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ORIGINAL ARTICLE

Muscle morphology and architecture of the medial gastrocnemius between typically developing children with different ancestral background

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Abstract

Muscle ultrasonography is frequently used to improve the understanding of musculoskeletal impairments in children with spastic cerebral palsy (SCP). So far, most studies on muscle morphology and architecture have included typically developing children and children with SCP with similar ancestry, being mainly Caucasian. Less is known about differences in muscle morphology between children with different ancestral backgrounds. Therefore, the aim of this study was to compare muscle morphology and architecture of the medial gastrocnemius in typically developing children with African, South Asian and Southeast Asian descent from Suriname. This explorative cohort study identified children as Maroon (Ghana, African descent), Hindustani (India, South Asian) or Javanese (Indonesia, Southeast Asian), aged 5–10 years. Using 3D freehand ultrasound with the subject prone, the following medial gastrocnemius parameters were defined: muscle tendon unit (MTU) length, muscle belly length, tendon length, muscle volume, muscle thickness, anatomical cross-sectional area (ACSA), fascicle length, pennation angle, and physiological cross-sectional area (PCSA). In addition, differences between ancestral groups were assessed for the length of the MTU, muscle, tendon and fascicles in two passive stretch conditions corresponding to an externally applied joint torque of 1Nm and 4Nm. One-way ANOVA with post hoc *t*-tests were used to investigate differences between the ancestral groups. In total, 100 Hindustani ($n=34$), Javanese ($n=34$) and Maroon ($n=32$) children were included. For statistical analyses, we matched the children by age, which resulted in groups of 25 children per ancestral group ($n=75$). There were no differences found in MTU length, muscle belly length, ACSA, PCSA and muscle volume. Tendon length, fascicle length and pennation angle were different between ancestral groups. Compared to Javanese children, tendon length was longer ($p=0.001$) and pennation angle ($p=0.001$) was larger in Maroon children and fascicle length was shorter in both Maroon and Hindustani children ($p<0.001$). While there was a difference found in MTU length at different conditions of passive stretch between ancestries, no differences were found in the muscle, tendon and fascicles. This is the first study that investigated macroscopic morphological and architectural parameters for the medial gastrocnemius in one extended cohort of typically developing children, stratified in three ancestral subgroups. The current results imply that ancestry-specific reference

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data for children are needed, especially for tendon length, fascicle length and pennation angle when investigating altered muscle morphology in neurological or neuromuscular pathologies, such as SCP. Future studies should report the ancestral background when describing muscle morphology and architecture of children and ancestral specifications should be included in normative databases.

KEYWORDS
ancestry, architecture, children, morphology, muscle

1 | INTRODUCTION

Muscle ultrasonography is increasingly used to improve the understanding of musculoskeletal impairments in children with spastic Cerebral Palsy (SCP) (Williams et al., 2021). During growth, adaptations in skeletal muscles, namely an increase in muscle length and anatomical cross-sectional area (ACSA), resulting in increases muscle volume, occur in both typically developing (TD) children and children with SCP (Bell et al., 2021; Benard et al., 2011; Handsfield et al., 2022; Modlesky & Zhang, 2019). However, these increases are lower in children with SCP compared to TD children (Barber et al., 2016), while tendons are found to be longer (Barrett & Lichtwark, 2010). For a good understanding of the musculoskeletal impairments in children with SCP, proper normative reference data of muscle morphology in TD children are important.

The current studies investigating muscle morphology of the medial gastrocnemius in TD children have been published in North America, Europe, Australia, two in Asia and one in Africa (Kunimasa et al., 2022; Williams et al., 2021). Although, the literature describes high variability of gastrocnemius morphology in adults from different populations (Butler & Dominy, 2016; Refsdal, 2017; Sano et al., 2013), less is known about the differences in muscle morphology of TD children with different ancestral backgrounds. To the best of our knowledge, only one study described differences in medial gastrocnemius morphology in children with different ancestry (Kunimasa et al., 2022). Kunimasa et al. (2022) reported smaller muscle thickness, shorter fascicle lengths, longer tendon lengths in Kenyan (East-African) compared to Japanese (East-Asian) children, whereas pennation angles did not differ (Kunimasa et al., 2022). Most of these morphological differences were already observed at an age of 4 years. Due to the limited number of studies on ancestry-specific paediatric muscle morphology, there is a need for more data on muscle morphology parameters of TD children with different ancestral backgrounds.

Morphological differences may also suggest that the tissue properties (e.g., compliance) of the medial gastrocnemius muscle and tendon differ between children with different ancestries. For example, muscles with a greater physiological cross-sectional area (PCSA) and thereby more fibres in parallel with a higher amount of connective tissue (Wang et al., 1991) present a higher resistance during muscle lengthening than muscles with smaller PCSA (Kruse et al., 2021). Previous literature has suggested that medial gastrocnemius muscle and tendon length at different passively applied ankle joint torques are different between SCP and TD children (Kalkman et al., 2018a; Weide et al., 2020). Therefore, an assessment, that combines relevant medial gastrocnemius morphological and architectural parameters assessed at different externally applied joint torques, can identify potential differences between ancestral groups and help to understand altered muscle properties in children with SCP.

The scarce results reported on ancestry-specific muscle morphology and architectural parameters question the validity of pooling muscle morphological and architectural data of children with different ancestral backgrounds and the eventual need for an ancestral-specific reference database. This is especially relevant for regions with a population including different ancestral backgrounds, such as Suriname, where the research on children with SCP inherently includes children of mainly Indian (South Asian), Ghanaian (West-African) and Indonesian (Southeast Asian) descent. It is crucial to know the differences between children from different ancestral populations, before comparing muscle morphology and architecture of SCP and TD children with ancestries other than already described in literature.

Therefore, the aim of this explorative cohort study was to compare the muscle morphology and architecture of the medial gastrocnemius of a large group of Surinamese TD children from African, South Asian and Southeast Asian descent, from 5 to 10 years old. The ancestral-specific morphological features for TD children were delineated and the comprehensive reference paediatric database was made available online to be used for future studies (Walhain et al., 2023).

2 | METHOD

2.1 | Study design and participants

This exploratory cohort study included TD children aged 5 to 10 years old. Children were included if the ancestral background of the child was identified as Maroon (Ghana, African descent), Hindustani (India, South Asian) or Javanese (Indonesia, Southeast Asian) if at least three of the grandparents had Javanese, Hindustani or Maroon background. We aimed for an equal age and gender distribution per ancestral group. Questionnaires were handed out to the parents of children to inquire about the ancestral

background of the grandparents, history of neurological or developmental disorders and frequency of weekly sport activities of the child. Children were excluded in case of (1) mixed ancestral background, (2) having a history of neurological problems or developmental disorders, (3) previous lower leg musculoskeletal injuries, such as fractures or (4) doing organised sports three times or more in a week.

Ethical approval was given by the Medical Ethical Committee of the Ministry of Health in Suriname (reference VG 003-17). Approval to perform the measurements at schools was given by the Ministry of Education, Science and Culture (NB/mz/Ag. 5653). Written informed consent was obtained from the parent/guardian of all children as well as the children's verbal consent before inclusion.

2.2 | Recruitment and sample size determination

Recruitment and measurements of the children were performed from March 2021 to April 2022. Children were recruited via primary schools ($n=93$) and via family and friends of co-workers of the Faculty of Medical Sciences of the Anton de Kom University of Suriname ($n=7$). All children were living in the capital city of Suriname, Paramaribo.

For sample size calculation, the effect size was estimated using the available literature data on differences in muscle mass, measured by dual-energy X-ray absorptiometry, in children with different ancestral backgrounds from the United States of America (effect size: 0.94 for girls and 0.41 for boys (Silva et al., 2010)) and the available literature data for the differences in tendon length among adults (effect size: 0.80 (Kunimasa et al., 2014) and 1.30 (Refsdal, 2017)). These previous studies suggest an averaged large effect size of 0.86. Based on a sample size calculation (independent *t*-test–power 0.80, alpha 0.0167 (corrected for 3 groups), effect size 0.86, G-power version 3.1.9.6) each ancestral group should include 30 children. To prevent bias caused by the fact that not the same number of children was recruited for all ages in each ancestral group, we matched the children by age for further analysis.

2.3 | Data collection and processing

Assessments took place at five primary schools or the Human Motion Laboratory at the Faculty of Medical Sciences of the Anton de Kom University in Suriname. The left or right leg was randomly selected by use of a coin toss. The duration of the measurement session lasted 45 min per child. All data were collected and processed by one assessor (first author, FW), who was trained and experienced in conducting clinical assessments and had 5 years of experience in performing ultrasound measurements, and was assisted by at least one physiotherapy student or colleague. During the data processing, files were anonymised for ancestry to ensure that FW was blinded for the ancestral background of the child.

2.4 | Anthropometric measurements

Anthropometric data were collected before the ultrasound assessment including measurement of body height, body weight and lower leg length. Body height was measured with a SECA 213 stadiometer (recorded in centimetres) and body weight was defined using a calibrated SECA 762 mechanical personal scale (Medical Measuring Systems and Scales, Chino, CA, USA) rounded up to the nearest 0.5 kg. Lower leg length was measured in supine with the knee in full extension by use of a measuring tape (recorded in millimetres) and was defined as the distance between the lateral epicondyle of the femur and the proximal tip of the lateral malleolus of the fibula (Baumbach et al., 2014).

2.5 | Muscle morphology and architecture assessments

2.5.1 | Measurement set up

Two sets of measurements were performed. The first set of measurements was to investigate the morphological and architectural parameters for the different ancestral groups. The second set of measurements was performed to investigate differences between ancestral groups in the length of the MTU, muscle, tendon and fascicles at different conditions of passive stretch by applying an external torque to the ankle joint. The measurement set-up is shown in Figure 1a.

2.5.2 | Comparison of the morphological and architectural parameters

The muscle morphological parameters were measured in prone position. Children were instructed to relax while reading a book or watching a video during the collection of muscle morphological data. The lower leg was placed on a triangular pillow that was adjustable to support different lower leg lengths, resulting in 30 degrees of knee flexion during all measurement conditions. This 30 degrees of knee flexion was chosen so that this method could also eventually be used in children with SCP who have limited knee extension and/or severe gastrocnemius contractures that would limit ankle motion in case of full knee extension. Since the data in this study were also acquired as a paediatric reference database for future studies in pathological groups, we chose to assess the parameters in an ankle position wherein the foot was hanging freely at the end of the cushion when the child was fully relaxed. This was in line with the majority of studies performed in SCP and TD children (Williams et al., 2021). In most children, this position corresponded to 10–15 degrees of plantar flexion. In this first set of measurements, all parameters such as MTU length, muscle belly length, tendon length, fascicle length, pennation angle, ACSA and PCSA were acquired. In case of body segment movements or muscle contraction of the participant, the acquisition was repeated.

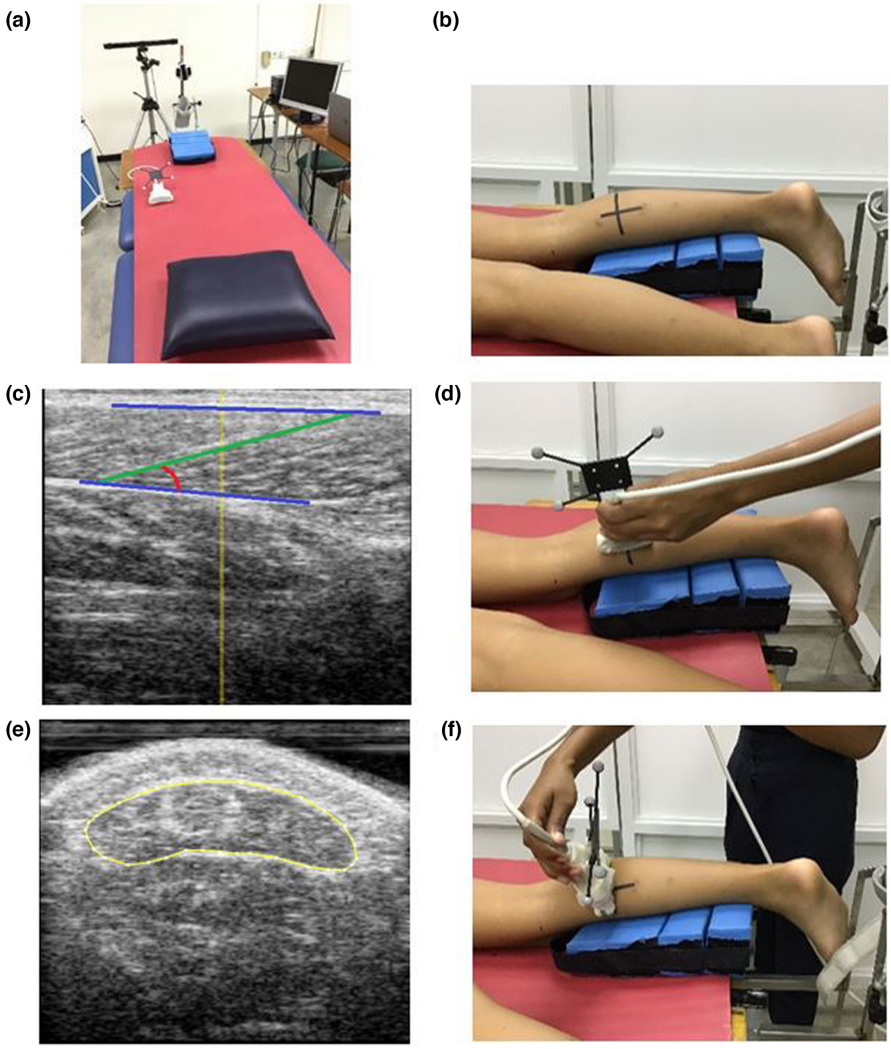


FIGURE 1 These figures show the 3D freehand ultrasound measurement set-up (a) and standardised position of the transducer for 2D ultrasound according to the longitudinal and transverse line (b). The position of the transducer on the longitudinal line (d) was to acquire the fascicle length and pennation angle and on the transverse line (f) to acquire ACSA and muscle thickness. The ultrasound images (c,d) show how fascicle length (green line, (c)) and pennation angle (red angle, (c) and anatomical cross-sectional area (yellow shape (e)) and muscle thickness (e) were acquirend.

2.5.2.1 | *2D ultrasound*

A conventional B-mode 2D ultrasound (Telemed EchoBlaster128, Vilnius, Lithuania) was used for all ultrasound measurements. The system included an echo blaster with 128 beamformer (cext-1z REV:C, serial: 2311-160818-0320) and a linear transducer with a 55 mm field of view (HL9.0/60/128Z-2, scanning frequency: 5.0–12.0 Mhz, serial: 31027-160823-0011). Data collection was performed using STRADWIN software with a sample frequency of 30hz. For standardisation of the 2D ultrasound measurements, two lines were drawn on the leg of the participant (Figure 1b). One line was drawn transverse to the leg at 50 percent of the muscle belly length that was measured from the medial epicondyle of the femur to the distal muscle tendon junction of the medial gastrocnemius. Another longitudinal line was drawn perpendicular to the transverse line in the midsagittal plane of the medial gastrocnemius. The crossing point of the transverse and longitudinal line was used for standardised placement of the transducer for the 2D ultrasound measurements that were acquired to define fascicle length and pennation angle (along the longitudinal line, Figure 1d) and ACSA (along the transverse line, Figure 1f). For the 2D measurements, ultrasound images were acquired while the transducer was tilted back and forth with the transducer in contact with de drawn line (close to the axis of rotation). From this series of images, the image wherein the probe was placed most perpendicular to the muscle, defined as the image wherein the deeper aponeuroses was most clearly visible and was not shown as a blurry or vague line, was used for further analysis of these parameters.

Fascicle length and pennation angle. Fascicle length (in cm) was defined as the length of a linear line parallel with the fibres, between the deep and superficial aponeuroses, crossing the midline of the ultrasound image. The pennation angle (in degrees) was defined

as the angle between the fascicle and the deeper aponeuroses. Both, fascicle length and pennation angle, were extracted from the same image and analysed with a custom-made MATLAB software (Mathworks, R2017b) (Figure 1c).

Anatomical cross-sectional area (ACSA) and muscle thickness. The ACSA and muscle thickness were measured in a transverse plane perpendicular to the length of the muscle. The ACSA was calculated by manually outlining the borders of the medial gastrocnemius using STRADWIN software (version 6.0; Mechanical Engineering, Cambridge University, Cambridge, UK) and was recorded in cm^2 (Figure 1e). In case the ACSA was not fully visible in the dimensions of the ultrasound image, the ACSA was extracted from the combination of two ultrasound images that overlap each other to visualise the borders of the ACSA. The muscle thickness was extracted in the midline of the ultrasound image and analysed with a custom-made MATLAB software.

2.5.2.2 | *3D freehand ultrasound*

For the 3D freehand ultrasound measurement, the ultrasound system was combined with an OptiTrack V120: Trio camera system with three cameras and a 1 mm spatial resolution (serial: #330030) to track four passive markers that were attached to the transducer. This method was shown to be reliable and valid to measure muscle volume, muscle belly length, tendon length and muscle-tendon unit (MTU) length (Cenni et al., 2016). An Aquaflex ultrasound gel pad placed in a concave-shaped portico was used for optimal contact and signal transmission between the skin and the transducer of the ultrasound (Cenni et al., 2018). Cenni et al. (2018) demonstrated that the use of a portico and gel pad is a more reliable and valid method to measure muscle volume compared to the approach with direct contact of the transducer on the skin (Cenni et al., 2018). The depth of the curve of the gel pad depended on the size of the participant's lower leg and Ultrasound Aquaflex gel was used to attach the gel pad to the transducer. The same gel was used as a conductive medium between the skin and gel pad. For muscle volume, muscle belly length, tendon length and MTU length, acquisitions of the medial gastrocnemius were taken starting from just above the femoral condyles to the distal end of the calcaneus. The ultrasound transducer was moved in a transverse position perpendicularly to the surface of the lower leg at an almost constant velocity (1 cm/s), while 30 ultrasound images per second were recorded. A multiple sweep was made, which is a reliable technique that combines the acquisitions of two separate acquisitions to capture the whole muscle, in case the borders of the muscle were not fully visible in the dimension of the ultrasound image (Budzikowski & Murray, 2023). The 3D reconstructions of the muscle and tendon, as well processing of the data, were performed with the open-source STRADWIN software.

Muscle, tendon and MTU length. Muscle belly length of the medial gastrocnemius was defined as the distance between the most superficial point of the medial femoral condyle and the first image where no muscle tissue of the medial gastrocnemius was visible, further labelled as the muscle tendon junction (MTJ). The distal tendon of the medial gastrocnemius, further referred to as tendon length, was defined from MTJ to the most proximal point on the calcaneus representing the tendon insertion. MTU length was recorded as the sum of the muscle and tendon length.

Muscle volume. Muscle volume was calculated by manually outlining the borders of the medial gastrocnemius starting at the medial condyle of the femur, and ending at the MTJ. Muscle segmentations were made each centimetre and an automatic cubic planimetry interpolation was applied to compute the muscle volume (in mL) (Treece et al., 1999).

Physiological cross-sectional area (PCSA). PCSA was calculated similar to other studies by the following equation (Barber et al., 2011; Herskind et al., 2016);

$$\text{PCSA} = \frac{\text{muscle volume} * (\text{cosinus}(\text{pennation angle}))}{\text{fascicle length}}$$

2.5.3 | Comparison of the length changes in morphological and architectural parameters during passive stretch

In the second set of measurements, the length of the medial gastrocnemius MTU, tendon, muscle and fascicles at different conditions of passive stretch were assessed by applying an external torque to the ankle joint. While the child was still positioned as during the first set of measurements, the foot of the child was placed in an orthosis of a custom-made set-up. Three different sizes (small, medium, large) of orthoses were available to ensure that the rotation axis of the set-up could be visually aligned with the axis through the malleoli (red dot, Figure 2a). A baseline measurement (i.e., 0 Nm) was performed, what corresponded to 10–15 degrees of plantar flexion in the majority of the children. A dynamometer (MicroFed2, Hogan Health Industries, Inc. 8020 South 1300 West, West Jordan, USA) was attached by an iron bar to the footplate of the orthosis and the ankle was passively moved to dorsiflexion. The amount of force (green arrow in picture Figure 2a) that was applied by the investigator on the dynamometer was calculated by dividing the desired torque by the moment-arm (yellow line, Figure 2a) of the dynamometer with respect to the ankle joint (red dot, Figure 2a). When the desired ankle torque was achieved (i.e., 0 Nm, 1 Nm, or 4 Nm), the axis of the custom-made set-up was fixated to that certain position, and the muscle morphology measurements for the MTU, muscle, tendon and fascicle length were performed in this static position (Figure 2b–d). The 1 Nm condition was chosen as a condition in between baseline and 4 Nm, wherein the 4 Nm condition was similar to the ankle torque applied by Weide et al. (2020) and was in most cases the maximal tolerated dorsiflexion angle.

2.6 | Statistical analysis

Statistical analysis was performed in Jeffreys's Amazing Statistics Program (JASP) version 0.14.1. The Pearson's Chi-Squared test was

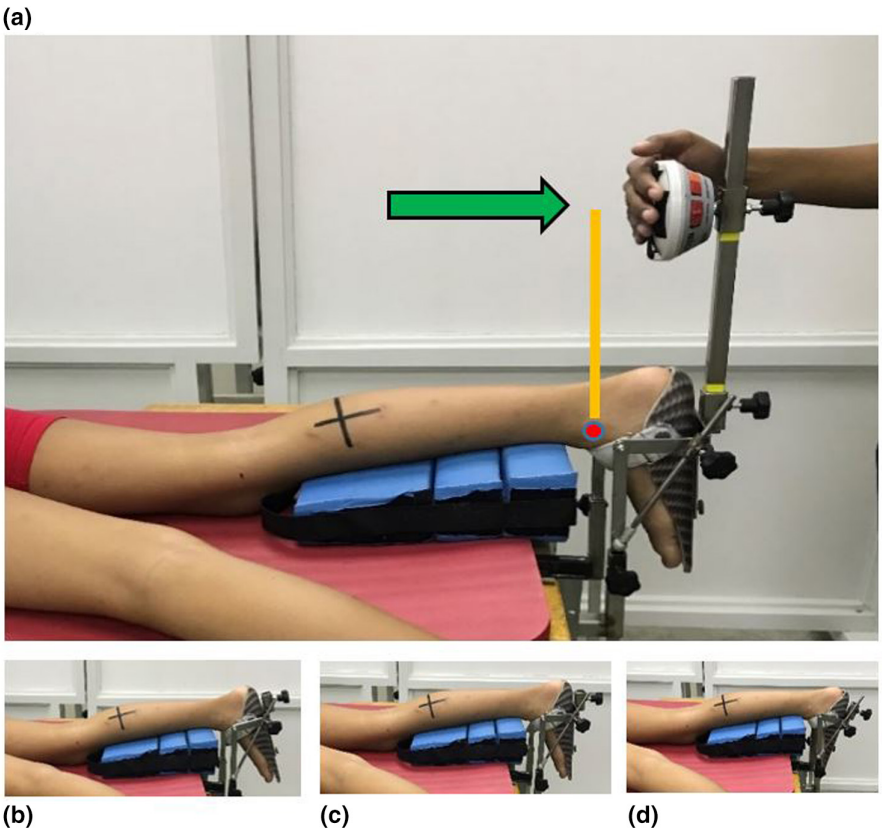


FIGURE 2 The set-up for fixation of the ankle joint for applying a torque on the ankle joint (a), to assess the length changes of the medial gastrocnemius MTU, tendon, muscle and fascicles during passive stretch, from baseline (0 Nm, (b) to 1 Nm (c) and 4 Nm (d)). Nm, Newton metre.

performed to evaluate the equal distribution of boys and girls between ancestral groups. Normal distribution of the data were assessed with the Shapiro–Wilk test and visual inspection of the plots for each ancestral group and each individual parameter. The data were described by means and standard deviation (SD) in case they were normally distributed, or by medians and interquartile ranges (IQR) in case they were not normally distributed. When data were normally distributed, the one-way ANOVA was used to investigate differences between ancestral groups, while the Kruskal–Wallis H test was used when the data of one of the ancestral groups was not normally distributed. If the one-way ANOVA or Kruskal–Wallis H test was significant, a post hoc *t*-test or Mann–Whitney *U*-test was used with a Bonferroni correction for multiple testing, to define differences between the three ancestral groups. A two-way repeated measures ANOVA was used to define if there were differences in the length of the MTU, muscle, tendon and fascicles at different conditions of passive stretch (0 Nm, 1 Nm and 4 Nm) between ancestral populations. The level of significance, after correcting for multiple testing was set to $p < 0.05$.

3 | RESULTS

In total, 100 Hindustani ($n = 34$), Javanese ($n = 34$) and Maroon ($n = 32$) children were included. For statistical analyses, we matched the children by age which resulted in groups of 25 children per ancestral group ($n = 75$). Participants were equally distributed over the age-range from 5 to 10 years and Pearson's Chi-Square (X^2 (2, $n = 75$) = 0.325, $p = 0.850$) showed equal distributions of boys and girls between ancestral groups (Table S1). There was no difference between ancestral groups for age, height, weight and lower leg length (Table 1).

3.1 | Comparison of the morphological and architectural parameters

While MTU length tended to be longer in Maroon children compared to Hindustani and Javanese children, there was no significant difference between groups (Table 2). The muscle length was not different between groups, while tendon length was significantly longer (≈21%) in Maroon compared to Javanese children ($p = 0.001$). The fascicle length of Javanese children was ≈19% longer compared to Maroon and ≈9% longer compared to Hindustani children (both *p*-values <0.001). The muscle thickness, ACSA, PCSA and muscle volume were not different between groups. The pennation angle showed differences between ancestral groups with a larger pennation angle in Maroon children compared to Javanese children ($p = 0.001$). Individual data of all included children ($n = 100$) and the individual data points of the matched children are visualised in Figure 3. The median, interquartile range, mean and standard deviation for all parameters of the matched in children were provided in Table S2 (anthropometric data) and 3 (morphology and architecture), to allow comparison with other studies in literature and to be used in future studies.

TABLE 1 Group data for the anthropometric parameters of the ancestral groups.

		Hindustani ($n=25$)	Javanese ($n=25$)	Maroon ($n=25$)	
		Median (IQR)/Mean (SD)	Median (IQR)/Mean (SD)	Median (IQR)/Mean (SD)	*p*-value
⊥	Age (years)	7.81 (2.7)	7.81 (2.7)	7.51 (3.3)	0.975
⊥	Body weight (kg)	22.00 (10.5)	24.00 (16.0)	27.00 (12.0)	0.719
|	Body height (cm)	127.98 (9.8)	127.60 (14.3)	131.05 (11.5)	0.498
|	Lower leg length (cm)	31.26 (3.1)	31.22 (4.5)	32.86 (3.8)	0.231

Note: | parametric analysis: mean and standard deviation are given and *p*-values were calculated by one-way ANOVA and post hoc independent *t*-tests, ⊥ non-parametric analysis: median and interquartile range are given and *p*-values were calculated by Kruskal–Wallis H and post hoc Mann–Whitney *U*-test.

Abbreviations: IQR, interquartile range; SD, standard deviation; kg, kilogram; cm, centimetre.

TABLE 2 Group data for the morphological and architectural parameters per ancestral group.

		Hindustani ($n=25$)	Javanese ($n=25$)	Maroon ($n=25$)	
		Median (IQR)/Mean (SD)	Median (IQR)/Mean (SD)	Median (IQR)/Mean (SD)	*p*-value
|	MTU length (mm)	311.67 (30.9)	304.58 (41.2)	326.70 (38.1)	0.104
|	Muscle length (mm)	164.89 (16.5)	173.03 (19.3)	167.93 (18.2)	0.279
|	Tendon length (mm)	146.78 (19.0)	131.55 (26.2)	158.77 (30.3)	0.002*
⊥	Fascicle length (cm)	3.91 (0.7)	4.31 (0.6)	3.66 (0.6)	<0.001*†
⊥	Muscle thickness (cm)	1.24 (0.3)	1.30 (0.3)	1.20 (0.2)	0.132
⊥	ACSA (mm^2)	516.27 (238.5)	554.80 (115.8)	497.99 (125.1)	0.763
⊥	PCSA (mL/cm)	14.26 (5.0)	13.42 (7.2)	15.04 (7.1)	0.259
⊥	Volume (mL)	53.26 (29.3)	62.34 (37.2)	55.34 (20.6)	0.344
⊥	Pennation angle (degrees)	19.61 (4.3)	17.47 (4.1)	21.67 (3.9)	<0.001*

Note: Group differences ($p<0.05$) are displayed with; * for significant difference between Maroon and Javanese children; † for significant difference between Hindustani and Javanese children, | parametric analysis: mean (SD) are given and *p*-values were calculated by one-way ANOVA and post hoc independent *t*-tests, ⊥ non-parametric analysis: median (IQR) are given and *p*-values were calculated by Kruskal–Wallis H and post hoc Mann–Whitney *U*-test.

Abbreviations: ACSA, anatomical cross-sectional area; cm, centimetre; IQR, interquartile range; mL, millilitre; mm, millimetre; mm^2, millimetre squared; MTU, muscle-tendon unit; PCSA, physiological cross-sectional area; SD, standard deviation.

3.2 | Comparison of the length changes in morphological and architectural parameters during passive stretch

The repeated measures ANOVA showed significant increases in MTU, muscle, tendon and fascicle length when applying increasing torques from 0 Nm to 1 Nm and 4 Nm, with significantly decreased pennation angle (all *p*-values <0.001). There was no significant difference for the interaction with increased torque × ancestry for the muscle, tendon and fascicles ($p=0.188$; $p=0.120$ and $p=0.637$ respectively), while there was a significant interaction with increased torque × ancestry for the MTU length ($p=0.005$) (Figure 4). The absolute and percentage increase in MTU, muscle, tendon and fascicle length for 0 Nm to 4 Nm, 0 Nm to 1 Nm and 1 Nm to 4 Nm for each ancestry are given in Table S4.

4 | DISCUSSION

This study investigated the differences in the morphology and architecture of the medial gastrocnemius between children from three ancestral groups, namely Maroon (Ghana, West African), Hindustani (India, South Asian) and Javanese (Indonesia, Southeast Asian). There were no differences found in MTU length, muscle belly length, muscle thickness, ACSA, PCSA and muscle volume, while tendon length, fascicle length and pennation angle were different between ancestral groups. Compared to Javanese children, tendon length was longer and pennation angle was larger in Maroon children and fascicle length was shorter in both Maroon and Hindustani children. While there was a difference found in the length change of the whole MTU between ancestries, no differences were found in the length changes of the muscle, tendon and fascicles when different torques on the ankle joint were applied to passively stretch the medial gastrocnemius muscle. Especially the differences in muscle morphology and architecture highlight the need to take ancestry into account when comparing the medial gastrocnemius in children from different ancestries and to use ancestry-specific norm-databases when studying alterations in children with neurological or neuromuscular pathologies. This is the first study that investigated macroscopic morphological parameters for the medial gastrocnemius, as well as the length changes of the medial gastrocnemius during passive stretch in one extended cohort of typically developing children, stratified in three ancestral subgroups.

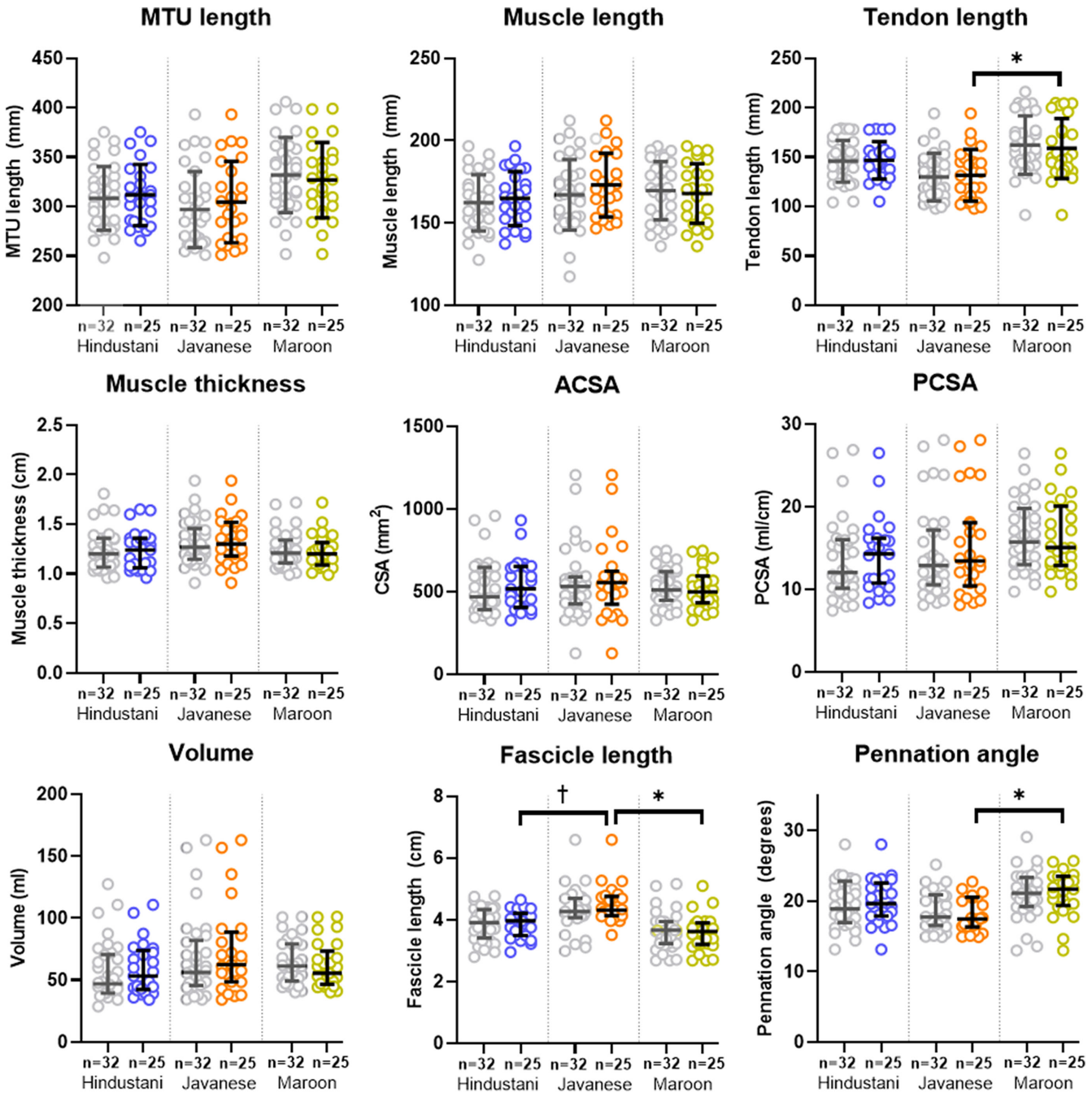


FIGURE 3 Individual data points for all children grouped by ancestry (grey) and the age-matched children (coloured) that were used for statistical analysis. Mean and standard deviation (Muscle tendon unit, muscle and tendon length) and median with interquartile range (fascicle length, anatomical cross-sectional area, physiological cross-sectional area, volume and pennation angle) are given (in black) for each ancestry. Group differences ($p < 0.05$) are displayed with; * for significant difference between Maroon and Javanese children; † for significant difference between Hindustani and Javanese children. cm, centimetre; mL, millilitre; mm, millimetre; mm^2, millimetre squared; ACSA, anatomical cross-sectional area; MTU, muscle-tendon unit; PCSA, physiological cross-sectional area.

4.1 | Comparison of the morphological and architectural parameters

This study was set up to compile an extended cohort of morphological parameters of the medial gastrocnemius in typically developing children with different ancestries. Therefore, the data of all children ($n = 100$) that were included, are reported. In this group of children ($n = 100$), the ancestral groups were not evenly distributed by age. Therefore, it appears essential to match groups based on age to interpret the results correctly. The group of age-matched children, resulted in 75 children (25 for each ancestral group) that were compared statistically. Although we matched the children with different ancestry by age, we still need to be careful in comparing absolute values for muscle belly length, tendon length and muscle

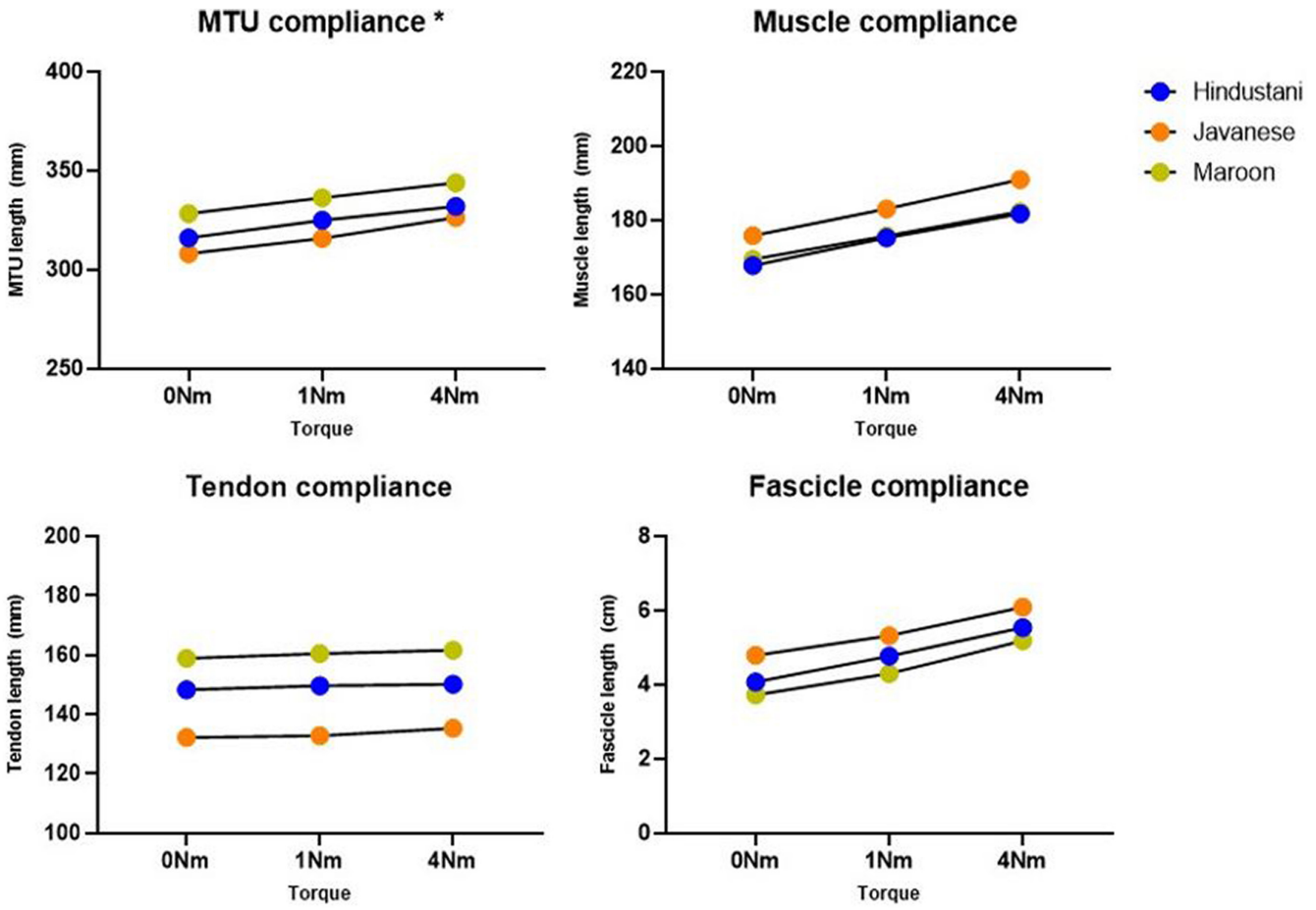


FIGURE 4 The increase in muscle-tendon unit length, muscle belly, tendon and fascicle length when an external torque was applied on the ankle joint. The mean for each ancestry is shown from 0 Nm to 1 Nm and 4 Nm. * significant interaction with increased torque × ancestry. cm, centimetre; mm, millimetre; MTU, muscle tendon unit; Nm, Newton metre.

volume since these parameters increase in growing children. Therefore, interpretations have to be made with caution, since standard deviations (and greater ICR) do increase by pooling data from children over a range of 5 to 10 years old, thereby decreasing the chance of finding differences between groups. For example, for muscle volume, no differences were found in absolute values, but if we normalise muscle volume by body weight, to adjust for growth, differences were found between Maroon and Hindustani children compared to Javanese children. Therefore, normalised muscle volume and the relative tendon length as a percentage of MTU length were added in the appendix (Table S5). Different normalisation strategies are mentioned in earlier studies, but it is unclear if these normalisation strategies could also be applied for different ancestral groups (Peeters et al., 2023; Williams et al., 2021). We want to advise other authors to take caution in the matching procedure when comparing groups of children who are inherently growing to prevent possible bias of the results.

When comparing current results with a recently published study of Kunimasa et al. (2022), where the muscle morphology and architecture of the medial gastrocnemius was assessed in children with West African and Southeast Asian ancestry, similar differences were present in the tendon length, fascicle length and pennation angle between ancestries. Kunimasa et al. (2022) reported the differences in these parameters in children with East African and East Asian ancestry, while in our study these differences were most pronounced between West African and Southeast Asian ancestry. Although the ancestry of the children in the study of Kunimasa et al. (2022) and our study were not similar to each other, both studies showed that the children with East- or West African ancestry had longer tendon lengths, shorter fascicles and a greater pennation angle compared to East-Asian or Southeast Asian ancestry.

In the current study, tendon length of the medial gastrocnemius muscle was significantly different and 21% longer in Maroon (West African) children, compared to Javanese (Southeast Asian) children. The difference in tendon length in the current study was found in children 5 to 10 years old, which is in line with the study of Kunimasa et al. (2022) where differences in tendon length were ≈20% and already found at the age of 4 years old. The current study results revealed that the difference in tendon length between South Asian and African descendants compared to Southeast Asian descendants exceeds the difference (i.e., ≈4%) found in tendon length between TD and SCP (Barrett & Lichtwark, 2010; Kruse et al., 2021). Therefore, when comparing the tendon length of a group of TD and SCP children including different ancestral groups (especially including the

Southeast Asian ancestry), group-matching needs to take ancestral-variance into account.

Fascicle length was lower for Maroon compared to Hindustani and Javanese children, while the pennation angle was greater in Maroon children compared to Javanese children. These findings were in line with the findings of Kunimasa et al. (2022), who also reported longer fascicle lengths and larger pennation angles in Kenyan (West African) children compared to Japanese (East Asian) children. Since differences in fascicle length and pennation angle were found, it was surprising that we did not identify differences in muscle thickness or muscle belly length between ancestral groups. Although, earlier studies performed in adults showed that simple muscle models cannot accurately predict the changes in shape of a muscle (Bolsterlee et al., 2017) since there is high regional variability of fascicle arrangement (Aeles et al., 2022; Takahashi et al., 2022). It remains unclear if the regional variability of fascicle length may be already present in children, and since fascicle length and pennation angle were measured at only one site of the muscle (in 2D at mid-belly length), the differences in fascicles length between ancestries need to be interpreted with caution.

In the study of Kunimasa et al. (2022), a smaller muscle thickness was found in West African compared to Southeast Asian ancestry from the age of 8 years old. While in our study muscle thickness, ACSA and muscle volume were higher in West African compared to Southeast Asian ancestry, our study showed no significant differences for these parameters. When comparing the muscle thickness for children 8 years and older ($n=36$), no significant differences were found between ancestral groups ($p=0.181$). So far, no other studies have evaluated the medial gastrocnemius volume between children with different ancestry. In Javanese children, there was a high variability for body weight (standard deviation of 11 kg compared to 8 kg in Maroon and Hindustani children) and muscle volume (standard deviation of 36 mL compared to 19 to 21 mL in Maroon and Hindustani). Since the variability of body weight may explain the variability found in muscle volume, muscle volume was normalised by body weight. Normalised muscle volume in Javanese children was higher compared to Hindustani and Maroon children ($p=0.034$ and $p=0.025$). As said before, these results showed that matching groups of children by age, but also by body weight, is essential to compare muscle morphological and architectural parameters. Matching procedures should be performed accurately and normalisation strategies should be further explored in ancestral groups.

Most of the current studies investigating muscle morphology and architecture in children with SCP compared to TD children have been published in North America, Europe, the UK, Australia and Korea (Williams et al., 2021), indicating that data have been primarily reported for Caucasian children. In Suriname, children with Caucasian (European) ancestry are scarce. Therefore, the data in the current study were compared with data from Caucasian children in Belgium, using exactly the same position of the child, equipment, method for data acquisition and data processing (Peeters et al., 2023). Data for muscle belly length, tendon length and muscle volume were extracted and additional data for muscle tendon unit length were requested. After matching the data, an age-matched group of 23 children with West-European ancestry was compared to Hindustani ($n=23$), Javanese ($n=23$) and Maroon ($n=23$) children that were included in this study. Comparison of the four different ancestry groups with a two-way ANOVA and post hoc testing showed that there was no difference in age, body height and body weight between the four ancestral groups (p-values between 0.557 and 0.988). Also, no differences were found for muscle belly length ($p=0.544$), muscle volume ($p=0.654$) and MTU length ($p=0.075$), while tendon length was significantly shorter in Caucasian compared to Maroon children ($p=0.002$). The difference in tendon length for Maroon compared to Caucasian children, may imply that caution is needed when pooling these parameters for children with West-African and West-European ancestry (besides the caution when pooling data from children with different ancestries included in this study). We advise that future studies report the ancestral background when describing muscle morphology and architecture of children and adults and that ancestral specifications are well-considered when establishing and using normative databases.

In studies that compared medial gastrocnemius morphology and architecture in adults, differences in tendon and fascicle length were also reported between ancestries (Refsdal, 2017; Sano et al., 2013). A longer tendon length was found in West-African compared to East Asian adults and for West-African compared to Caucasian adults (Refsdal, 2017; Sano et al., 2013). Also, a shorter fascicle length was found for West-African compared to Caucasian adults (Refsdal, 2017). Based on the study of Kunimasa et al. (2022) and the results of current study, differences in medial gastrocnemius morphology and architecture found in adults with different ancestry, seem to be already present in young children. Moreover, the recent published cross-sectional study, wherein different age groups were investigated, showed that differences in medial gastrocnemius morphology and architecture appeared from 4 years old and continued into adulthood (18 years old) (Kunimasa et al., 2022). This may imply that the differences between ancestries, that are already present from a young age, remain during adolescence and adulthood.

Compared to the study of Kunimasa et al. (2022), who performed assessments in a standing upright position, our data were assessed in prone with a standardised torque in a relaxed position. Positioning with no tension applied on the muscle is recommended for assessing muscle morphology and architecture between subjects (Aeles et al., 2017; Williams et al., 2021). While we do not know the exact tendon slack length for the enrolled children, previous literature (Veerkamp et al., 2022) suggested that the positions, wherein there was no passive torque applied on the joint, included tendon slack. Moreover, we aimed to investigate an extended TD database that can be used for comparison with the already published data in children with SCP. Since most of the published data in children with SCP has been acquired in resting position, we also acquired the muscle morphology and architecture in resting position. For further studies comparing muscle morphology and architecture in children with different ancestries, standardisation of methods during data acquisition need to be well-considered.

4.2 | Comparison of the length changes in morphological and architectural parameters during passive stretch

In line with other literature, the results showed a significant increase in MTU, muscle, tendon and fascicle length when applying increasing torques from 0 Nm to 1 Nm and 4 Nm, with significantly decreased pennation angle (Bolsterlee et al., 2017; Kalkman et al., 2018b). While there was a significant difference between ancestries in the MTU length (muscle and tendon) at the different conditions of passive stretch, no significant differences were found for muscle, tendon and fascicle length. Only the cumulative length of the muscle and tendon (i.e. MTU length) appears to be different between ancestries when applying a torque from 0 to 4 Nm, which may imply that the differences in muscle and tendon length separately seem too small to detect significant differences between ancestries. Morphological differences, such as a greater ACSA or PCSA may present a higher resistance during muscle lengthening (Kruse et al., 2021). While there were no significant differences in PCSA, the fascicle length and pennation angle by which PCSA was calculated, were significantly different between ancestries. In Maroon compared to Javanese children, the fascicle length was shorter and the pennation angle was higher, which may result in higher PCSA and thereby higher resistance. It may be possible that the combination of these parameters with a lower muscle volume (not significant) in Maroon compared to Javanese children, equals out the change in muscle length, resulting in similar compliance of the muscle between these two ancestries. As far as we know, no other studies investigated the length of the medial gastrocnemius muscle at different conditions of passive stretch in a cohort of TD children, stratified in three ancestral subgroups. The current study data created a unique norm-database for the change in length of the muscle belly, tendon and fascicle length that can be used as a reference when investigating these parameters at different stretch conditions in for example SCP children.

4.3 | Limitations

The focus of the current study was not to evaluate muscle functional differences by investigating morphological parameters. Nevertheless, it can be hypothesised that the found differences in muscle morphology and architecture, that seem to already be present at an early age, may imply that children with Maroon or Javanese ancestry have functional advantages such as greater force production or muscle contraction shortening velocity. The longer tendon in Maroon children may have an advantage in efficient storage of energy and recoil of elastic energy (Sano et al., 2013). The shorter fascicles and larger pennation angle in Maroon children, result in a greater PCSA that is related to a greater force production (Kruse et al., 2021). Whilst Javanese children, with longer fascicles and thereby more sarcomeres in series, may create a greater maximal velocity during contractions (Hill, 1938). We advise that further research in children with different ancestries also include functional measures to relate differences in muscle morphology and architecture to functional outcomes.

Besides ancestral differences, also nutritional and other environmental factors can influence muscle growth. Therefore, we included only children living in the capital city of Paramaribo to minimalise cultural differences between ancestral groups (e.g., believes and traditional food) and living circumstances (e.g., housing, possibility of schools, access to sports clubs). Although we do not expect great differences in these factors from 5 to 11 years old, it would be interesting to take these factors in account in further studies or studies with older children.

5 | CONCLUSION

The current study investigated the differences in the morphology and architecture of the medial gastrocnemius in children 5 to 10 years old from three ancestral groups, namely Maroon (Ghana, West African), Hindustani (India, South Asian) and Javanese (Indonesia, Southeast Asian). There were no differences in MTU length, muscle belly length, ACSA, PCSA and muscle volume, while tendon length, fascicle length and pennation angle were different between ancestral groups. While there was a significant difference between ancestries in the MTU length at the different conditions of passive stretch applied by an external torque on the ankle joint, no significant differences were found for muscle, tendon and fascicle length. Current results imply that ancestry-specific reference data for children are needed, especially for tendon length, fascicle length and pennation angle when investigating altered muscle morphology and architecture in neurological or neuromuscular pathologies, such as SCP. We recommend that future studies report the ancestral background when describing muscle morphology and architecture of children and that ancestral specifications are included in normative databases.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the Research Data Repository (RDR) of the KU Leuven at https://doi.org/10.48804/MYJQE2.

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