Gender-specific regional changes in genetic structure of muscularity in early adolescence

Loos, R., M. Thomis, H. H. Maes, G. Beunen, A. L. Claessens, C. Derom, E. Legius, R. Derom, and R. Vlietinck. Gender-specific regional changes in genetic structure of muscularity in early adolescence. J. Appl. Physiol. 82(6): 1802–1810, 1997.—Genetic and environmental influences on muscle circumference measurements of the extremities were estimated in 105 pairs of twins between 10 and 14 yr of age. Four circumferences, extended upper arm (EAC), forearm (FC), thigh (TC), and calf (CC), were measured. Univariate model fitting revealed that the largest part (87–95%) of the variance for all circumferences at most ages was explained by additive genetic factors. Sex differences were observed for some age categories. Multivariate analyses showed a different pattern evolving according to age and gender. In boys from 10 to 12 yr of age, one general genetic factor influenced all four circumferences. With increasing age, an arm-leg model emerged, one genetic factor influencing the arm and another genetic factor the leg circumferences. In young girls one genetic factor loaded on the proximal (EAC,TC) and another on the distal (FC,CC) circumferences. With subjects at age 14 yr, an arm-leg model was observed. High genetic correlations indicated that genetic factors related to EAC, FC, TC, and CC did not act independently. The age- and gender-specific changes in the genetic structure suggest pubertal influences. This study shows that muscle circumferences are highly heritable characteristics and are therefore a promising starting point at which to locate their genes. Gene mapping could validate the gender-specific change of the genetic structure with age and region.

Skeletal muscle circumferences; twins; genetic model fitting; univariate and multivariate genetic analysis; gender and age effects

Skeletal muscle size is an indicator for undernutrition, aging, and muscular and neuromuscular diseases and is important in a variety of sports disciplines (9, 10, 19). Circumferences of extended upper arm (EAC) and calf (CC) are the most commonly used sites to estimate muscularity, followed by the circumferences of the forearm (FC) and thigh (TC). Most often, they are investigated by means of anthropometric or radiographic measurement. Muscle is the major tissue composing the circumferences.

Genetic influence on extremity circumferences is suggested by family studies, most often by comparing the correlations between parents and offspring and between full and half-siblings, controlling for age, gender, and rearing conditions (1, 13–15, 20, 27). It needs to be emphasized that the comparison of results of these studies is complicated by differences in sample characteristics (type of relationship, age, gender, size) and differences in the methods used to estimate heritability.

Methods

Subjects. One hundred and five twin pairs volunteered to participate in the Leuven Longitudinal Twin Study (16). All twins were reared together and were selected from the East Flanders Prospective Twin Study (6). The parents gave informed consent, and the project was approved by the Local Committee of Medical Ethics and the Committee of the National Scientific Fund. From 1985 to 1991, 10-yr-old twin pairs entered the study, and anthropometric data were collected longitudinally for 6 yr. At present, data are nearly complete until the age of 14 yr. Table 1 gives the sample size for the five yearly data points by age, gender, and zygosity. One pair skipped age 11 yr, one dropped out at age 11 yr, another dropped out at age 12 yr, and five pairs had not yet reached age 14 yr.

Little et al. (15) found evidence for different genetic influence depending on body region. However most studies (13, 14, 20, 27) found only small differences between the correlations of upper and lower extremity circumferences. Gender differences were observed by Kaur and Singh (14), Mueller and Malina (20), and Little et al. (15).

Comparison of monozygotic (MZ) and dizygotic (DZ) twins also provides insight into the possible genetic control of the trait. Twin studies indicate strong evidence for a genetic component of circumference measurements, with a heritability generally ranging between 0.53 and 0.75 (2, 4, 5, 8). Heritability coefficients are higher for CC than for upper arm circumference in adults (4), whereas only little difference was found in schoolchildren (2). Gender differences were found by some authors (5) but not by others (8).

All these studies investigated the genetic influence of each circumference separately. Until now, apparently no study ever examined the covariation between circumferences in a multivariate way nor assessed whether the genetic structure was similar in either gender or changed over time.

The purpose of this study was to estimate the genetic and environmental contributions to the variation of skeletal muscle characteristics, as assessed by four anthropometric circumferences of the extremities. The specific questions to be answered were 1) how large are the genetic and environmental influences on each of the four circumferences? 2) are the same genes influencing the circumference in different regions? 3) how stable is this pattern over time? and 4) is the heritability the same in men and women and are the same genes expressed in men and women?

These questions were answered by univariate and multivariate model fitting.
The contribution of genetic and environmental influences to the individual differences in the circumference measurements was estimated by the method of genetic model fitting (22). With this method, the variation in the observed phenotype is decomposed into genetic and environmental variance. The genetic variance may be due to additive (A) or dominant (D) genetic influences. The environmental variance can be decomposed into common environmental factors (C), which are shared by both twins reared in the same family (e.g., diet, family attitude toward sports) and to the specific environmental factors (E), unique for each individual (e.g., subject-specific sports participation). Their influence on the phenotype is given by parameters a, d, c, and e, which are equivalent to the standardized regression coefficients of the phenotype on A, D, C, and E, respectively. The amount of variance is expressed as a proportion of the total phenotypic variance, explained by the main sources: additive ($a^2$) or dominant ($d^2$) genes and common (c²) and unique (e²) environment.

Hypotheses, based on a priori knowledge, were translated into mathematical models. Parameter values, predicted by the models, were optimized by maximum likelihood method. These values were compared with the observed data. When differences between predicted and observed data are small, it does not necessarily indicate that the hypothesis is correct but that it is accepted until an alternative model better fits the data.

In univariate analyses, the genetic and environmental influences responsible for variation in each of the four circumferences (EAC, FC, TC, CC) taken separately were identified. In the multivariate approach, the underlying genetic structure of the covariation between these four circumferences was determined, i.e., it was tested whether there is only one general genetic and/or environmental factor that contributes to the variation and covariation in the different circumference measures or whether two or three common genetic and/or environmental factors are needed. In the latter case two hypotheses were distinguished: 1) one common factor loads on proximal limbs (EAC and TC) and the other common factor on distal limbs (FC and CC), and 2) one common factor loads on the upper extremity (EAC and FC) and the other common factor on the lower extremity (TC and CC). All hypotheses tested whether additional genetic and/or environmental factors with a localized action were needed to explain the residual variance.

Univariate analysis. For each circumference measurement, correlations were computed for MZ male and female (M2M, M2F) and dizygotic male, female, and opposite-gender twin pairs (D2M, D2F, D2O). The comparison of the correlations of these five groups gave a first idea of the importance of the genetic and environmental influences. They also allowed us to compare the results of the present study with those reported in the literature.

Univariate genetic models were fitted separately to each of the four circumferences (EAC, FC, TC, CC). The goodness of fit of different models, each representing an alternative hypothesis, was tested. These models included A and/or D genetic factors and C and/or E environmental factors. The AE model assumes that variance is explained by only two factors, i.e., an additive genetic and/or a unique environmental factor. If dominance or shared environment is included, the models were referred to as ADE or ACE, respectively. It should be noted that the power of the sample is sufficient to detect additive genetic influences but may be too low to detect dominance or shared environmental effects, unless their effect accounts for >50% of the total variance. The CE and E models assume no genetic influence at all.

Gender differences can result from differences in magnitude of the genetic and/or environmental influences. This hypothesis was tested by comparing a model that equated the genetic and environmental influences for boys and girls with a model that allowed for different estimates in either gender. Neale and Cardon (22) provide a detailed description of univariate model fitting.

Multivariate analysis. It is known that most of the somatic characteristics are phenotypically correlated to some degree. Multivariate analysis estimates the genetic and environmental contributions to the covariance between the circumference measurements.
Multivariate models, called general models, tested whether only one genetic and only one environmental factor were responsible for the covariation between the four circumference measurements (EAC, FC, TC, CC) or whether two genetic and environmental factors better explained the observed covariation. To test the latter hypothesis, two types of models were tested: the proximal-distal models assume that one genetic and/or environmental factor explains covariation of the circumferences taken at the proximal regions of the limbs (EAC, TC) and one explains the covariation of the circumferences taken at the distal region of the limbs (FC, CC); the arm-leg models assume one factor is responsible for covariation of the circumferences of the upper extremity (EAC, FC) and one for covariation of the circumferences of the lower extremity (TC, CC). In summary, seven multivariate models were fitted to the data: the first model assumed the presence of only one general genetic (G) and one E factor to explain the covariation; the other six models included, alternatively, one G factor, one E factor, two common genetic factors [1 factor loading on proximal and 1 on distal circumferences or 1 on arm and 1 on leg circumferences (GpGd or GpGl)], and/or two common E factors [1 factor loading on proximal and 1 on distal circumferences or 1 on arm and 1 on leg circumferences (EpEd or EaEl)]. Additional variable-specific genetic (Gv) and environmental (Ev) latent factors were included in all models to account for residual site-specific contributions of variation. In models with at least two common factors, factors were allowed to correlate. Alternative models, in which this correlation was constrained to zero, were also tested. Models for multivariate analysis were applied separately for men and women.

Assessment of models. Two criteria were used to evaluate models derived from different hypotheses: the goodness of fit and the parsimony.

For the univariate models, the overall goodness of fit of a model was assessed by the $x^2$ goodness-of-fit statistic: the lower the $x^2$, the better the fit. The significance of each latent factor (A, D, C, and E) was tested by comparing the full model to the model leaving out this factor. A significantly worse fit of this submodel compared with the full model indicated the significance of this factor. The second criterion was the parsimony, as measured by Akaike's Information Criterion (AIC) (22) of the models, which combines the $x^2$ with the degrees of freedom (df) (22)

$$
\text{AIC} = x^2 - 2df
$$

Models that fit data exactly have $x^2$ values equal to zero, but the most parsimonious model (with the most df) will have the lowest AIC. Alternative univariate and multivariate models were compared by the AIC (22).

Because $x^2$ does not provide an adequate assessment of the goodness of fit for multivariate models, the Tucker-Lewis Index (TLI) (22) was calculated, which takes parsimony into account. For this purpose, the traditional "null" model was fitted; this assumes no covariance at all but estimates only the variances of each variable of each individual (TLI\_null). Because of the special character of twin data, an additional null model, the diagonal model (TLI\_diag), was fitted. This model estimates the within-variable across-twin covariances, in addition to the variances of the variables. Parsimony is accounted for by dividing the $x^2$ values of the tested model and the null model by their df; the higher the index the better the fit. The goodness of fit was further evaluated by fitting a saturated model, i.e., a double Cholesky model (22) (triangular decomposition for A and for E factors). Because this model is fully saturated, it provides an indication for the lowest limit of $x^2$ obtainable with the data. Because multivariate models can be complex, alternative models were also compared by the AIC.

The statistical modeling package Mx (21) was used to evaluate the fitting of univariate and multivariate models. Results with a probability level $<0.05$ were considered as statistically significant.

RESULTS

Means and variances. All variables showed positive skewness. After a logarithmic transformation, all had a Gaussian distribution. Means and variances were not significantly different between boys and girls (Table 2), between MZ and DZ, or by birth rank. Compared with a singleton control group we examined (23, 26), subjects were within the expected range for their respective ages.

Univariate genetic analysis of each circumference. TABLE A1 shows the twin correlations for each variable and age of the five groups (MZM, MZF, DZM, DZF, DZO). MZ correlations for circumference measurements ranged, respectively, between 0.81 and 0.90 for EAC, 0.84 and 0.89 for FC, 0.86 and 0.95 for TC, and 0.72 and 0.93 for CC. The DZ correlations varied between 0.30 and 0.71 for EAC, 0.44 and 0.65 for FC, 0.37 and 0.76 for TC, and 0.33 and 0.65 for CC, respectively. The MZ correlations were quite stable across age, whereas the DZ correlation tended to vary more. Because MZ correlations were very high, a strong genetic component was expected. As DZ correlation exceeded one-half of the MZ correlation, a common environmental influence could be present as well.

The goodness-of-fit statistics of the most parsimonious models were satisfactory; the probability levels (P) of the $x^2$ and the standardized parameter estimates are summarized in Table 3. The AE-model that incorporated additive genes and environment, unique to the individual members of a twin pair, provided the best explanation of the variation. Additive genes explained 87–95% of the variation of all circumferences, except for CC at age 14 yr, where a C factor explained 26%, and only 64% was left to be explained by genes. E factors accounted for 4–14%. Incorporation of differences due to gender (gAE) improved the fit in several cases, especially at younger ages. Between 10 and 12 yr of age,
the variance in girls was 1.2 times greater than in boys (gender heterogeneity).

Multivariate genetic analysis of covariation between circumferences taken at different regions of limbs. Three models emerged: general, proximal-distal, and arm-leg (Fig. 1). For each age, one model was selected on the basis of its goodness of fit and its parsimony (Table 4).

All the selected common factor models showed an acceptable multivariate fit, as indicated by their TLI sub and the TLI diag, ranging from 0.90 to 0.98 for TLI sub and from 0.88 to 0.94 for TLI diag. At each age level and for both genders, Gs and Es factors were present. In boys between 10 and 12 yr old, the general model fitted best; i.e., one common genetic factor loaded on the four variables. At these ages, however, the arm-leg model with two common genetic factors (GaGl), one loading on the arm circumferences and the other on the leg circumferences, did not differ significantly (P > 0.05) from the selected, more parsimonious, general model, according to the χ2 difference test. In girls 10–13 yr of age, two correlating common genetic factors were required, one for the proximal (EAC and TC) and one for the distal (FC and CC) limb circumferences. The χ2 of the proximal-distal model was significantly (P < 0.05) lower than the χ2 of the general model with only one common genetic factor. The correlation between the two common genetic factors, however, was high (0.95–0.97).

At an older age, the best fitting model was the same in both genders. The arm-leg model contained two genetic factors, one common to the arm circumferences (EAC and FC) and one to the leg circumferences (TC and CC). Although the correlation between those two common genetic factors was high (0.93 and 0.92 in boys, 0.94 in girls), the fit was significantly (P < 0.05) better than for the general model with only one common genetic factor. At all age levels and for both genders, there was only one environmental factor common to all four variables.

Table A2 shows the standardized estimates for the best-fitting multivariate model by age and gender. The contribution of the common genetic factor(s) (parameters 1–4) was systematically lower in boys (0.60–0.79) than in girls (0.72–0.93). Inversely, the contribution of the specific genetic factors (parameters 5–8) was higher in boys (0.09–0.29) than in girls (null to 0.07). Summing the common genetic with the specific genetic factors, the overall genetic determination varied between 0.82 and 0.91 in boys and between 0.76 and 0.91 in girls. Total environment (summing specific and common factors) was responsible for 9–18% of the variance in boys and for 4–24% in girls. Similar to the genetic contribution, the environmental common factor (9–12) accounted for the largest part of the total environmental contribution, ranging for boys between 0.01 and 0.17 and for girls between 0.02 and 0.21.


<table>
<thead>
<tr>
<th>Age, yr</th>
<th>EAC</th>
<th>CC</th>
<th>AFC</th>
<th>CAC</th>
<th>DFC</th>
<th>DCAC</th>
<th>DFCAC</th>
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<td>14</td>
<td>15</td>
<td>17</td>
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<td>17</td>
<td>19</td>
<td>21</td>
<td>23</td>
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</table>

DISCUSSION

The main findings of this longitudinal study are the high genetic determination of the circumferences and the gender-specific change of the multivariate genetic structure with age.
The striking similarity of all circumferences in MZ twins, indicated by the correlations ranging between 0.72 and 0.95, approaches the test-retest reliability of 0.97–0.99 we obtained. MZ twins resemble each other nearly as much as one individual measured on different occasions. This suggested a very strong genetic determination. The correlations in DZ twins, ranging from 0.30 to 0.76, showed also a familial relatedness. Because some of these DZ correlations exceeded one-half of the corresponding MZ correlations, environmental influences.

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Table 4. Explanatory factors of covariation among EAC, FC, TC, and CC and goodness-of-fit statistics of corresponding multivariate models by age and gender

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age, yr</td>
<td>Age, yr</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Genetic factors</td>
<td></td>
<td></td>
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<tr>
<td>Number of specific</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Type of common factor between common</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Environmental factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of specific</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Type of common model</td>
<td>General</td>
<td>E</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>74.80</td>
<td>86.27</td>
</tr>
<tr>
<td>$P$</td>
<td>0.047</td>
<td>0.006</td>
</tr>
<tr>
<td>df</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>$TLI_{null}$</td>
<td>0.95</td>
<td>0.93</td>
</tr>
<tr>
<td>$TLI_{diag}$</td>
<td>0.94</td>
<td>0.91</td>
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</tbody>
</table>

G, 1 general genetic factor loading on all 4 circumferences; Gs, 2 genetic factors, 1 loading on arm circumferences and 1 on leg circumferences; Gd, 2 genetic factors, 1 loading on proximal circumferences and 1 on distal circumferences; general model, 1 genetic and 1 environmental factor; arm-leg model, 1 factor explains covariation at upper extremity and 1 covariation at lower extremity; prox-dist, 1 factor explains covariation at proximal limb region and 1 at distal limb region; $TLI_{null}$ and $TLI_{diag}$, traditional null model and diagonal model fitted to Tucker-Lewis Index, respectively.
ences, common to both twin members, could be suspected. The presence of a strong genetic influence (87–95%), suggested by comparison of the MZ and DZ correlations, was confirmed by the model fitting. This genetic influence remained stable over age in both genders. A suspected common environmental factor, however, could not be demonstrated, except for CC at the age of 14 yr. In structural equation modeling, common environmental factors can only be identified when the effect or the sample size is relatively large.

The comparison of heritability estimates from previous studies with the present ones is difficult because of differences in sample composition and in the method of heritability calculation.

The high genetic contribution confirms the results found in most earlier twin studies (2, 4, 5). Heritabilities obtained from the family studies are known to be often lower than those obtained from twin studies. Because DZ twins can be considered to be similar to other first-degree relatives, the DZ correlations were used to compare our results with the sibling-sibling and parent-offspring correlations of previous family studies.

The parent-offspring correlations (13, 14, 27) of circumference measurements ranged between 0.17 and 0.42, which are lower than our DZ correlations. This might be due to a difference in generational and in environmental conditions between parents and offspring. The DZ correlations in this study, ranging between 0.30 and 0.76, are similar but somewhat higher than the sibling-sibling correlations of schoolchildren (15, 20) and young adults (13, 14), ranging between 0.35 and 0.45.

The narrow heritability for the EAC (0.30) in 10-yr-old siblings estimated by Bouchard et al. (1), on the basis of path analysis, is much lower than the genetic contribution (0.87) in this study and in other twin studies (2, 4). Susanne (27) found also a low heritability coefficient (estimated with the method of Fisher) of only 0.47 for arm circumferences and CC. The genetic component was of the same magnitude for the circumferences of the upper and lower extremities. This confirmed the findings of most investigators (2, 13, 14, 20, 27). Only Clark (4) and Little et al. (15) found a higher genetic component for the lower extremity circumferences compared with the upper extremity circumferences. They did not mention, however, whether this difference was statistically significant. Gender differences were mainly apparent at younger ages. Additive genes contributed equally to the variance in both boys and girls, but because girls had a larger total variance, the genetic variance was higher in girls than in boys. This may be due to the difference in individual and gender-related timing of puberty. While the girls were already in puberty, most boys were still in preadolescence. This might result in a larger variance in girls than in boys. The gender difference may also be caused by the marked increase of subcutaneous fat in the extremities in girls (28). This influences the utility of limb circumference as a measure of muscularity for girls. A higher genetic influence for women on CC and EAC is also reported in adolescents (5) and in schoolchildren (15, 20). Little et al. (15) ascribe the gender difference to the environmental impact, which might be larger in boys than in girls. Because no common environmental factor was identified in this study, this hypothesized environmental impact could not be confirmed. Hewitt (11) and Hoshi et al. (12) did not observe any gender difference in calf muscularity by radiographic analysis, either.

Because of the method used, none of the earlier studies could identify a shared environmental component, although many of them (1, 8, 13–15, 20, 25, 27) assume that environment contributes to the variance of these variables. In the present study, the presence of a shared environmental factor was only apparent at age 14 yr.

To our knowledge, no comparable multivariate path analysis of the covariation between limb circumferences has been reported. This multivariate analysis revealed the importance of genetic factors, common to different regions. With increasing age, there is evidence that the genetic architecture that underlies the variation and covariation in limb circumferences changes. This is not identical in both genders.

In boys between 10 and 12 yr of age, covariation and variation of the variables were explained by the general model, assuming one common genetic factor influencing the circumferences taken at the four regions. The genetic architecture changes with age. This change could already be suspected because the arm-leg model, assuming two correlated common genetic factors, one for the arm circumferences and one for the leg circumferences, fitted the data equally well but was less parsimonious. At ages 13 and 14 yr, this tendency was confirmed because the fit of the arm-leg model was statistically significantly better. This might suggest that a different set of genes influences arm vs. leg circumferences. The strong correlation between the two common genetic factors indicated, however, that the same genes had a different influence on arms than on legs. The fact that the presence of two common genetic factors was more pronounced at ages 13 and 14 yr than at earlier ages, possibly reflected an age-associated effect of genes at different maturational stages, as suggested by Mueller and Malina (20) and Little et al. (15). Phenotypic observation might indicate this genetic structure because boys gain relatively more muscle mass in the arms compared with the legs (28).

In girls of all ages, two common genetic factors were observed, although the loading of these factors at ages 10–13 yr differed significantly from the loading at age 14 yr. At ages 10–13 yr, the proximal-distal model fitted data best; one factor loaded on the proximal limb circumferences and another on the distal limb circumferences, whereas at age 14 yr, as in boys at this age, there was evidence for the arm-leg model; one factor loaded on the two arm circumferences and one on the two leg circumferences. This switch in girls was rather unexpected because the proximal-distal model in the younger subjects did not suggest a strong covariation between arm and leg circumferences. The accumulation of fat in the extremities may influence the circumference as an indicator for muscularity (28), which complicates the interpretation. Like in boys, the two factors correlated highly at all age levels. This again
Suggested that nearly the same set of genes was responsible for variation and covariation of the four variables but that they acted differently depending on the body region.

These age-associated changes and gender differences in genetic control could be influenced by several factors. First, the validity of circumference measurements as indicators of muscle tissue may result in gender differences; fat accumulation may be a confounding factor in girls. However, the pattern of age- and gender-associated variation in anthropometric estimates of limb musculature is similar to that for radiographic measurements (17). Second, genders may differ because of gender-specific changes in muscle mass and fat accumulation during puberty. Boys experience a large increase in muscle mass, more in the upper arm than in the calf, resulting in a maximum increase in muscle mass approximately 3 to 6 months after peak height velocity (PHV). Girls accumulate relatively more fat on the extremities and boys more on the trunk, especially during puberal years, when the typical gender-specific subcutaneous fat is accentuated (17, 18, 28). Furthermore, the difference in biological maturation of boys and girls at the same ages may result in age-associated and gender-specific differences. On the average, girls experience PHV at approximately 12 years of age and boys at approximately 14 years of age (18). Consequently, girls are biologically two years more advanced than boys at the same age.

Given the timing of the changes in genetic architecture, especially in boys, it is tempting to associate these changes with hormonal secretions and the concomitant changes in muscle tissue. To demonstrate the common genetic architecture, multivariate analyses need to be carried out in which both hormonal secretion and muscle dimensions are incorporated (18).

Because circumferences are mainly determined by muscle (17, 29), our findings could be explained by the biology of this tissue. Two questions arise: 1) can the regional difference in genetic structure be explained by genes known from the literature to influence muscle tissue in different regions?; and 2) what factors could explain the temporal changes of the genetic structure in either gender?

Heritable neuromuscular diseases in childhood (7) show that the action of genes controlling skeletal muscle development is neither stable in time nor do these genes influence different regions in the same way. Some of these genes affect predominantly the muscles in certain body regions. Autosomal dominant myotonic dystrophy and different types of hereditary motor and sensory neuropathies have a more severe effect on the distal musculature than on the proximal. Spinal muscular atrophy, the Duchenne-type of muscular dystrophies, and limb-girdle muscular dystrophies affect the proximal musculature and, only in a later stage, the distal musculature. The facioscapulohumeral muscular dystrophy affects predominantly the muscles of the face, neck, and shoulder girdle. To some extent, this is what the proximal-distal model assumes. Furthermore, several genes on the X-chromosome have an impact on neuromuscular function, i.e., genes for Duchenne muscular dystrophy, spinobulbar muscular atrophy, and myotubular myopathy. Abnormalities or polymorphisms in these genes will have a different effect in male vs. female children (7). There is thus ample evidence from genetic diseases that several genes influence muscles in different regions of the body.

In both boys and girls, additive genetic factor(s), common to several regions, explained the largest part of the phenotypic variation. The genetic influence, specific for each region, was low, especially in girls. This suggested that there was no clear evidence for “circumference”-specific genes and even strengthened the assumption that genes coding for muscle circumferences did not act completely independently. Total genetic contribution (common and specific) to the variance of each phenotype accounted for up to 94% of the total variance, which confirmed the univariate results and suggested a strong genetic basis for the phenotypic variation in different muscle areas. The contribution of unique environment was low.

In summary, this study confirmed that muscle circumferences are some of the most heritable characteristics in humans and are therefore a promising starting point to locate their genes. The environmental influence, shared by both twin members, was too small to be demonstrable. Multivariate analysis of the covariance between different muscle regions showed that additive genetic factors influenced the muscle circumferences differently according to the region in boys and girls at different ages. The high correlation between the genetic factors suggested that muscle circumferences might be mediated to some extent by the same genes that act not completely independently. Gene mapping should validate this gender-specific change of the genetic structure with age and region.

### APPENDIX

Table A1. Correlation of each circumference measurement between both twin members for each zygosity, gender, and age

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>EAC</th>
<th>FC</th>
<th>TC</th>
<th>CC</th>
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<td>0.70</td>
<td>0.58</td>
<td>0.61</td>
<td>0.62</td>
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</table>

MZM, monozygotic male; MZF, monozygotic female; DZM, dizygotic male; FZF, dizygotic female; DZO, dizygotic opposite sex.
### Table A2. Standardized parameter estimates for best fitting multivariate model by age and gender, with each number representing a parameter referred to in Fig. 1

<table>
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<tr>
<th>Age, yr</th>
<th>Model</th>
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<th>Girls</th>
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<td>Arm-leg</td>
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<td>Arm-leg</td>
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</tr>
</tbody>
</table>

Bold values indicate paths due to same factors.

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