Anthropometric, biochemical and hormonal profiles of the partially admixed pygmoid group in Rampasasa (Flores, Indonesia)

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Abstract

Objectives: We performed a cross-sectional study on anthropometric and laboratory characteristics of inhabitants of Rampasasa (Flores, Indonesia). Adults were categorised according to ancestry into three groups: pygmoid (P/P, offspring of pygmoid parents, n=8), mixed pygmoid (P/N, offspring of pygmoid and non-pygmoid parents, n=12) and non-pygmoid (N/N, n=10). Children (n=28) were P/N.

Methods: Measurements included height, weight, sitting height, arm span, head circumference, haematological analysis and serum albumin, calcium, vitamin D, insulin-like growth factor-I (IGF-I) and IGF binding protein 3 (IGFBP-3). Pubertal stage and bone age was assessed in children. Anthropometric data were expressed as standard deviation score (SDS) for age. IGF-I, IGFBP-3 and IGF-I/IGFBP-3 ratio were expressed as SDS for age, bone age and pubertal stage.

Results: Mean height SDS showed a gradient from P/P (–4.0) via P/N (–3.2) to N/N (–2.3) (–3.4, –3.1 and –2.2 adjusted for age-associated shrinking). Sitting height and head circumference showed similar gradients. Serum IGF-I SDS was similar among groups (approximately –1 SDS). IGFBP-3 SDS tended toward a gradient from P/P (–1.9) via P/N (–1.5) to N/N (–1.1), but IGF-I/IGFBP-3 ratio was normal in all groups. In P/P and P/N, mean head circumference SDS was >2 SD greater than mean height SDS. Children showed a progressive growth failure and bone age delay, delayed female pubertal onset and an initial low serum IGF-I, normal IGFBP-3 and low IGF-I/IGFBP-3 ratio.

Conclusions: P/P showed proportionate short stature with relative macrocephaly and relatively low IGFBP-3; P/N presented an intermediate pattern. P/N children were progressively short, showed delayed skeletal maturation, delayed puberty in girls and low IGF-I and IGF-I/IGFBP-3.

Keywords: growth; head circumference; IGF-I; IGFBP-3; negritos; pygmies; short stature; vitamin D deficiency.

Introduction

In several parts of the world, small isolated human populations can be found in which all members are short compared to mean stature in the remaining part of the region, using various cut-offs for average male height (<150, <155 or <160 cm) [1–3]. Some investigators use the term “pygmies” for all such tribes irrespective of the geographical location [4, 5]. Others prefer to restrict this term to short tribes in Africa [6], and use the term “negritos” for short populations in Asia (e.g. the Andaman Islands, the Philippines, Malaysia and Papua New Guinea) [7, 8]. Negritos are thought to be descendants of the earliest migrants to the Southeast Asian region, along with Papuans and Australian Aboriginals [7]. The more generic terms like “pygmoid phenotype” [8, 9] or “pygmoid group” [5, 10] have also been suggested for an isolated short population anywhere in the world, and we decided to use the latter term in this paper.

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Hypotheses about the underlying mechanism of the short stature observed in pygmoid groups can be divided into evolutionary and genetic origins. None of the evolutionary hypotheses (e.g. adaptation to food scarcity, hot and humid climate, density of the environment) have proven valid, so most investigators agree that phenotypic plasticity alone cannot be the explanation, and that (epi) genetic factors are likely involved [11–13]. However, social differences between pygmoid groups and their neighbours may also play a role [14].

The phenotypic, hormonal and genetic characteristics of several pygmy tribes have been studied in some detail. Virtually all pygmies have normal body proportions [15]. In several pygmy tribes the biochemical evaluation of the growth hormone (GH) – Insulin-like growth factor-I (IGF-I) axis was suggestive for an impaired function of the GH receptor (GHR), in view of a normal GH secretion, low circulating IGF-I and GH binding protein (GHBP), decreased expression of the GH receptor (*GHR*) and normal circulating IGF-II [15–24]. However, in some other pygmy tribes serum IGF-I was not decreased [15, 25, 26] and so far genome-wide studies have not identified functional aberrations in the coding and non-coding regions of the gene encoding GHR (*GHR*) [12, 21] or other genomic regions related to GH sensitivity [4].

In Indonesia, a small pygmoid group lives in the rural village of Rampasasa, located in the Manggarai district, Flores, East Nusa Tenggara, located 12 km from the closest town. At the time of this study, Rampasasa had no electricity, water sources were scarce, and there were no local schools and community religious or health centres. In the past, the pygmoid group in Rampasasa practiced endogamy in order to preserve family assets (so their farm and crops were kept within their clan only). When they started practicing religion (mostly Christianity), mixed marriages started to become common. Consequently, the present population of Rampasasa consists of a combination of three groups: a pygmoid group (offspring of parents who both belong to the five pygmoid families), a mixed pygmoid group (offspring of one parent belonging to a pygmoid family and one parent originating from a neighbouring village ["non-pygmoid"]), and a non-pygmoid group. These groups will further be abbreviated to P/P, P/N and N/N.

Remarkably, this Rampasasa pygmoid community is located in the immediate neighbourhood of the cave Liang Bua where the remains of *Homo floresiensis* were discovered [27, 28]. However, there is no sign of any association between the phenotype of the pygmoid group and the archaeological findings. A recent study on the evolutionary history and adaptation of this pygmoid community through single nucleotide polymorphism (SNP)-array analysis and whole genome sequencing provided evidence that polygenic selection acting on standing genetic variation was an important determinant of short stature in Rampasasa [29].

The first purpose of the present paper was to provide an extensive description of the anthropometric, haematological, biochemical and endocrine characteristics of the three groups living in Rampasasa. Secondly, the same characteristics plus bone maturation and pubertal development were investigated in children, all belonging to the P/N group.

Methods

Study design

This cross-sectional study, conducted from December 2011 to April 2014, was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo Hospital (Approval Letter no. 472/PT02.FK/ETIK/2012). Ruteng District General Hospital, located 1,212 m above sea level, was used as research centre during the study. Subjects or their parents were informed about the purpose and design of the study and provided informed consent.

Study population

The study population consisted of all available inhabitants of Rampasasa during the study period. The subjects' pedigrees were documented up to three generations.

Study procedures

From subjects who met the inclusion criteria we collected information on date of birth, sex, birth weight and length, family history and pedigree. Physical examination was performed to obtain data on body height, weight, sitting height, arm span, head circumference and any dysmorphic features. Laboratory investigations were performed regarding haematological status and serum levels of albumin, calcium, vitamin D, IGF-I and IGFBP-3. The choice of reference data for auxological parameters was based on the authors' estimations about their applicability for the Indonesian population; reference data for laboratory parameters were primarily based on the availability of the respective assays. In children, bone age was also determined and pubertal status was assessed in most adolescents.

Standing height (Ht) was measured with a stadiometer (GEA, Indonesia) against the wall, with footwear taken off and expressed as Standard Deviation Score (SDS) for age and sex according to the Centers for Disease Control and Prevention (CDC) reference data [30]. For illustrative purposes, height SDS (HtSDS) was also calculated based on the recent Indonesian growth reference [31]. In children below 2 years, supine length was measured with an appropriate device. Sitting height (SH) was measured with a stadiometer (GEA, Indonesia), with subjects sitting straight on a 40 cm-tall wooden chair.

Sitting height/height ratio (SH/Ht) was calculated and expressed as SDS for age and sex, based on Turkish reference data [32]. Arm span was measured using a wooden measuring stick. In children, arm span/height ratio was expressed as SDS for age based on a Dutch reference [33]. Mean arm span/height in adults was compared with the mean for age in Asian populations [34]. Head circumference (HC) was measured with a tape measure and expressed as SDS for age and sex [35]. Weight was measured with a scale for adults and children who were able to stand up, and with a table-top baby scale for infants. Body mass index (BMI, weight [kg]/height [m^2]) of adults was compared with age references from WHO Western Pacific Region – International Association for The Study of Obesity (IASO) – International Obesity Taskforce (IOT)'s Asian adults classification in order to calculate SDS. Underweight and overweight were defined as <18.5 and ≥23 kg/m^2, respectively. For children, BMI was plotted on CDC reference charts according to age and sex, expressed as SDS, and classified as normal (P5–P85), underweight (<P5) or overweight/obesity (>P85) [30]. Bone age was determined according to Greulich and Pyle and delayed bone age was defined as <–2 SDS [36].

Haematological and biochemical analyses were carried out in the Prodia laboratories in Jakarta and Bali, Indonesia, and compared with in-house reference data. Serum albumin was measured with the bromocresol green assay (reference range 2.8–4.4 g/dL). Serum calcium was measured with the O-cresolphthalein complexone assay (reference range 8.3–10.6 mg/dL for adults, cut-off for hypocalcaemia <9.4 mg/dL for 1–4 years and <9.2 mg/dL for 5–20 years). Serum vitamin D was measured using chemiluminescent immunoassay and cut-off limits for vitamin D deficiency and insufficiency were <10 and 10–30 ng/dL, respectively. Serum IGF-I was measured using the chemiluminescent immunoassay (Immulite-2000) (Prodia Laboratories, Kupang, Indonesia). Results for adults were expressed as SDS for sex and age based on Chinese reference data [37]. IGF-I results for children were expressed for sex and age or bone age, using an in-house lambda, mu, and sigma (LMS) transformation of German references [38]. Furthermore, for children from 9 years of age we also calculated SDS for sex and pubertal stage [38]. Serum IGF binding protein 3 (IGFBP-3) was measured using Immulite-2000 by Quest Diagnostics Laboratory (California, USA) and expressed as SDS for sex and age or bone age, as well as for sex and pubertal stage in children ≥9 years [38]. Serum IGF-I/IGFBP-3 ratio (μg/mg) was expressed as SDS for sex and age or bone age [38].

Data analysis

Before statistical analysis, patient data first underwent a quality control step, including double data entry, manual checking of data transfer to excel files, and checks for outliers. One female subject in the P/N group (C.IV.3) was 39 weeks pregnant, and one non-pygmy subject as well (B.III.1), so their data regarding weight, BMI, IGF-I, IGFBP-3, IGF-I/IGFBP-3 ratio and haematological status were excluded from group analysis. One individual (E.IV.2) had severe bowing of the legs, so his height, SH/Ht, arm span/height ratio and BMI were excluded from group analysis.

In the general population, adult height shows a gradual decline by age, presumably due to progressive osteoporosis. Age is therefore a potential confounder if height is compared among groups with a different mean age. To adjust for this confounder we used the mathematical equations to estimate height at 21 years of age for both sexes based on current height and age, in order to adjust for age-associated shrinking [39].

All analyses were conducted using SPSS software. Mean (SD) values were calculated for anthropometric and laboratory data in the three groups. Comparison between groups was analysed using t-tests or one-way analysis of variance (ANOVAs), which were univariate, bivariate, or multivariate with bootstrapping analysis.

Results

After exclusion of three subjects because of incomplete data, 58 remained for analysis. The 30 adults were divided into 8 P/P, 12 P/N and 10 N/N according to information on their ancestry. There were 28 children, all P/N. The pedigrees of the five families (A–E) are shown in Supplementary Figures 1–5.

Table 1 shows the mean data for anthropometry, nutritional status and serum IGF-I, IGFBP-3 and IGF-I/IGFBP-3 ratio SDS for the three adult groups and children. Sexes were distributed nearly equally.

Anthropometry

Birth size

Information on birth weight could be collected for six children (two males) with an age range from 1 day to 5.3 years, but no reliable information could be obtained about gestational age. Mean birth weight was 2.75 kg (range 2.0–3.5 kg). Birth length was measured in two female newborns (45.8 and 46.5 cm, equivalent with –1.5 and –1.2 SDS, assuming term deliveries) [30].

Height

Mean HtSDS of P/P and P/N were significantly lower than that of N/N ($p<0.001$) (Table 1), with a gradient from P/P (–4.0 SDS) via P/N (–3.2 SDS) to N/N (–2.3 SDS) [30]. Because the mean age of P/P individuals was higher than of the other groups, we estimated height at 21 years of age for all adults based on a mathematical formula for shrinking by age [39], which showed that the difference between the mean adjusted height of P/P (–3.4 SDS) and P/N (–3.1 SDS) did not reach statistical significance (Table 1).

Using recent Indonesian references [31], mean (SD) unadjusted HtSDS was –2.7 (0.4), –2.0 (1.1) and –0.9 (0.7) and the adjusted HtSDS was –2.1 (0.5), –1.6 (0.7) and –0.7 (0.7) in P/P, P/N and N/N, respectively. This illustrates a 1.3 SD difference between height of the Indonesian and US reference populations. The average (SD) height of male

Table 1: Mean (SD) findings on anthropometry, nutritional status and IGF-I, IGFBP-3 SDS and their ratio for adults in the three groups (P/P, P/N and N/N) and children (all P/N).

Parameter	Adults			Children	Total	p-Value (Adults)
	P/P	P/N	N/N	P/N		
Number	8	12	10	28	58	
Age, yrs	56 (15)	34 (16)	37 (11)	7.4 (4.1)	25 (21)	
Sex: n (%) males	3 (38%)	9 (75%)	3 (33%)	12 (43%)	27 (47%)	
Height SDS	−4.0 (0.5)	−3.2 (0.6)[c]	−2.3 (0.6)	−4.0 (1.2)	−3.5 (1.1)	<0.0001[d]
Adjusted height SDS[a]	−3.4 (0.6)	−3.1 (0.6)[c]	−2.2 (0.6)	–	−2.9 (0.8)	<0.0005[e]
SHSDS	−4.2 (0.5)	−3.2 (0.6)[c]	−2.7 (0.8)	−3.5 (0.9)	−3.4 (0.9)	<0.0005[f]
SH/height SDS	0.5 (0.7)	1.3 (0.9)[c]	1.0 (1.3)	0.5 (1.0)	0.7 (1.0)	0.23[g]
Arm span/height	1.035 (0.0273)	1.038 (0.0286)[c]	1.031 (0.0210)	0.9984 (0.0228)	1.0168 (0.0300)	0.82[g]
Head circ SDS	−1.6 (0.7)	−1.1 (1.7)	−0.7 (0.8)	−1.7 (0.8)	−1.4 (1.1)	0.3[g]
BMI[b]	19.7 (2.3)	18.7 (2.3)[c]	20.5 (2.3)	15.2 (1.6)	17.5 (3.1)	0.25[g]
BMI SDS[b]	−0.9 (1.0)	−1.4 (1.2)[c]	−0.46 (1.1)	−0.9 (1.5)	−0.9 (1.3)	0.16[g]
n (%) underweight	3 (38%)	5 (46%)	2 (22%)	8 (31%)	18 (31%)	
n (%) overweight	0 (0%)	1 (9%)	1 (11%)	2 (8%)	4 (7%)	
IGF-I SDS	−1.1 (1.1)	−1.2 (0.9)	−0.7 (1.6)	−2.6 (0.7)	−1.7 (1.3)	0.60[h]
IGFBP-3 SDS	−1.9 (1.0)	−1.5 (1.3)	−1.1 (1.1)	−0.9 (1.2)	−1.2 (1.2)	0.39[h]
IGF-1/IGFBP-3 ratio SDS	−0.4 (1.1)	0.2 (1.0)	0.2 (1.8)	−2.9 (1.0)	−1.4 (1.9)	0.39[i]

BMI, body mass index; circ, circumference; IGF-I, insulin-like growth factor-I; IGFBP-3, insulin-like growth factor binding protein-3; SH, sitting height. [a]Height SDS based on estimated height SDS at 21 years of age, thus adjusted for age-associated shrinking (Niewenweg et al. [39]). [b]Nutritional status was assessed according to Asian adults classification of weight by BMI (WHO/WPR/IASO/IOT) in adults and according to CDC in children. Nutritional status was not assessed in pregnant women, nor in two children below 2 years of age. [c]One subject was excluded from height SDS calculation due to extremely bowed legs (rickets). [d]One-way ANOVA test; post hoc analysis (Tukey's multiple comparisons test) show significant differences for P/P vs. P/N (p=0.04), P/P vs. N/N (p<0.0001), and P/N vs. N/N (p=0.007). [e]One-way ANOVA test; post hoc analysis (Tukey's multiple comparisons test) show significant differences for P/P vs. N/N (p<0.0005), and P/N vs. N/N (p<0.005). [f]One-way ANOVA test; post hoc analysis (Tukey's multiple comparisons test) show significant differences for P/P vs. P/N (p=0.012) and P/P vs. N/N (p=0.0002). [g]One-way ANOVA test. [h]Kruskal-Wallis test. Two pregnant subjects were excluded because of the reported effect of pregnancy. [i]One-way ANOVA test. Two pregnant subjects were excluded because of the reported effect of pregnancy.

adult N/N [159.2 (4.0) cm] is close to the mean height of 18.5 year old males living in Papua and Nusa Tenggara Timur (159.6–160.2), the provinces with the shortest mean height in Indonesia [31].

Table 2 shows data on growth and endocrine status for males and females separately. In both sexes a similarly increasing gradient of HtSDS was noticed. In males, the difference among P/P and N/N was statistically significant (ANOVA, p<0.05) with significant differences between P/P vs. P/N (p=0.04) and vs. N/N (p=0.005). Also in females, statistically significant differences among the three groups were observed (ANOVA, p<0.05), with a statistically significant difference between P/P and N/N (p=0.005). When height was adjusted for age-associated shrinking, the difference between the P/P and P/N groups remained statistically significant, but height SDS in females in the P/P and P/N groups was similar (Table 2).

The difference between mean height of the three male and five female P/P (5.4 cm unadjusted, 6.0 cm adjusted for shrinking) was smaller than in P/N and N/N (unadjusted 9.1 and 10.9 cm; adjusted 9.9 and 11.8 cm, respectively), but the low numbers did not allow for a meaningful statistical analysis. This low sex-related height difference was caused by a relatively low male HtSDS (unadjusted −4.4 vs. −3.7 in females, p=0.03, adjusted −3.9 vs. −3.1, respectively). In the other two groups mean HtSDS was similar for males and females (Table 2).

Individual HtSDS data of P/P and N/N adults are shown in Figure 1. In P/P (Figure 1a), HtSDS ranges for males and females were −4.7 to −4.3 and −4.3 to −3.3, respectively, without a significant correlation with age. In N/N (Figure 1b), HtSDS for adult males and females ranged from −2.9 to −1.8 and −3.4 to −1.6, respectively, with a negative correlation with age (Pearson correlation test, p=0.038). Subjects >35 years were substantially shorter than 20–35 year olds (p<0.0001). After adjustment for age-associated shrinking, the negative correlation lost statistical significance (p=0.15).

Individual data of all P/N subjects (adults and children) are shown in Figure 2a. In the 28 children, linear regression of HtSDS by age showed a statistically significant inverse correlation (p=0.010 in boys, p=0.014 in girls).

Table 2: Comparison of mean (SD) height and serum IGF-I and IGFBP-3 in male and female adult subjects in the three groups (P/P, P/N and N/N).

	Males				Females			
	P/P	P/N	N/N	p-Value	P/P	P/N	N/N	p-Value
n	3	9	3		5	3	7	
Height, cm	144.7 (2.0)	152.9 (5.0)[a]	159.2 (4.0)	0.0066[d]	139.3 (2.6)	143.8 (3.1)	148.3 (4.7)	0.0060[e]
Height SDS	−4.4 (0.3)	−3.3 (0.7)[a]	−2.5 (0.6)	0.0066[f]	−3.7 (0.4)	−3.0 (0.5)	−2.3 (0.7)	0.0062[g]
Estimated height at 21 years	148.9 (3.6)	153.8 (4.6)	161.1 (3.2)	0.014	142.9 (2.6)	144.1 (3.2)	149.3 (4.2)	0.0219
Adjusted height SDS[c]	−3.9 (0.5)	−3.2 (0.6)	−2.2 (0.4)	0.014	−3.1 (0.4)	−3.0 (0.5)	−2.2 (0.6)	0.0222
IGF-I, ng/mL[b]	101.3 (34.5)	155.9 (72.6)	174.7 (33.8)	0.34[h]	87 (40)	157 (73)	159 (80)	0.21[h]
IGF-I SDS[b]	−1.01 (1.07)	−1.16 (0.93)	−0.07 (0.42)	0.15[h]	−1.17 (1.27)	−1.35 (1.31)	−1.03 (1.85)	0.97[i]
IGFBP-3, mg/L[b]	3.0 (0.3)	3.7 (1.0)	3.9 (0.7)	0.41[h]	3.2 (0.7)	4.5 (2.2)	4.2 (1.1)	0.32[h]
IGFBP-3 SDS[b]	−1.72 (0.91)	−1.35 (1.23)	−1.29 (0.82)	0.87[h]	−2.04 (1.20)	−2.32 (1.59)	−1.0 (1.23)	0.21[i]
IGF-I/IGFBP-3 ratio SDS[b]	0.06 (0.84)	−0.02 (1.08)	1.25 (0.33)	0.18[i]	−0.66 (1.25)	0.75 (0.57)	−0.32 (2.01)	0.48[i]

[a]One subject was excluded from height analysis due to extremely bowed legs (rickets). [b]Two subjects were excluded from calculation due to pregnancy. [c]Height SDS based on estimated height SDS at 21 years of age, thus adjusted for age-associated shrinking (Niewenweg et al. [39]). [d]One-way ANOVA test. Post hoc analysis (Tukey's multiple comparisons test) showed significant difference between P/P vs. P/N (p=0.04) and P/P vs. N/N (p=0.005). [e]One-way ANOVA test. Post hoc analysis (Tukey's multiple comparisons test) showed significant difference between P/P and N/N (p=0.0046). [f]One-way ANOVA test. Post hoc analysis (Tukey's multiple comparisons test) showed significant difference between P/P vs. P/N (p=0.04) and P/P vs. N/N (p=0.005). [g]One-way ANOVA test. Post hoc analysis (Tukey's multiple comparisons test) showed significant difference between P/P vs. P/N (p=0.0047). [h]Kruskal-Wallis test. [i]One-way ANOVA test.

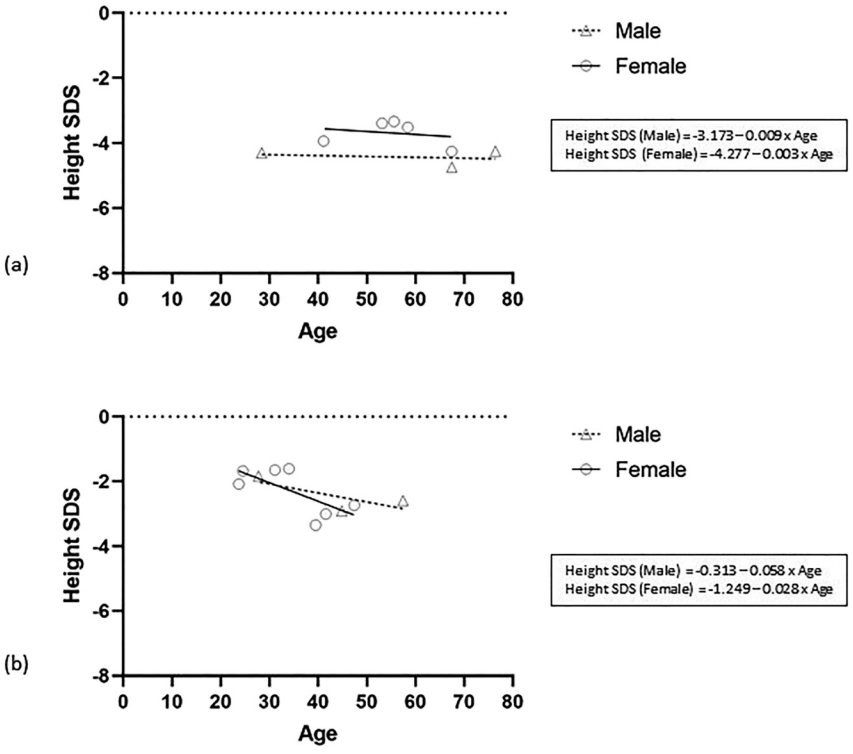

Figure 1: Individual HtSDS data in the pygmoid group (panel a) and non-pymoid group (panel b) plotted against age. In both panels regression lines are shown for males and females, as well as the pertinent equations. When height SDS in N/N (panel b) was adjusted for age-associated shrinking, mean (SD) was −2.16 (0.56), and regression equations changed to height SDS = −1.627 − 0.01307 × age for males (p=0.705) and height SDS = −0.6429 − 0.04361 × age for females (p=0.153).

Mean HtSDS of children (−4.0 SDS) tended to be lower than in adults (−3.2 SDS) (p=0.069).

One female adolescent (B-III-4), aged 14.3 years, caught our special attention because of an extremely short stature (117.8 cm, −6.7 SDS) and prepubertal status (thus severe pubertal delay). At re-examination at 20.7 years the medical history revealed developmental delay and an extremely late menarche (at 19 years), compared with a mean age at menarche of 11.9 (0.8) years in the Indonesian population [40]. Her height was 137 cm (−4.0 SDS), weight 32 kg (−5.8 SDS) and BMI 17 kg/m^2 (−2.2 SDS). Laboratory investigations including indicators of pituitary function

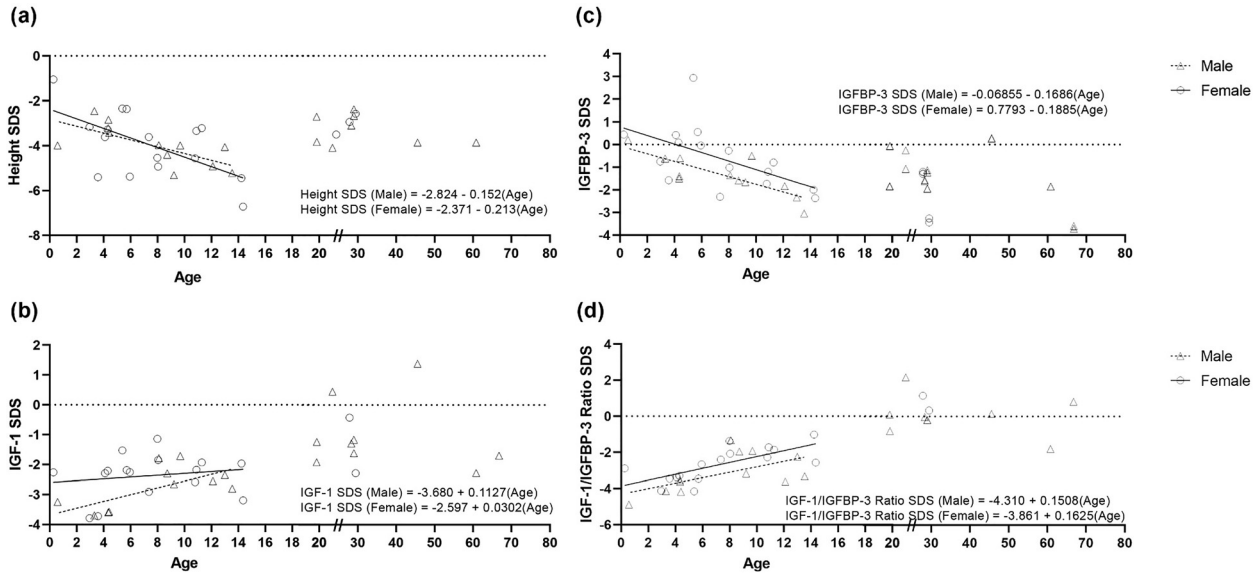

Figure 2: Individual data of individuals belonging to the P/N group plotted by age.
Panel a: Height SDS for age plotted by age. Separate regression lines are shown for boys (p=0.01) and girls (p=0.01) (<18 years). Panel b: Serum IGF-I SDS for age plotted by age, with regression lines for boys (p=0.02) and girls (=0.53). Panel c: Serum IGFBP-3 SDS for age plotted by age, with regression lines for boys (p=0.002) and girls (0.022). Panel d: IGF-I/IGFBP-3 ratio SDS for age plotted by age, with regression lines for boys (p=0.045) and girls (0.002).

showed no abnormalities (Supplementary Table 1). Serum IGF-I results at 14.3 and 20.7 years were 105 (–3.2 SDS for age, –1.0 for pubertal stage) and 249 ng/mL (–0.4 SDS). IGFBP-3 increased from 3.0 (–2.4 SDS for age, –1.7 SDS for pubertal stage) to 4.8 mg/L (0.2 SDS). IGF-I/IGFBP-3 increased from –2.6 to –1.2 SDS (Supplementary Table 2).

Indicators of body proportions

SHSDS showed a similar increasing gradient between the three groups as HtSDS (Table 1). SH/Ht in subjects older than 6 years (n=45) was consistent with the reference range for Turkish individuals (Table 1), taking into account that in the general population mean SH/Ht SDS is >0 in short individuals [41].

Arm span/height ratio in adults was not significantly different between groups (p=0.82) (Table 1) and the mean values were slightly higher than reported for Asian adults [34]. In children, mean (SD) arm span/height ratio SDS compared with Dutch children [33] was 0.667 (1.088), significantly higher than 0 SDS (p=0.0045, one sample t-test).

Head circumference

In adults, there was a similar tendency for a gradient of HCSDS between groups as observed for HtSDS, though not reaching statistical significance (Table 1). Mean HCSDS was >2 SD larger than mean HtSDS in P/P and P/N groups, indicating relative macrocephaly. In P/N individuals, HCSDS tended to be lower in children than in adults (–1.7 vs. –1.1 SDS, p=0.29).

Bone age, pubertal development and body mass index

Bone age was assessed in 26 of the 28 children (two subjects of 3 and 6 months were excluded) (Supplementary Table 2). In 62% of subjects bone age was <–2 SDS for age. Bone age showed a progressive delay up to 10 years and then stabilised (Figure 3). Delayed bone age was more evident in children older than 5 years (2.9 ± 1.1 years, n=18) than in younger children (0.45 ± 026, n=8, p=0.001, unpaired test).

Tanner stages were assessed in all five female teenagers above 9 years of age and in three out of five males (Supplementary Table 2). Breast stage and pubic hair were at Tanner stage 1 in girls of 10.8, 10.9, 11.3 and 14.3 years, and at stage 2 in a 14.2 years old girl, suggesting delayed puberty. Tanner stage 1 was observed in two nine year old boys and stage 2 in one 12.1 year old boy; it was not assessed in two other 12–13 year old boys.

Body mass index (BMI) and percentages of under- and overweight subjects are presented in Table 1. Mean BMI SDS was approximately –1 SDS for all groups [30].

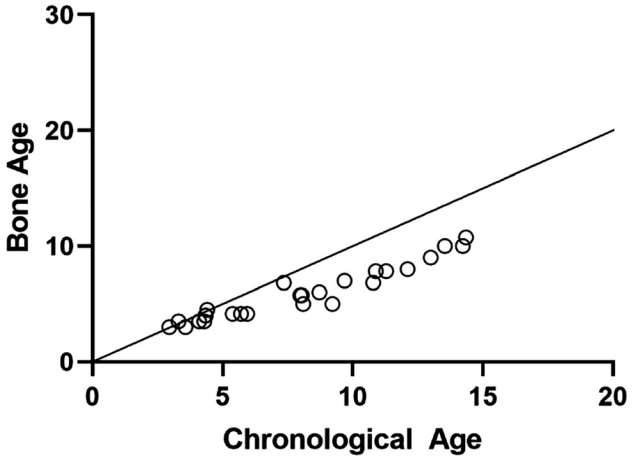

Figure 3: Bone age vs. chronological age in P/N subjects aged <18 years.

Haematological and biochemical findings

Haematological data showed no significant differences between the three adult groups (Table 3). Less than 10% of adult subjects were anaemic, compared to 50% in children. No between-group differences were observed for serum albumin, calcium or 25-OH vitamin D level. Low serum calcium was observed in one subject (P/N). No vitamin D deficiency (<10 ng/mL) was observed, but vitamin D insufficiency (10–30 ng/mL) was found in ≈60%, without statistically significant between-group differences.

Serum IGF-I, IGFBP-3 and IGF-I/IGFBP-3 ratio

Adults

For adults, mean circulating IGF-I in P/P and P/N was –1.1 and –1.2 SDS, not significantly lower than in N/N (–0.7 SDS) (Table 1). IGF-I in ng/mL was lowest in P/P compared with the other two groups (Table 2), but this could be explained by an effect of age. Serum IGFBP-3 SDS showed a similar tendency as observed for HtSDS, with a gradual increase from P/P to P/N and N/N, though not statistically significantly (p=0.39). IGF-I/IGFBP-3 ratio SDS was close to zero in all groups. There was no statistically significant correlation between height SDS vs. serum IGF-I or IGF-I/IGFBP-3 ratio SDS (data not shown).

Children

Children (all P/N) had a significantly lower mean IGF-I SDS for age than P/N adults (–2.6 vs. –1.2, p<0.0001), a tendency to a higher IGFBP-3 SDS (–0.9 vs. –1.5, p=0.18) and much lower IGF-I/IGFBP-3 SDS (–2.9 vs. 0.2, p≤0.0001) (Table 1). Individual data for the two measurements and their ratio as SDS for age, bone age and pubertal stage are shown in Supplementary Table 2. Mean IGF-I SDS for age and bone age were –2.6 and –2.2, respectively. IGF-I SDS for age in children <9 years was –2.7 (0.9) and IGF-I SDS for pubertal status in children of ≥9 years mean (SD) was –1.7 (0.7) (assuming Tanner stage 2 in the two undocumented teenage boys) compared with –2.4 (0.5) for age. Mean (SD) IGFBP-3 SDS for age and bone age was –0.9 (1.2) and –0.4 (1.1), respectively. Mean (SD) IGFBP-3 SDS for age in children <9 years was –0.5 (1.2) and for pubertal stage in children of ≥9 years –1.2 (0.8). Mean (SD) IGF-I/IGFBP-3 SDS was –2.9 (1.0) for age and –2.6 (1.3) SDS for bone age.

Scatterplots of serum IGF-I, IGFBP-3 and IGF-I/IGFBP-3 ratio SDS by age are shown in Figure 2b–d. While serum IGF-I SDS was very low in children below 5 years of age, it plateaued at about –2 SDS in later childhood and adolescence. IGFBP-3 SDS was normal in infancy followed by a steady and statistically significant decrease. Correspondingly, IGF-I/IGFBP-3 SDS was extremely low in the first 5 years of life, followed by a steady increase in later childhood and adolescence and a normal mean value in adulthood. There was no statistically significant correlation between height SDS vs. serum IGF-I or IGF-I/IGFBP-3 ratio SDS (data not shown).

Discussion

This is the first detailed report on the anthropometric, haematological, biochemical and endocrine status in the small pygmoid population living in the village of Rampasasa. In P/P, mean (SD) male height was 144.7 (2.0) cm, only 5.4 cm taller than female height [139.3 (2.6) cm], in contrast to a difference of 9.1 and 10.9 cm in P/N and N/N, respectively. Similarly low differences between male and female adult height have been observed in African pygmies [42, 43]. We speculate that this may be associated by a delayed puberty in females, as we demonstrated for female P/N adolescents, resulting in low circulating oestrogen concentrations for a longer time period. The concomitant delay of skeletal maturation would be expected to allow for more pubertal height gain. A similar effect of sex on pubertal height gain was observed in a study on the effect of delaying puberty with a GnRH analogue in combination with growth hormone, which only had a positive effect in girls [44].

There is an apparently progressive admixture between members of the five pygmoid families and individuals from

Table 3: Mean (SD) findings on haematological and biochemistry parameters for adults in the three groups (P/N, P/N and N/N) and children (all P/N).

Parameter	Adults			Children	Total	p-Value (Adults)
	P/P	P/N	N/N	P/N		
n	8	12	10	28	58	
RBC, 10^{12}/L[a]	4.56 (0.50)	4.75 (0.50)	4.67 (0.32)	4.37 (0.46)	4.52 (0.47)	0.69[d]
MCV, %[a]	86.45 (4.22)	86.04 (4.45)	83.72 (6.38)	78.11 (4.51)	81.76 (6.00)	0.59[e]
MCH, %[a]	29.68 (1.75)	29.55 (2.22)	28.82 (2.38)	26.88 (1.76)	28.12 (2.29)	0.77[e]
MCHC, %[a]	34.31 (0.53)	34.35 (0.88)	34.40 (0.84)	34.40 (0.61)	34.37 (0.68)	0.80[e]
Platelets, 10^9/L[a]	246.4 (60.99)	259.7 (66.49)	263.1 (59.88)	361.86 (77.36)	309.43 (86.95)	0.85[d]
Hb, g/dL[a]	13.45 (0.94)	14.09 (2.04)	13.42 (1.06)	11.70 (1.09)	12.70 (1.64)	0.12[e]
n (%) anaemia[a,b]	0 (0%)	1 (9%)	0 (0%)	10 (35.7%)	11 (18%)	
WBC, 10^9/L[a]	7.74 (1.29)	6.74 (1.22)	7.79 (2.49)	11.63 (3.34)	9.49 (3.41)	0.33[d]
HCT, %[a]	39.31 (2.92)	40.96 (5.34)	39.08 (3.27)	34.08 (3.23)	36.98 (4.68)	0.18[e]
Calcium, ng/dL	9.3 (0.3)	9.3 (0.35)	9.3 (0.5)	9.5 (0.2)	9.4 (0.3)	0.99[d]
Albumin, g/dL	4.44 (0.22)	4.46 (0.41)	4.48 (0.50)	4.38 (0.20)		0.97[d]
Vitamin D, ng/mL	28.5 (6.0)	30.2 (8.3)	29.1 (3.6)	29.6 (6.9)	29.5 (6.5)	0.84[d]
n (%) vitamin D insufficiency[c]	5 (62.5%)	7 (58%)	5 (60%)	17 (61%)	34 (59%)	

RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; Hb, haemoglobin; WBC, white blood cells; HCT, haematocrits. [a]Subjects who were pregnant (one from the P/N group and one from the N/N group) were excluded from haematological parameter analysis. [b]Anaemia is defined as haemoglobin below 11.5 g/dL. [c]Vitamin D insufficiency is defined as vitamin D levels of 10–30 ng/mL. [d]One-way ANOVA test. [e]Kruskal-Wallis test.

neighbouring villages. P/N adults had a mean height, SH and HCSDS about halfway those of P/P and N/N groups, as previously observed for height in other pygmoid populations [4, 8, 9, 15, 45]. However, if height SDS was adjusted for potential age-associated shrinking, this pattern was only seen in males, while P/P and P/N females had a similar adjusted height SDS. Mean body proportions as assessed by SH/Ht ratio and arm span/height ratio were normal in all adult groups and slightly increased in children. Mean IGF-I SDS was similar in P/P and P/N groups (close to –1.0), and not significantly lower than in N/N (–0.7). In contrast, mean serum IGFBP-3 SDS was close the lower limit of the reference range in P/P and showed a similar (though not statistically significant) between-group gradient as HtSDS, SHSDS and HCSDS. IGF-I/IGFBP-3 ratio SDS was normal in the three adult groups.

Regarding the low IGF-I concentrations presented in previous reports [12, 16–20] (for review, see [13]) it is noteworthy that these were not adjusted for age and sex. Furthermore, in the interpretation of serum IGF-I in subjects living in low-middle income countries, one has to consider that in such countries BMI is substantially lower than in western countries [31], which may explain the relatively low mean serum IGF-I SDS (–0.7) in N/N individuals in our study.

The gradient of height, SH and HCSDS among the three adult groups, as well as the absence of differences in BMI and haematological and biochemical parameters, is consistent with the hypothesis that the short stature in the adult P/P and P/N groups is caused by one or more genetic variants with a gene-dose effect, a dominant inheritance or epigenetic variant. Interestingly, a similar gradient was observed in serum IGFBP-3 SDS. A decreased serum IGFBP-3 has previously been reported in pygmy children in the Ituri Forest in Congo [46]. The proportionate short stature, relative macrocephaly and bone age retardation are consistent with a downregulation of the GH-IGF-I axis in childhood, with an apparent discrepancy with the observations of a normal IGF-I/IGFBP-3 ratio in adulthood. The absence of a statistical difference of serum IGF-I SDS between groups (also observed in three previous studies [15, 25, 26]) and equal IGF-I/IGFBP-3 ratio argues against a direct role of IGF-I deficiency in adults.

Children in the village were all offspring of a P/P or P/N parent and N/N parent, so that no comparisons could be made with children belonging to the other two groups. The limited data on birth weight suggest that the growth failure started prenatally, as observed in various pygmy tribes [13, 47, 48]. The cross-sectional design of our study did not allow for any data on the shape of the adolescent growth spurt, so that previous speculations on a weak or absent growth spurt in Baka or Aka pygmies [43, 49] could not be tested.

In our study, 96.4% children were short compared to CDC references [30]. The only exception was a three months' old infant with a length of 57 cm (–1.7 SDS). As

illustrated in Figure 2a, HtSDS significantly decreased with age in children and adolescents, and adult HtSDS was 0.8 SDS taller than HtSDS in children and adolescents. Such phenomenon is compatible with delayed bone age and pubertal development. The available data on Tanner stages in female adolescents suggest indeed that pubertal delay is common in girls in Rampasasa, and clearly different from population data even if adjusted for BMI SDS (a mean menarcheal age 12.4 years for a BMI of −0.9 SDS) [40]. Delayed bone age has been observed in several pygmy populations [6, 13, 43, 48].

It is tempting to analyse the cross-sectionally collected data of subjects in the P/N group in a longitudinal fashion. If doing so, there appears to be change with age in P/N individuals with regards to height, bone age and endocrine parameters. While HtSDS progressively decreases and bone age delay increases from birth to 10 years, IGF-I SDS for age is very low in the first 5 years and subsequently stabilises. Female adolescents have a delayed puberty, and when IGF-I is expressed as SDS for bone age or Tanner stage in both sexes, there is slight increase by age. Height SDS of P/N adults is higher than that of P/N adolescents, compatible with a late pubertal growth spurt, and they have a serum IGF-I similar to N/N subjects.

In contrast, serum IGFBP-3 SDS in young children is normal and decreases with age to approximately −1.5 SDS, and remains so in adulthood. As a result, the IGF-I/IGFBP-3 ratio SDS in infants and toddlers is extremely low, subsequently increases to −2 SDS in adolescence, and reaches the mean of the reference population in adulthood. These observations suggest that there is a disbalance between hepatic IGF-I vs. IGFBP-3 secretion in young children, which is apparently restored in adults. Such discrepancy between serum IGF-I and IGFBP-3 restricted to childhood and adolescence cannot be explained by classical growth hormone deficiency or insensitivity. We speculate that the low IGF-I/IGFBP-3 ratio SDS in childhood and adolescence plays a causative role in the progressive growth failure and delayed skeletal and pubertal maturation in P/N children, but acknowledge that further studies are needed to confirm this.

In a recent study on the evolutionary history and adaptation of the pygmoid population in Rampasasa, their genomes revealed a complex history of admixture with Denisovans and Neanderthals, but no evidence for gene flow with other archaic hominins [29]. The authors also noticed that the genomes bear the signatures of recent positive selection encompassing the FADS (fatty acid desaturase) gene cluster, likely related to diet, and polygenic selection acting on standing variation that contributed to their short stature phenotype [29]. Also in other pygmoid populations such increased percentage of archaic introgression has been documented [50, 51]. In this context, it is noteworthy to refer to the paper on Mapping Human Genetic Diversity in Asia [52] which showed that genetically, people from Manggarai (the regency in East Nusa Tenggara where Rampasasa is located) have a high genetic similarity to Melanesians (e.g. people in Papua).

Conclusion

P/P and P/N individuals are proportionally short. The gradient of HtSDS, SHSDS and HCSDS from P/P to P/N and N/N adults is consistent with a genetic or epigenetic effect. Indicators of nutritional status (BMI, serum albumin, calcium and vitamin D) were not different from N/N controls, which make an environmental cause of short stature unlikely. The low serum vitamin D concentrations are in line with a previous report on South East Asian Nutrition Surveys on South Asian populations including Indonesia [53]. All children included in our study belong to the P/N group and show a so far unique and unexplained age-dependent pattern of progressive growth failure and bone age delay, increasing serum IGF-I SDS from −4 to −2 SDS in contrast with a decreasing IGFBP-3 SDS from 0 to −1.5 SDS. We speculate that the initially extremely low IGF-I/IGFBP-3 SDS plays a role in the growth pattern of P/N children, and is suggestive for a novel kind of transient dysregulation of the GH-IGF-I axis in childhood. The increasing percentage of admixture with people from neighbouring villages is expected to lead to a less typical phenotype in the future, as noticed in several African pygmy populations [54].

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Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' institutional review board (Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo Hospital) or equivalent committee (Approval Letter no. 472/PT02.FK/ETIK/2012).

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