute value) was considered acceptable.

Table 1. The equations of regression for age prediction

Feature	Males	Females
en-ex	–	–
eye height	$\frac{0.68291 - [eye\ height]}{0{,}0253}$	–
pu-sto	$\frac{[pu - sto] - 0.49883}{0.03324}$	$\frac{[pu - sto] - 0.62375}{0.01844}$
pu-prn	$\frac{[pu - prn] - 0.41247}{0.01967}$	$\frac{[pu - prn] - 0.44807}{0.01301}$
pu-gn	$\frac{[pu - gn] - 0.90978}{0.03492}$	$\frac{[pu - gn] - 0.89280}{0.02675}$
en-en	$\frac{[en - en] - 0.46760}{0.00767}$	–

The good estimation accuracy (Ac ≤ 1) was observed in 44% (n=38) of males and 17% (n=15) of females (table 2). The acceptable estimation accuracy (Ac >1 and ≤ 2) was observed in 23% (n=22) males and 18% (n=16) of females. To summarize, the age of 67% of males and 35% of females was estimated with error no higher than 2 years. For each regression separately, the best results were observed for the measurement *pu-sto*, with almost 1/3 of the analyzed material (31%) classified correctly, that is within ± 1 year range. Slightly worse results (27%) were given by age estimation based on *pu-gn* feature.

There were statistically significant differences in the incidence of chin shapes in the three age categories (in males only) (table 3, Fig. 4). A significant over-representation of the square shape was observed in the oldest age category (16–18); this shape was not present in any other group, which may make it an indicator of the male character >16 years of age. Moreover, the round and triangular shape was observed more often in younger males.

Table 2. Age prediction accuracy

Age prediction accuracy (Ac)	Males		Females	
	N	%	N	%
Good (Ac ≤ 1)	38	44	15	17
Acceptable (Ac >1 and ≤ 2 years)	20	23	16	18
Unacceptable (Ac >2 years)	28	33	56	65
TOTAL	86	100	87	100

Table 3. Chin shapes in three age categories of males (*Fo* – frequency observed, *Fe* –frequency expected); $\chi^2 = 10.47$; $df = 4$, $p = 0.03$

Age group		Chin shape			Total number
		Triangular	Round	Square	
10–12	*Fo*	21	13	0	34
	Fo – Fe	+1.88	+1.31	– 3.19	
13–15	*Fo*	19	10	2	31
	Fo – Fe	+1.56	– 0.57	– 0.90	
16–18	*Fo*	14	10	7	31
	Fo – Fe	– 3.44	– 0.66	+ 4.09	
Total number		54	33	9	96

Fig. 4. Types of chin shape in males: A – triangular (source: *Bakuman*), B – round (source: *Inuyashiki*), C – square (source: *Haikyū!!*).

Discussion

In the present study, good (± 1 year) predictive accuracy was obtained for 44% of males and 17% of females. Taking into account the cases where the accuracy of the estimate was within the limit value (± 2 years), the estimated biological age for 67% of males and 31% of females oscillated around the acceptable value, with a departure of no more than two years. It should be noted that in the case of animated characters it is not possible to accurately determine the age (date of birth), which reduces the likelihood of accurately predicting at the age of a living child.

It is noteworthy that the accuracy of age prediction with the proposed method was much more precise for males. This may be due to female faces undergoing less modifications during sexual maturation; secondly, female faces share more pedamorphic features with childen's faces, while male faces show more differences with the latter. Furthermore, the morphological neoteny of females is associated with their perception as being physically attractive (Palumbo et al. 2017). These features have been invariably depicted in artistic works spanning millenia. Therefore, it may be more difficult to assess the age of young female animated characters.

It should also be noted that the results obtained for individual regression equations were not convergent – better

results were obtained for both sexes by using equations including measurements of the lower face (*pu-gn, pu-sto, pu-prn*). Interestingly, the estimates based on *pu-gn* and *pu-sto* ratios were more accurate for male characters. This coincides with the knowledge of the maturation and facial morphology of living people – higher testosterone levels, especially pubertal (Hodges-Simeon et al. 2016), for males imply stronger masculinization, as well as a higher mandibular shaft, with subsequent relatively longer lower face. This conclusion corresponds with the results of the χ^2 test relating to diverse male chin morphology. The square shape – clearly suggesting higher masculinization (Sayegh et al. 2019) – was significantly more frequent in older males (over 16 years of age). This type of chin presentation was absent in many female and younger male images, which suggests that this feature could be a feasible indicator of male characters who have already sexually matured. A similar feature (not included in this study) may be the marking of the laryngeal cartilage in the neck outline, which is more pronounced in sexually mature men (Franklin 2015).

The issue of assessing biological age is problematic not only in relation to fictitious images, but also to real persons. Łabęcka et al. (2011) (in a study of more than 200 children patients) attempted to develop a comprehensive age assessment method (based on the structure of the face, body, degree of laryngeal cartilage development and hair development and many others). However, its accuracy was unsatisfactory (9% males and 39.4% females classified correctly). In a study by Cattaneo et al. (2011) on the assessment of age based on facial morphology 69.9% of German children, 69.4% of Lithuanian children and 80.5% of Italian children were classified correctly. However, the individual age of study subjects was not taken into account, and only four age groups were considered: 6, 10, 14 and 18 years old. According to Ferguson and Wilkinson (2017), the visual age estimation of children by various groups of judges revealed an inadequate accuracy (33%). It is noteworthy, that even methods involving advanced algorithms may be able to provide moderate results in automatic age estimation. For instance 58.4% hitting ratios in a method proposed by Iga et al. (2003).

Research involving subjective expert judgments may be biased by past experience, individual beliefs, or socialization. The use of digital technologies can reduce this bias, as exemplified in Demonstrator software, which was more successful in assessing the age of 10–19 years old females in comparison with forensic experts (Retnayake et al. 2013).

In forensic practice, the biological age of the shown individuals is often assessed based on the degree of development of secondary sexual characteristics. However, this method is subject to a significant risk of error, which has been controversial. While incorrect classification of a child as an adult is rare, reverse errors can be made approximately 67% of cases (Franklin et al. 2015). This problem was highlighted by Cattaneo et al. (2009), where 11 photographs of adult pornographic actresses (of known age) were shown for evaluation to a group of experts composed of pediatricians, forensic experts and gynecologists. Only 22% of pediatricians, 25.5% of gynecologists and 60% of forensic doctors working in Italy and 5% of pediatricians, 9% of gynecologists and 51% of German forensics made a proper assessment. Such discrepancies may result from differences

in educational levels, but also from inter-population differences widely documented in literature during puberty. However, Franklin et al. (2015) state: "inter-population differences in age of puberty that are widely documented in the literature". It has been suggested that it is easier to mistake an adult as a child than vice versa (especially if it is before the onset of puberty).

Inter-individual and ethnic diversity in relation to the rate puberty challenges the reliability of secondary sexual characteristics as feasible indicators of biological age. Characteristics of Japanese anime hair style and sporadic presence of so-called *futari* (representation of hermaphrodites with often overdeveloped sexual characteristics) may exclude this method out of assessing the age of images from the productions of *shotacon* and *lolicon*.

Informational sources on the age of depicted individuals may also be secondary elements resulting from the context of the comic, such as specific school uniforms (characteristic males' *gakuran* and females' *sērā-fuku*) suggesting educational stage (Arunrangsiwed 2015), speech patterns (e.g. personal pronouns '*boku*' and '*ore*' used by males of different age) and other elements belonging to the cultural region. In addition, given the popularity and universality of *doujinshi* (so-called 'fan art'), character recognition and indication of a source that was a prototype for pornographic material could allow verification of an author's information on the character (including age).

Interest in art forms, such as anime and manga, is steadily increasing in the West (Hinton 2014), which is manifested in their popularity in mass media, such as TV or Netflix. The popularity of manga and anime have been instrumental in the spread of comic based eroticism via the mass media. As Arunrangsiwed (2015) indicates, the phrase *'hentai'* has been googled with an average frequency of 13.6 million times a month, which may contribute to the spread of child animated pornography. These facts indicate a sharp shift in perceptions of eroticism and pornography, and the growing importance of fictional works. Equally disturbing are current robotic trends in developing sexualised child-shaped robots or dolls (Chatterjee 2020). This has created a 'gray area' in which there is a greater possibility for child sexual abuse related to the production of fictional child pornography. Due to the illegal status of animated pornography internationally, creating and improving methodologies for assessing it is an important research area.

In conclusion, while this study's method does not allow for a highly precise assessment of the age of animated characters, the results achieved can be comparable with the accuracy of the estimation documented for the images of living persons. Consequently, this method should be treated as a basis for further development of established protocols.

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2D:4D digit ratio and its relationship to BMI, sporting choices and physiological predispositions among women

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Abstract: The 2D:4D digit ratio has been established as a biomarker of the level of exposure to prenatal sex hormones' balancebetween prenatal testosterone (PT) and estrogenne levels. Higher 2D:4D indicates lower PT exposure and vice versa. Data suggests that PT exposure is linked to a risk-taking attitude and physical aggressiveness, both of which are requirements in contact sport. A possible correlation between 2D:4D and human body mass index has also been identified. The aim of the study was to examine the relation between 2D:4D ratio and choice of sport. It was assumed that female soccer players who choose a contact sport would have a lower 2D:4D ratio (thus experiencing higher exposure to PT) than female volleyball players (selecting non-contact sport). The analysis was also aimed at identifying whether a correlation between prenatal testosterone level and BMI exists. The participant sample consisted of 103 women – 36 volleyball players, 33 soccer players and a control group (N=34). Measurements were collected in 2019–2020. The results suggest that 2D:4D was significantly different in women practicing various sports (contact and non-contact sports). Women engaged in contact sports had lower 2D:4D than women engaged in non-contact sports, and vice versa ($p<0.05$). 2D:4D correlated positively with BMI and body weight – the higher the 2D:4D ratio, the higher the BMI and body weight (and vice versa) ($p<0.05$). Low 2D:4D (high PT exposure) may predict the choice of more risky, aggressive contact sports, and vice versa. High 2D:4D may predict a higher BMI and body weight, and *vice versa*.

Key words: contact sport, non-contact sport, volleyball, soccer, prenatal testosterone, weight

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Introduction

The digit length ratio 2D:4D has repeatedly featured as an object of study. The latter is a ratio of the index finger length (2D) and ring finger length (4D) (Manning et al. 2014). The value of digit length ratio is a biomarker of prenatal sex hormones' balance between testosterone and estrogene levels. A high level of prenatal exposure to testosterone decreases the value of the 2D:4D ratio, while the low level of prenatal testosterone boosts the value of this index (Lutchmaya et al. 2004; Manning and Fink 2011; Manning et al. 2014; Bovet 2019). During the fetal stage, the synthesis of prenatal testosterone is one of many factors that influence development of gender and reproductive glands. If prenatal testosterone dominates prenatal estrogen, it directs the development towards the male gender, and vice-versa (Lichtenberg-Kokoszka 2016). For this reason, male individuals have a lower digit ratio (and at the same time a higher level of testosterone) than women (Manning et al. 1998). An abundance of scholarly work confirms the dimorphic character of this index (Putz et al. 2004; Rahman et al. 2005; Malas et al. 2006; Galis et al. 2010; Manning et al. 2014; Mularczyk et al. 2014; Zaleska et al. 2017; Manning and Fink 2020).

The 2D:4D ratio is established whilst still in the prenatal development phase, identifiable during the fetal stage *in utero* and is constant across all stages of ontogenetic development (Manning et al. 1998). The difference between the 2D:4D ratio in male and female individuals can already be observed at the end of the first trimester of pregnancy (Malas et al. 2006; Galis et al. 2010). In order to explain the mechanism responsible for the establishment of the length ratio between the ring and index digit, as well as its correlation with the level of exposure to hormones during the prenatal stage, it should be noted that the development of digits correlates with the formation of the genitourinary system through common control of their development by homeotic genes (Hox) (Kondo et al. 1997; Manning et al. 1998; Manning 2011). This accounts for the correlation between the process of the prenatal sex steroid production and fetal digit formation patterns. It appears, however, that this correlation is limited only to prenatal androgens, as no reliable evidence has been identified to explain the correlation between the 2D:4D ratio values and the level of testosterone in adults (Manning et al. 2004; Hönekopp et al. 2007). Since the 2D:4D ratio is a sex dimorphic trait that develops *in utero* (Manning et al. 1998; Manning 2002; Malas et al. 2006), this suggests a correlation with many other traits that are determined during prenatal development. Intrauterine hormone balance influences also adult behavioral traits (Hines 2006). Proneness to aggressive behavior is one of the differences between genders in humans – men exhibit a greater tendency for aggressive behavior than women (Campbell 2006). Male individuals tend to make risky decisions more often than women (Hersch 1996). Researching the correlation between the 2D:4D ratio and aggressiveness, Schwerdtfeger et al. (2010) identified a negative correlation in male individuals, indicating that the lower the 2D:4D ratio (and concurrently a higher level of PT), the higher the level of aggressiveness in the examined cases. In research involving a large study group (N=2200), Hönekopp (2011) confirms a negative correlation between the

2D:4D ratio and physical, as well as verbal, aggressiveness. Millet (2011) and Millet and Dewitte (2007) have linked low values of this ratio with a greater tendency for aggressive behavior in competitive circumstances. Hönekopp and Watson (2011) confirm these results for men, yet failed to identify a similar correlation in examined female individuals. Kociuba et al. (2015) and Koziel et al. (2018) noticed the relationship between 2D-4D with choices of relatively risky professions such as military and police officers.

Tamiya et al. (2012) and Perciavalle et al. (2013) consider aggressiveness to be one of the potential factors for success in sport, suggesting it may be useful in certain sports that involve high risk (of, for instance, injuries). For example, soccer is classified as one such sport since it involves a high risk of a body collisions between players (Mitchell et al. 2005). Perciavalle et al. (2013) note that soccer players ranked higher, in terms of tendency for aggressive behavior, than other sportsmen from the control group. Moreover, soccer players exhibited a lower 2D:4D ratio than other surveyed individuals. In their study, Kim and Kim (2016) argue that low 2D:4D values might positively impact the success rate in aggressive sports. Additionally, they demonstrate that low 2D:4D values might serve as a predictor of the choice of competitive sport, involving high levels of aggressiveness and risk. Hönekopp (2011) also noted a significant positive correlation between 2D:4D values and a tendency to risky behavior (in females). Reed and Meggs' (2017) research sought to measure testosterone levels during the prenatal development as a predictor of the choice of sport. Their research introduces a categorization of sports as either openly aggressive (contact sports) or non-aggressive (non-contact). As a result, it has been demonstrated that athletes training for contact sports display significantly lower 2D:4D values by comparison to athletes training for non-contact sports. These conclusions have been corroborated by Kociuba et al.'s (2017) research, which determined that women who chose contact sports that involved risky and aggressive behavior (boxing and judo) possessed significantly lower 2D:4D values than women who chose non-contact sports.

One of the sex dimorphic features *in utero* is BMI (Body Mass Index) (Broere-brown et al. 2016; Galjaard et al. 2019). It may be assumed that sex hormones are the main regulators of male and female dimorphic morphological sex characteristics during puberty (as well as in adult individuals). Contrary to body fat deposition, which changes its character as a dimorphic trait across development stages (the differences between males and females are in certain stages evident, while in others barely visible), characteristics such as body mass index, waist to hip perimeter ratio and waist to chest perimeter ratio, are stable dimorphic traits (Fink et al. 2003). In this regard, the differences between males and females are constantly visible, as they are not dependent, to such a degree, on postnatal sex hormones (which are ontogenetically changeable) (Fink et al. 2003). For this reason, the changeability of these traits may, at least partly, depend on the effect of masculinization or feminization during the fetal stage. For instance, a low waist-hip ratio (WHR) in females usually results from a high level of exposure to prenatal testosterone (which is characteristic of males) (Evans et al. 1983, Manning et al. 1998), however there is new evidence that the

2D:4D is not a reliable indicator of the levels of testosterone (Hollier et al. 2015; Whitehouse et al. 2015; Apicella et al. 2016). Female BMI is also usually lower than that of males (Erkec 2019). Drawing on digit length index as a biomarker of prenatal exposure to sex hormones, Fink et al. (2003) explore the hypothesis that typically male (low) values of the 2D:4D ratio correlate with typically male (androgenized) BMI values. They confirm a significant positive correlation between BMI and 2D:4D values in males (higher BIM, higher 2D:4D value). Bagepally et al. (2020) reach similar conclusions. Although research has not attested similar correlations in surveyed women (Manning 2002; Fink et al. 2003; Erkec 2019), some studies argue for the existence of a correlation between a higher estrogen level and obesity, in both males and females (Kley et al. 1980; Kirschner et al. 1981), which prompts further studies and research of the issue.

In addition to the biological importance of the feature under the study, the finger length index can be a good tool to select the best, most predisposed to specific disciplines Athletes, and it can therefore be used in coaching work.

Material and Methods

The research sample for this study consisted of 103 women: 36 soccer players athletes training on a semi-professional level, 33 volleyball players training volleyball on a semi-professional level and 34 non-athletes. Their average age was 22 years (SD=6.38). The survey was conducted in 2019–2020. The inclusion criterion for participation in the study for female athletes was practicing a given sport for at least three years or more.

The length of digits was measured in one part of surveyed individuals: index and ring fingers were measured using an electronic caliper gauge accurate to 0.01 mm. The surveyed individuals placed their hands on a table palm up and with outstretched fingers. Digits were measured from epiphysis (*pseudophalangion* II and IV) to tip (*dactylion* II and IV) (Manning et al. 1998). Each of the measurements was repeated thrice and the arithmetic mean of all these measurements was subsequently calculated, which was then used to calculate the 2D:4D ratio. When direct measurements were not possible, measurements were conducted by means of an electronic hand scanner where measurements were conducted in ImageJ software in the same way as measurements taken by electronic caliper.

Student's t-test did not reveal any statistically significant differences between measurements taken by electronic caliper gauge (Mean=0.99; SD=0.03) and those taken using an electronic hand scanner (Mean=0.99; SD=0.03) (Table 1).

Table 1. Comparison of right hand measurements conducted by electronic scanner and electronic caliper gauge.– not significant, *p* – significance level, *t* – Student's t-test result for relevant groups

Trait	Electronic scans (N=10)	Electronic caliper gauge (N=10)	Difference volleyball – soccer players	
	Mean	mean	*t*	*p*
2D:4D	0.988	0.988	0.053	ns

Respondents provided their age, body height and mass. Based on these data, their BMI was calculated. Participation in the survey was voluntary. In order to assess the significance of the difference between three mean values of the 2D:4D index (interval features) taken separately for the left and right hand, a one-way analysis of variance (ANOVA) was first used, and then a *post-hoc* analysis was conducted. In order to assess the existence of a correlation between 2D:4D and BMI, Pearson r correlation was used.

Results

Descriptive statistical data of the 2D:4D ratio – such as the mean, median, minimum, maximum and standard deviation for left and right hand respectively – are presented in Table 2.

Average values of the 2D:4D ratio for non-athletes (N=34), volleyball players (N=33) and soccer players (N=36), were compared using the category of left and right hand. This analysis did not reveal any statistically significant differences ($p>0.05$) (Table 3).

During the course of further analysis, the *post-hoc* analysis for the left hand demonstrated a statistically significant difference between two surveyed groups – volleyball players and soccer players. Significantly higher 2D:4D values were noted in the case of volleyball players (Mean=0.987) than in the case of soccer players (Mean=0.968). Female soccer players exhibited significantly lower

Table 2. Basic descriptive characteristics of the 2D:4D index in groups of women engaged in volleyball, soccer and non-athletes

Trait	Control group		Volleyball		Soccer	
	2D:4DL	2D:4DR	2D:4DL	2D:4DR	2D:4DL	2D:4DR
N of valid measurements	34	34	36	36	33	33
Mean	0.984	0.994	0.987	1.001	0.968	0.983
Median	0.987	0.999	0.989	1.000	0.971	0.979
Minimum	0.916	0.914	0.916	0.929	0.915	0.926
Maximum	1.099	1.121	1.084	1.051	1.030	1.064
SD	0.044	0.040	0.039	0.030	0.028	0.031

SD – standard deviation

Table 3. Comparison of average 2D:4D values in female volleyball and soccer players and non-athletes, for left and right hand respectively

Study group	2D:4DL		2D:4DR	
	Mean	*SD*	Mean	*SD*
Control group (N=34)	0.984	0.044	0.994	0.040
Volleyball (N=36)	0.987	0.039	1.001	0.030
Soccer (N=33)	0.968	0.028	0.983	0.031
Difference	F=2.331; p=ns		F=2.276; p=ns	

F – variance analysis result, p – significance level, *SD* – standard deviation, ns – not significant.

values of the index in question (Table 4). The *post-hoc* analysis for the right hand also revealed a statistically significant difference between the two surveyed athlete groups – volleyball players and soccer players. As in the case of the left hand, significantly lower values of the 2D:4D index were noted in the soccer players (Mean=0.983) than volleyball players (Mean=1.001), and vice versa (Table 5).

Descriptive statistics of BMI (mean, median, minimum, maximum and standard deviation) for individual survey groups are presented in Table 6.

The conducted analysis attested to the existence of a statistically significant positive correlation between BMI and the value of the 2D:4DL index (r=0.332; p=0.001). The index 2D:4DR also displayed a statistically significant positive correlation (r=0.406; p<0.001) with BMI. These results demonstrate a directly proportional correlation – the lower the BMI values, the lower the 2D:4D ratio values, and *vice versa* (Table 7).

Table 4. *Post-hoc* test – differences between average 2D:4DL values in specific study groups

Study group	[control group] $\bar{x}$ =0.984	[volleyball] $\bar{x}$ =0.987	[soccer] $\bar{x}$ =0.968
Control group (N=34)		ns	Ns
Volleyball (N=36)	ns		0.045
Soccer (N=33)	ns	0.045	

ns – not significant, $\bar{x}$ – mean

Table 5. *Post-hoc* analysis – differences between average 2D:4DR values in specific study groups

Study group	[1] $\bar{x}$ =0.994	[2] $\bar{x}$ =1.001	[3] $\bar{x}$ =0.983
Control group [1] (N=34)		ns	Ns
Volleyball [2] (N=36)	ns		0.036
Soccer [3] (N=33)	ns	0.036	

ns – not significant, $\bar{x}$ – mean

Table 6. Basic descriptive characteristics of BMI in groups of women engaged in volleyball, soccer and non-athletes

Trait	Control Group	Volleyball	Soccer
N of valid measurements	34	36	33
Mean	23.492	21.274	21.951
Median	22.026	20.659	22.308
Minimum	15.987	16.529	18.750
Maximum	41.522	26.881	24.974
SD	5.730	2.223	1.628

SD – standard deviation

Table 7. Pearson's r correlation between BMI and 2D:4D index for left and right hand

Trait	BMI	
	r	*p*
2D:4DL	0.332	0.001
2D:4DR	0.406	<0.001

r – correlation value, *p* – significance level.

Discussion

The research conducted aimed to establish whether a correlation exists between ratio 2D:4D – a biomarker of exposure to testosterone during the prenatal stage – and the choice of sport in the case of female athletes. Two athlete groups, volleyball players and soccer players, were examined. Volleyball is perceived as a sport with a significantly lower risk of injury by contrast with soccer (Kujala et al. 1995). The results suggest that 2D:4D was significantly different in women practicing various sports (contact and non-contact sports). Women engaged in contact sports had lower 2D:4D than women engaged in non-contact sports, and vice versa ($p<0.05$). 2D:4D correlated positively with BMI and body weight – the higher the 2D:4D ratio, the higher the BMI and body weight (and vice versa) ($p<0.05$). Low 2D:4D (high PT exposure) may predict the choice of more risky, aggressive contact sports, and vice versa. High 2D:4D may predict a higher BMI and body weight, and vice versa. Predominantly, the latter results from the difference in the rate of body contact of the sports in question. In this regard, soccer is considered a sport with a high risk of injury (Złotkowska et al. 2015). Additionally, soccer has been classified as a sport involving a high risk of body clash, while in the case of volleyball, which is a non-contact sport, this risk is very low (Mitchell et al. 2005). Given the high risk of injury inherent to soccer, it may be assumed that the choice of this sport is indicative of a congruent awareness of the risk of injury.

The high risk of injury characteristic of soccer correlates with aggressiveness, which is significant to contact sports. Aggressiveness allows players to achieve specific aims; for instance, scoring a goal during a soccer match, intercepting the ball and blocking the opponent's influence on the course of the game (Soroka 2011). In order to block the opponent and prevent their domination in the field, soccer players often opt for risky and aggressive behavior, such as fouls, for instance. Moreover, not all aggressive behavior is considered a foul and penalized with a red card, a punishment reserved for the most brutal actions. Taking all of the latter into account, it may be concluded that women who choose and train for soccer should exhibit a higher tendency towards risky and aggressive behavior, by comparison with women who choose a non-contact sport such as volleyball.

It has been established that the tendency to risky and aggressive behavior correlates with low, masculine 2D:4D values, indicative of high exposure to testosterone during prenatal development (Millet and Dewitte 2007; Schwerdtfeger et al. 2010; Hönekopp 2011; Hönekopp and Watson 2011; Millet 2011). Brañas-Garza et al. (2018) also connect low 2D:4D values in women with a tendency towards risky behavior. Perciavalle et al. (2013) further conclude that soccer players with low 2D:4D values were more prone to aggressive behavior than players with higher values

of this index. Moreover, Mailhos et al. (2016) note that the most aggressive soccer players (receiving red cards or penalties for more brutal actions) exhibited a significantly lower 2D:4D ratio than the rest of the players. Manning et al. (2014) suggest a correlation between the 2D:4D values and physical aggressiveness in sports as circumstances of challenge. Following this reasoning, this study hypothesizes that female soccer players who select a contact sport, entailing both aggression and higher risk, exhibit lower values of the 2D:4D ratio than volleyball players. Female athletes engaged in soccer exhibit lower values of both the 2D:4DL, as well as 2D:4DR ratio, than women engaged in volleyball (Tables 4 and 5). This suggests that women with a high exposure to testosterone during prenatal development choose contact sport, while women with less exposure to testosterone during this period, choose non-contact sport.

These results align with Kociuba et al.'s (2017) research, which highlights that women who chose contact sports (boxing and judo) exhibited lower 2D:4D ratio values than women who chose safer sports. It should be noted that, as with soccer, boxing and judo are classified as sports with a high risk of injury (Złotkowska et al. 2015). Kim and Kim's (2016) research also corroborates these results, concluding that a low 2D:4D index is indicative of a greater predisposition for more competitive sports and may positively impact the success rate in sports that require an aggressive attitude. Additionally, Ribeiro et al.'s (2016) research with men concludes that the level of testosterone rises (triggering an increase in aggressiveness and strength) as the result of aggressive challenges (for instance, a body clash in a contact sport) more often in individuals with a low 2D:4D ratio, predisposing them to contact sports. Similar conclusions are evidenced in Kociuba et al. (2019) and in Joyce et al.'s (2013) research, where they determine that more aggressive athletes (both men and women) exhibit a significantly lower 2D:4D ratio. Drawing on the results of a survey involving 200 men, Reed and Meggs (2017) note that athletes who had chosen contact sports had a lower 2D:4D ratio than athletes engaged in non-contact sports.

Bailey and Hurd (2005) confirm the negative correlation between the 2D:4D ratio and aggressive tendencies in men, yet failed to obtain similar results for women. Similarly, Hönekopp and Watson (2011) do not note this correlation in females, prompting the need for further studies on the existence of this correlation in women. bo Bagepally et al. (2020) also confirm this correlation. However, all of these studies were conducted on male individuals. In the case of women, Manning (2002), Fink et al. (2003) and Erkec (2019) fail to observe any significant correlations between the discussed indexes. Nevertheless, it should be noted that BMI is a stable dimorphic trait. The latter is contrary to, body fat for instance, which displays a dimorphic character that changes across different development stages (the differences during infancy, early and later childhood are negligible, while they significantly increase during puberty), thus depending on the relationship of (postnatal) estrogen and testosterone (Nelson et al. 1999). The main reason behind the stability of BMI's sex dimorphism is the individual profile of sex hormones (Fink et al. 2003), which indicates that this trait can be dependent on the effect of

masculinization or feminization during the fetal stage. These results provide an incentive for further research.

A small number of surveys conducted on women who train for specific sports, as well as discrepancies across some studies that have been conducted thus far, encourage further exploration of this issue. If the hypothesis put forward by this study is correct, further evidence will be forthcoming in the future. Digit measurements, as well as the establishment of length index values, do not pose any research difficulty and may be easily obtained for future useful studies – for instance, during a selection of young athletes – which may be helpful in the training of new generations of better (physiologically predisposed to specific sports) players.

The authors are aware of the limitation, concluding that such an approach and possible biases resulting from the small sample size and possible hormonal disorders may affect the obtained results. None of the participants declare hormonal disorders, but it was only based on personal declarations.

A small number of surveys conducted on women who train for specific sports, as well as discrepancies across some studies that have been conducted thus far, encourage further exploration of this issue. If the hypothesis put forward by this study is correct, further evidence will be forthcoming in the future. Digit measurements, as well as the establishment of length index values, do not pose any research difficulty and may be easily obtained for future useful studies – for instance, during a selection of young athletes – which may be helpful in the training of new generations of better physiologically predisposed to specific sports players.

Authors' Contribution

JL collected the data, performed statistical computations and drafted the manuscript.

AT was project supervisor, drafted the manuscript, edited the final version of the manuscript.

All authors carefully read and accepted the final version of the manuscript.

With the submission of this manuscript I would like to undertake that the above mentioned manuscript **is without any conflict of interest** and has not been published elsewhere.

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Prevalence and Factors Associated with Overweight/ Obesity in Adolescent School Girls: A Cross-Sectional Study in Kolkata, India

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Abstract: Overweight and obesity in adolescent girls are considered a leading global public health issues in recent times. There is a need to evaluate the potential socioeconomic and behavioural factors behind adolescents' overweight and obesity in different environmental settings. The present study aims to understand the prevalence of overweight and obesity among urban adolescent school girls and to determine the association between selected socioeconomic and behavioural factors and overweight/obesity. This is a cross-sectional study using a multistage stratified cluster sampling with a sample size of 1041 adolescent girls aged 10 to 18 years from schools of Kolkata, India. Overall prevalence of overweight and obesity were 18.9% and 23.7%, respectively. The prevalence of overweight and obesity was higher among those adolescent girls whose parents had completed higher education (49.5%) and had higher monthly per capita household expenditure (48.4%). Stepwise binary logistic regression analysis confirmed that the probability of being overweight/ obese tended to be in adolescents who slept less than 7 hours per day ($p<0.001$). Overweight /obesity was also higher among those children whose fathers were fatty ($p=0.002$), taken medicines three months before the survey ($p=0.008$), and watched television and mobile phones for more than 1 hour a day ($p=0.039$). Rapid change in modern lifestyles is seemingly decreasing sleep duration in adolescents with subsequent negative impact on their health.

Key words: Prevalence, Obesity, Body Mass Index, Sleep, Adolescents, India

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Introduction

Obesity, a "pandemic", has potential consequences for human health (Chincholikar et al. 2016). It generally results from an imbalance between energy intake and energy expenditure. Several international and regional studies indicate that several factors are responsible for developing obesity; these include genetic, behavioural, environmental, cultural, and socioeconomic factors (Okour et al. 2019).

Adolescent obesity is growing very fast and leads to a wide range of health issues regardless of weight during adulthood. It has become increasingly important to identify the risk factors predisposing to overweight and obesity from childhood onwards (Viswambharan et al. 2020). Adolescence is a period of high physical growth and reproductive maturation that generally requires optimal macro or micronutrients or both (Egidaw and Gebremariam 2019; Kumar et al. 2021). It is also the peak time when individuals start to decide on their lifestyle, take fast food, develop sedentary behaviours, etc. (Bjorntorp 2001).

Examining the factors involving weight gain and obesity in developing countries is very sensitive because socioeconomic changes in those countries lead to the problem of managing obesity and have a significant concern about malnutrition (Bharati et al. 2008). Therefore, it is essential to determine which factors are associated most with obesity among adolescents. Chincholikar and Sohani (2016) stated that adolescents belonging to the upper socioeconomic class may be at higher risk of becoming obese than those in lower classes. Several studies found that economic difficulties, educational level, lifestyle behaviours, low cost of energy-dense foods etc., are potential variants in determining the physical health status of adolescent girls (Pigeyre et al. 2012). Ghosh (2014), who studied adolescent girls of Kolkata, found that family monthly income, taking too many fast foods and junk foods, and spending time with computers, were factors in increasing BMI.

In adolescents, sleep is considered an essential factor for physical health. However, adolescent lifestyles often curtail sleep time. For example, adolescents need to wake up early to go to school. There are also social pressures for shortening the amount of sleep, as well as playing with mobile phones, etc. Healthy sleep depends upon the quality and regularity of sleep and proper duration. When sleep is disrupted, health risks ensue (Chaput and Dutil 2016). Reduced sleep time up-regulates appetite by reducing leptin, an increase in ghrelin, and a reduction in insulin sensitivity; these changes increase the risk for obesity (Van Cauter et al. 2008). Adolescent girls experience weight gain when there is insufficient sleep, especially if they sleep less than 6 hours per night (Berkey et al. 2008).

Therefore, this study examines the prevalence of overweight and obesity among urban adolescent school girls, and in determining the association between selected socioeconomic and behavioural factors and overweight/obesity.

Material and Methods

Study Population and area: In this cross-sectional study, data was collected from the adolescent girls aged 10–18 years from Government and Government-sponsored higher secondary schools in

Kolkata, West Bengal, India. West Bengal is India's fourth most populous state and is located in the eastern region of India. Kolkata is the capital city of West Bengal and is often known as the cultural capital of India.

Sample size and sampling: The total study sample was 1041 adolescent girls. We calculated the sample size for estimating a reported prevalence of 35.5% (Ghosh 2014) with a margin of error of 5 percentage points and 98% confidence interval, assuming a design effect of two, approximately 5% non-response rate. Therefore, approximately 1041 girls were selected using multistage stratified cluster sampling. Firstly, four sampled schools were selected out of all Bengali medium higher secondary schools in Kolkata, including Government and Government-sponsored schools. The primary differences between Government and Government-sponsored schools are the management system. Government schools are owned and controlled by the local state government in India. In contrast, government-sponsored schools have their private management system but get funds from the state government. However, in the present study these reflected small differences in the students' socio-economic status between Government and Government-sponsored schools. Then from each group, two schools were selected randomly. Equal weightage was given to all the groups, and two schools were selected from every group. Adolescent girls who were Hindu by religion, whose mother tongue was Bengali and were within the age group 10 to 18 years were selected for this study. We were given prior informed consent from the School Education Directorate, the head of the respective schools, the students' parents, and the students. Data were collected from September 2018 to January 2020.

Data collection

Socioeconomic data: Socioeconomic data like age (date of birth), caste, education, and occupation of the parents, household size, and monthly household expenditure data were collected. The data was cross verified from school records and also from the respective parents. A schedule was formulated for the students' parents. Students filled in information from their parents. Mobile phone numbers were collected from the students and also from the schools for a future response if needed.

Anthropometric data: The height (cm) and weight (kg) of the subjects were measured according to the standard procedures (Weiner and Lourie 1981). We had used an anthropometer and a portable weighing machine for the measurements of height and weight. Before taking the measurement, each subject was asked to stand erect without footwear. Height was recorded to the nearest 0.1 cm and weight to the nearest 0.5 kg. Body mass index (BMI) was calculated by dividing weight in kilogram by square of height in meter. Calculated BMI was categorized using the BMI for Age Girls (5 to 19 years) World Health Organization percentiles guidelines (WHO 2006): normal (<5th – 85th percentile), overweight (<85th – 95th percentile), obese (>95th percentile). The individual with physical deformities was excluded from the study. Technical error of measurements (TEM) was incorporated and found within accepted limits (Ulijaszek and Kerr 1999).

Behavioural data: Physical activity (do you exercise regularly? Yes or No.), sedentary behaviour data (Do you watch television and mobile? If yes, how many hours do you spend on television and mobile in a day?), sleeping duration data (What time do you go to sleep? What time do you wake up?) were collected. We also collected health-related data such as taking medicine in the last three months before the survey and subjective perception of their each parent's body physique (obese, slim, or normal?).

Categorisation of socio-economic and demographic and also habits related characteristics: The ages of the adolescent girls were categorised into two groups, i.e. 10–13 years and 14–17 years. The family type was classified into two groups, i.e. nuclear (1–4 members) and joint (Above 4 members) families. Father's and mother's education status was combined to form a new category as parental educational status. Four quartile ranges (1st, 2nd, 3rd and 4th) were calculated to understand the household per capita monthly expenditure patterns. Sleeping duration (≤ 7 hours vs > 7 hours) and watching TV and Mobile (≤ 1 hour vs > 1 hour) per day were categorised by using the median values (50th percentile).

Statistical Analysis: Overall prevalence and age-specific prevalence rates of overweight and obesity were estimated. We calculated the percentages of socioeconomic, demographic, and habit-related data. We evaluated the association between overweight and obesity and other of socioeconomic, demographic, and habit-related variables through the chi-square ($\chi 2$) test. Lastly, stepwise binary logistic regression analysis (forward confidential) was performed to get the most significant predictors of overweight and obesity in studied adolescent girls. In the stepwise binary logistic analysis, the dependent variable as overweight and obesity status among the adolescent girls was considered binary and coded as "Overweight and obese adolescent girls" = 1 and "rest of the children" = 0. In the stepwise binary logistic analysis, all significantly distributed socioeconomic, demographic, and habit-related variables in the chi-square ($\chi 2$) test were chosen as independent variables except mother education and father education variables. We selected only our new combined variable parental education status as an independent variable in the stepwise binary logistic analysis. Nagelkarke R^2 was calculated for the final step of stepwise binary logistic regression analysis to find out the amount of variation in the overweight/obesity explained by the independent variables in the model. Data were analyzed using software named IBM SPSS (Statistical Package for Social Sciences) statistics version 16.0. A p-value <0.05 and <0.001 were considered statistically significant.

Results

Table 1 shows the age-specific prevalence of overweight and obesity among the studied adolescent girls. The overall prevalence for overweight was 18.92%, and obesity was 23.73%. The maximum prevalence of overweight girls (29.17%) was 16 years, while obesity was 31.58% at 17 years. In general, the prevalence of overweight and obesity rose with increasing age. The combined prevalence of overweight and obesity was 42.65% among the studied adolescent girls of Kolkata (Fig. 1).

Table 1. Age specific prevalence of overweight and obesity among the studied girls

Age (years)	Total (n)	BMI for age (percentiles)							
		Underweight (<5th)		Normal (5th – 85th)		Overweight (>85th)		Obese (>95th)	
		n	%	n	%	n	%	n	%
10	84	13	15.48	33	39.29	19	22.62	19	22.62
11	173	28	16.18	73	42.20	30	17.34	42	24.28
12	174	26	14.94	73	41.95	33	18.97	42	24.14
13	167	28	16.77	79	47.31	34	20.36	26	15.57
14	112	10	8.93	53	47.32	18	16.07	31	27.68
15	116	13	11.21	56	48.28	17	14.66	30	25.86
16	120	10	8.33	48	40.00	35	29.17	27	22.50
17	95	13	13.68	41	43.16	11	11.58	30	31.58
Total	1041	141	13.54	456	43.80	197	18.92	247	23.73

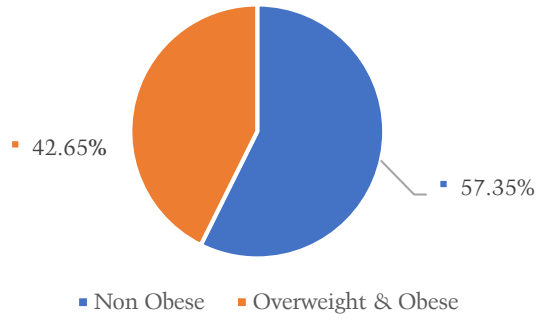

Fig.1. Overall prevalence of obesity (overweight and obese) among the studied girls.

Table 2 represents the socioeconomic and demographic and also habits-related characteristics of the studied participants. The result showed that the majority of the participants (57.4%) were within the age group of 10–13 years, studied in Government schools (52.8%), staying in nuclear families (1–4 members) (67.1%), and belonged to a general caste group (67.5%). The majority of the parents among the participants had graduated and undertaken higher education (39.0%), engaged in government or private services (40.6%), and the per capita monthly expenditure was Rs ≥ 5001 (29.6%, 4th quartiles). It also showed that 28.7% of students sleep was ≤ 7 hours per night (71.3%), did not engage in any physical exercise (81.2%), and consumed regular fast food (54.9%). Nearly 24% of girls watched television and mobile phones for more than one hour per day, and 37.4% had taken medicine three months before the survey. The perception of studied participants towards their parent's body physiques showed that 13.5% of fathers and 18.2% of mothers looked "fatty".

Table 2. Socio-economic and demographic and also habits related characteristics of the studied participants

Characteristics	Adolescent girls (n = 1041)	
	n	%
Age group (years)		
10–13	598	57.4
14–17	443	42.6
School status		
Government sponsored	491	47.2
Government	550	52.8
Family type		
Nuclear (1–4 members)	698	67.1
Joint (Above 4 members)	343	32.9
Social group		
General Caste	703	67.5
Others (SC, ST & OBC)	338	32.5
Father educational status		
Up to Higher Secondary	432	41.5
Graduation and above	609	58.5
Mother's educational status		
Up to Higher Secondary	565	54.3
Graduation and above	476	45.7
Parental educational status		
Both of them studied upto Higher Secondary	362	34.8
One of them studied graduation and above	273	26.2
Both of them studied graduation and above	406	39.0
Father occupation		
Self employed	40	3.8
Service	423	40.6
Business	395	37.9
Others	183	17.6
Household per capita monthly expenditure		
1st quartile (≤ Rs. 2250)	266	25.6
2nd quartile (Rs. 2251 – Rs. 3333)	231	22.2
3rd quartile (Rs. 3334 – Rs. 5000)	236	22.7
4th quartile (≥ Rs. 5001)	308	29.6
Sleeping duration per day		
≤ 7 hours	299	28.7
> 7 hours	742	71.3
Doing regular exercise[a]		
No	845	81.2
Yes	196	18.8
Consume regular fast-food[b]		
No	571	54.9
Yes	470	45.1
Watching TV and Mobile in a day		
≤ 1 hour	790	75.9
> 1 hour	251	24.1

$\bar{x}$	Adolescent girls (n = 1041)	
	n	%
Taking medicine 3 months prior to survey[c]		
No	652	62.6
Yes	389	37.4
Children perception on father's body physique		
Slim	134	12.9
Normal	766	76.6
Fatty	141	13.5
Children perception on mother's body physique		
Slim	101	9.7
Normal	751	72.1
Fatty	189	18.2

a = Bicycling, running and dancing, b = Drinking cold drink, cake and pastry and potato chips
c = medicines related to cold and cough, fever, thyroid, menstrual problem, etc
SC = Scheduled Caste, ST = Scheduled Tribe, OBC = Other Backward Caste

Table 3 depicts the prevalence of overweight and obesity among adolescent girls and the association with different socioeconomic and habit-related characteristics. Out of all factors, parental educational status found a significant association with overweight and obesity. The prevalence of overweight and obesity was higher among parents who both had graduated and undertaken higher education (49.5%, $p<0.001$) than other educational groups. Overweight and obesity were higher among the participants whose fathers engaged in service (44.9%) and business sectors (45.3%), and where the distribution was significant ($p<0.05$). In household per capita monthly expenditure, the 4th quartile group had the highest prevalence of overweight and obesity (48.4%, $p<0.05$). It found that children who slept ≤ 7 hours per night had a higher prevalence of overweight and obesity (52.8%) than the ≥ 7 hours group (38.5%). Here the distribution was highly significant ($p<0.001$).

Table 3. Percentage distribution of overweight and obesity according to characteristics of students

Characteristics	Adolescent girls (n = 1041)		Chi-square values
	n	%	
Age group (years)			
10–13	245	41.0	1.624[ns]
14–17	199	44.9	
School status			
Government sponsored	210	42.8	0.005[ns]
Government	234	42.5	
Family type			
Nuclear (1–4 members)	289	41.4	1.347[ns]
Joint (Above 4 members)	155	45.2	
Social group			
General Hindu	308	43.8	1.193[ns]
Others (SC, ST & OBC)	136	40.2	

Table 3 (cont.)

Characteristics	Adolescent girls (n = 1041)		Chi-square values
	n	%	
Father educational status			
Up to Higher Secondary	158	36.6	11.150**
Graduation and above	286	47.0	
Mother's educational status			
Up to Higher Secondary	212	37.5	13.290***
Graduation and above	232	48.7	
Parental educational status			
Both of them studied upto Higher Secondary	127	35.1	16.283***
One of them studied graduation and above	116	42.5	
Both of them studied graduation and above	201	49.5	
Father occupation			
Self employed	14	35.0	
Service	190	44.9	9.488*
Business	179	45.3	
Others	61	33.3	
Household per capita monthly expenditure			
1st quartile (≤ Rs. 2250)	112	42.1	
2nd quartile (Rs. 2251 – Rs. 3333)	99	42.9	8.971*
3rd quartile (Rs. 3334 – Rs. 5000)	84	35.6	
4th quartile (≥ Rs. 5001)	149	48.4	
Sleeping duration per day			
≤ 7 hours	158	52.8	17.813***
> 7 hours	286	38.5	
Doing regular exercise[a]			
No	370	43.8	2.367[ns]
Yes	74	37.8	
Consume regular fast-food[b]			
No	234	41.0	1.443[ns]
Yes	210	44.7	
Watching TV and Mobile in a day			
≤ 1 hour	323	40.9	4.174*
> 1 hour	121	48.2	
Taking medicine 3 months prior to survey[c]			
No	257	39.4	7.461**
Yes	187	48.1	
Children perception on father's body physique			
Slim	44	32.8	12.612**
Normal	324	42.3	
Fatty	76	53.9	
Children perception on mother's body physique			
Slim	35	34.7	6.063*
Normal	316	42.1	
Fatty	93	49.2	

a = Bicycling, running and dancing, b = Drinking cold drink, cake and pastry and potato chips
c = medicines related to cold and cough, fever, thyroid, menstrual problem, etc.
*$p<0.05$; **$p<0.01$; *$p<0.001$

On the other hand, the girls who watched television and mobile phones for more than 1 hour a day had a higher prevalence of overweight and obesity (48.2%, $p<0.05$) than the opposite group. It further identified that girls who had taken medicines related to cold and cough, fever, thyroid, menstrual problem, etc., had higher overweight and obesity (48.1%) than those girls who had not taken medicine three months prior to the survey. When the participants were asked about their perception of the patient's body physique, it was interesting to find that overweight and obesity were higher among girls whose father looked "fatty" (53.9%) and whose mother looked "fatty" (49.2%). Both the distributions were significantly varied.

Table 4 shows the stepwise binary logistic regression (forward conditional) analysis of significant socioeconomic and other habit-related predictors on overweight and obesity among adolescent girls. In this analysis, highly significant predictors were included, which directly affected overweight and obesity. In Step 1, the first predictor found was sleeping duration which indicates the maximum significance level ($\beta=0.580$; $p<0.001$). This step observed that adolescents who slept ≤ 7 hours per night had higher likelihood of being overweight and obese (OR=1.787, 95%CI = 1.363–2.343, $p<0.001$) than ≥ 7 hours sleeping duration group. In the next step, parental education ($\beta=0.529$; $p<0.001$) was considered. The analysis indicates that girls who slept ≤ 7 hours per day and had parents with higher education (both of them studied graduation and above) showed a higher prevalence of overweight and obesity. In step 3, children's perception of their father's body physique ($\beta=0.797$; $p=0.002$) was detected. This step revealed that adolescents who had perceived their father's body physique and parents were highly educated and slept ≤ 7 hours per day, had a higher risk of getting overweight and obese. The next step (Step-4) found that taking medicine three months before the survey ($\beta=0.349$; $p=0.008$) was added as a new predictor. Medicines included, menstrual related analgesics, fever, thyroid etc. In step 5, watching television and mobile phones in a day was included as a predictor. Results showed that adolescents who watched television and mobile phones for more than 1 hour per day showed a higher prevalence ($\beta=0.308$; $p=0.039$) of being overweight and obese. This analysis shows that the afore mentioned predictors had conjugated predictors on overweight and obesity among adolescent girls. In step 5, low sleeping duration (≤ 7 hours per day) was still a significant risk factor (OR=1.684, 95% CI = 1.280–2.216, $p<0.001$) for developing overweight and obesity among the studied girls. The step 5 model explained 10% (Nagelkerke R^2) of the variance of overweight and obesity in the studied adolescent girls and correctly classified approximately 59.5% of cases.

Discussion

The prevalence of overweight and obesity increases daily in Indian adolescents, specifically those living in urban areas. Socioeconomic and behavioural factors play an essential role in developing overweight and obesity risk. This study considers the overall prevalence of overweight and obesity among adolescent school girls of Kolkata. Ghosh (2014) observed that the prevalence of overweight and obesity was 35.5% among 12 to 15-year and 30.4% among 16 to 18-year-old girls in Kolkata. The present result showed that the overall prevalence is 42.65% which is relatively high and concerning.

Table 4. Step-wise binary logistic regression (forward confidential) analysis of significant socio-economic and habits related predictors on overweight and obesity among the urban adolescent girls

Step	Characteristics	B	S.E.	Wald	df	Sig.	OR	95.0% C.I.	
								Lower	Upper
Step 1	Sleeping duration per day ≤ 7 hours (Ref > 7 hours)	0.580	0.138	17.623	1	0.001	1.787	1.363	2.343
	Constant	-0.467	0.075	38.250	1	0.001	0.627		
Step 2	Parental educational status			12.415	2	0.002			
	One of them studied graduation and above (Ref = Both of them studied upto Higher Secondary)	0.299	0.166	3.256	1	0.071	1.349	0.975	1.866
	Both of them studied graduation and above (Ref = Both of them studied upto Higher Secondary)	0.529	0.150	12.411	1	0.001	1.697	1.264	2.278
	Sleeping duration per day ≤ 7 hours (Ref > 7 hours)	0.521	0.140	13.845	1	0.001	1.684	1.280	2.216
	Constant	-0.738	0.116	40.502	1	0.001	0.478		
Step 3	Parental educational status			11.770	2	0.003			
	One of them studied graduation and above (Ref = Both of them studied upto Higher Secondary)	0.276	0.167	2.748	1	0.097	1.318	0.951	1.827
	Both of them studied graduation and above (Ref = Both of them studied upto Higher Secondary)	0.518	0.151	11.768	1	0.001	1.678	1.248	2.255
	Sleeping duration per day ≤ 7 hours (Ref > 7 hours)	0.496	0.141	12.362	1	0.001	1.642	1.245	2.164
	Children perception on father's body physique			10.018	2	0.007			
	Normal (Ref = slim)	0.376	0.200	3.513	1	0.061	1.456	0.983	2.157
	Fatty (Ref = slim)	0.797	0.254	9.878	1	0.002	2.219	1.350	3.648
	Constant	-1.107	0.208	28.455	1	0.001	0.330		

Step 4	Parental educational status			12.149	2	0.002			
	One of them studied graduation and above (Ref = Both of them studied upto Higher Secondary)	0.286	0.167	2.917	1	0.088	1.331	0.959	1.847
	Both of them studied graduation and above (Ref = Both of them studied upto Higher Secondary)	0.528	0.151	12.149	1	0.001	1.695	1.260	2.282
	Sleeping duration per day ≤ 7 hours (Ref > 7 hours)	0.485	0.141	11.754	1	0.001	1.624	1.231	2.143
	Taking medicine 3 months prior to survey (Yes, Ref = No)	0.349	0.132	6.976	1	0.008	1.418	1.094	1.837
	Children perception on father's body physique			9.836	2	0.007			
	Normal (Ref = slim)	0.399	0.202	3.924	1	0.048	1.491	1.004	2.214
	Fatty (Ref = slim)	0.797	0.255	9.790	1	0.002	2.219	1.347	3.655
	Constant	-1.261	0.217	33.662	1	0.001	0.283		
Step 5	Parental educational status			12.303	2	0.002			
	One of them studied graduation and above (Ref = Both of them studied upto Higher Secondary)	0.281	0.168	2.802	1	0.094	1.324	0.953	1.839
	Both of them studied graduation and above (Ref = Both of them studied upto Higher Secondary)	0.532	0.152	12.299	1	0.001	1.703	1.265	2.283
	Sleeping duration per day ≤ 7 hours (Ref > 7 hours)	0.501	0.142	12.432	1	0.001	1.650	1.249	2.179
	Watching TV and Mobile in a day> 1 hour (Ref = ≤ 1 hour)	0.308	0.150	4.239	1	0.039	1.361	1.015	1.825
	Taking medicine 3 months prior to survey (Yes, Ref = No)	0.325	0.133	5.984	1	0.014	1.384	1.067	1.797
	Children perception on father's body physique			9.845	2	0.007			
	Normal (Ref = slim)	0.403	0.202	3.969	1	0.046	1.496	1.007	2.223
	Fatty (Ref = slim)	0.799	0.255	9.804	1	0.002	2.224	1.348	3.667
	Constant	-1.335	0.221	36.389	1	0.001	0.263		
R^2 = 0.10 (Nagelkarke); Correct Percentage = 59.5, Model chi-square = 51.627 (df=7), $p < 0.001$									

OR = Odd ratio, CI = Confidence interval, Ref = Reference category

The present study also investigated socioeconomic and behavioural determinants of overweight and obesity among adolescent girls. The results showed that the prevalence of obesity was higher among those who live in joint families. In supporting the finding, many studies have noted that children belonging to larger families were more likely to become overweight and obese. One reason could be that children from more prominent families receive less parental instruction regardingtheir food choices. Accordingly, smaller families may be more attentive regarding their child's dietary behaviour and physical activity patterns (Brown et al. 2004; Khader et al. 2009; Lindsay et al. 2006).

Furthermore, socioeconomic status directly influences the nutritional status of adolescents. According to Okour et al. (2019), the main factor influencing children's BMI was the parental economic status. The present findings showed that parents with the highest education graduation and above and highest household per capita monthly expenditure families reflected a higher prevalence of overweight and obesity among adolescents. The study also suggested that family economic status informed children's total calorie intake, dietary behaviour and physical activity (Khader et al. 2009). Schoolchildren raised within families of higher economic status had a more comprehensive range of food choices, including food served at restaurants. Moreover, we speculate that a higher daily children's pocket expenditure could have resulted in less strict control of children's dietary behaviour by the parents, a factor that might have led to higher consumption of calorie-dense food as noted in this study also (Ahmed et al. 2018; Zhang et al. 2018; Whitaker and Orzol 2006).

Another vital factor significantly associated with obesity among adolescents was sedentary behaviour like television and mobile watching and sleeping duration. The study suggested that obesity was higher among those who watched television and mobile and suffered from sleeplessness. Obesity was associated with television viewing in many studies, and the more the duration of television watching, the higher the prevalence of obesity (Viswambharan et al. 2020). A review by Mech et al. (2016) also found that television viewing mediated the relationship between socioeconomic status and overweight and obesity in children (Gatjens et al. 2020). Other mediation analyses also revealed that the effect of socioeconomic status on overweight and obesity assessed by BMI percentiles was partly mediated by media exposure (Morgenstern et al. 2009). Other than television watching and mobile browsing, sleeping duration directly affected the BMI of adolescents. Less sleep was associated with a more significant increase in BMI from age 14 to 18 years (Mitchell et al. 2013). This consequence provides strong evidence that sleep duration was an im portant risk factor in factoradolescent obesity. It is imperative to ensure that adolescents in the upper half of the BMI distribution at age 14 years accumulate sufficient hours of sleep throughout adolescence. It has also been proposed that less sleep increases adolescent BMI by decreasing physical activity due to fatigue and changes in hormones that regulate energy expenditure (Taheri 2010; Knutson et al. 2008). The reason could be adolescents with short sleep duration may be more likely to be awake at night and be exposed to light during the dark cycle. Such biological consequences could affect the periph-

eral clock in adipose tissue by releasing adipokines (Bass et al. 2010; Johnston et al. 2009). The secretion of several hormones, including growth hormone, prolactin, cortisol, thyrotropin, and insulin, are influenced by sleep (Copinschi 2005; Hart et al. 2011). In support of this, a cross-sectional study observed that adolescents going to bed late tend to have higher BMIs, independent of sleep duration, compared with adolescents who go to bed early (Olds et al. 2011). However, the present study found the additive impact of socioeconomic and behavioural factors in overweight and obese adolescent children. The adolescents who slept ≤ 7 hours per day, educated parents studied graduation and above, affluent families in terms of household expenditures, spend more time watching television, browsing mobile phones, taking medicines for health problems, and perceiving their fathers as being "fatty" showed a higher prevalence of overweight and obesity. Besides, adolescents with higher educated parents felt pressure in performing well at school, which could be one reason for sleeping late at night and gaining weight.

The study was limited to school-going adolescent girls in Kolkata, India. Thus, it did not represent all adolescents in the area. One should not generalize the results of all adolescent girls of West Bengal. As this was a cross-sectional study, the causal relationship between socioeconomic and behavioural factors and overweight and obesity could not be established. In-depth food habits and physical activity data were not considered in this study. Breakfast skipping data was also not taken into account, but missing breakfast had been associated with an increased tendency to have snacks and fast foods, resulting in weight gain.

Conclusion

This study concludes that socioeconomic status such as family size, father's occupation, parental education, and total family expenditure influence the increasing prevalence of overweight and obesity among adolescents. Other than these factors, television, mobile watching, and sleeping duration have become critical factors for increasing overweight and obesity in this study. Socioeconomic factors have detrimental effects in developing obesity, but behavioural factors like sleep duration may also significantly influence it. The rapid change in modern lifestyles influences adolescents from having adequate sleeping duration, negatively impacting their health. Therefore, adolescent girls should take preventive measures to reduce overweight/obesity. The government should implement competent nutrition and health promotion strategies towards adolescent girls and their respective parents/guardians to build proper dietary habits, physical activity, and sleeping habits to maintain a healthy lifestyle and reduce the risk of weight disorders..

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Author Contributions

PR developed the aim of the study. PR, SC, PB designed and assisted in drafting the methodology. PR oversaw the data collection. SC did the statistical analysis. PR and SC wrote the final draft of the paper. PB and DC helped in revising the final version of the manuscript. All the authors read and agreed to the final manuscript.

Conflict of Interest

The authors have no conflict of interest to declare.

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Erratum

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The affinities of *Homo antecessor* – a review of craniofacial features and their taxonomic validity

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Notes for Authors

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