AN INFLAMMATORY GENETIC DETERMINANT OF HANDGRIP STRENGTH

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Variants of the *IL10* gene associate with muscle strength in elderly from rural Africa: a candidate gene study.
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Background • It has recently been shown that the capacity of the innate immune system to produce cytokines relates to skeletal muscle mass and muscle strength in older persons. The *interleukin-10* (*IL10*) gene regulates the production capacities of IL10 and tumour necrosis factor α (TNFα). In rural Ghana, *IL10* gene variants associated with different production capacities of IL10 and TNFα are enriched compared with European populations. In this setting, we explored the association between these gene variants and muscle strength.

Methods • Among 554 Ghanaians aged 50 years and older, we determined 20 single nucleotide polymorphisms (SNPs) in the *IL10* gene, production capacities of IL10 and TNFα in whole blood upon stimulation with lipopolysaccharide (LPS), and handgrip strength as a proxy for skeletal muscle strength. We distinguished proinflammatory haplotypes associated with low IL10 production capacity and anti-inflammatory haplotypes with high IL10 production capacity.

Results • We found that distinct haplotypes of the *IL10* gene associated with handgrip strength. A proinflammatory haplotype with a population frequency of 43.2% was associated with higher handgrip strength (p = 0.015). An anti-inflammatory haplotype with a population frequency of 7.9% was associated with lower handgrip strength (p = 0.006).

Conclusion • In conclusion, variants of the *IL10* gene contributing to a proinflammatory cytokine response associate with higher muscle strength, whereas those contributing to an anti-inflammatory response associate with lower muscle strength. Future research needs to elucidate whether these effects of variation in the *IL10* gene are exerted directly through its role in the repair of muscle tissue or indirectly through its role in the defence against infectious diseases.

Interleukin-10 (IL10) is an anti-inflammatory cytokine with important regulatory effects on inflammatory responses. It downregulates the antigen presenting function and inhibits the production of proinflammatory cytokines like tumour necrosis factor α (TNFα) by various immune cells.[1] In mice, immune cells producing cytokines are crucial for the repair of skeletal muscle tissue.[2-5] We have recently shown that a higher TNFα production capacity of immune cells is positively related to muscle mass and muscle strength in a middle-aged Dutch population.[6]

The capacity to produce IL10 and TNFα upon whole-blood stimulation with lipopolysaccharide (LPS) varies between individuals. This variation is for more than 50% attributable to genetic determinants.[7-9] The *IL10* gene is highly polymorphic[10-12] and its haplotypes are transcribed differently.[13] This interindividual variation is extended by variation in the *IL10* hap-

lotype structure and distribution between ethnicities.[10,14] We have earlier reported that specific *IL10* gene variants are enriched in Ghanaian elderly living under adverse conditions.[12] These variants have functional significance: some are related to a proinflammatory cytokine production capacity, with lower IL10 and higher TNFα levels upon whole-blood stimulation with LPS, while others are related to an inverse anti-inflammatory response.[12,15,16] Interestingly, this *IL10* haplotype structure is less present in European populations living under affluent conditions,[12,14] possibly because balanced selection has conserved this haplotype structure in populations under adverse conditions. The functional variation in the genetic determinants of cytokine production capacity forms a meaningful instrument to study the effects of different cytokine production capacities largely free from confounding and reverse causality.[17]

For this study, we had the unique opportunity to study handgrip strength of individuals aged 50 years and older in the Ghanaian population of which we have characterised the *IL10* gene variants and their effects on cytokine production capacity.[12,15,16] This study aims to investigate the relation between the pro- and anti-inflammatory *IL10* gene variants, which are not present in European populations, and handgrip strength as a proxy of overall muscle strength. To account for the possible effects of ill health on handgrip strength, the analyses were also performed after exclusion of individuals with underweight.

Methods

Research area

This study was performed in a remote, rural, and underdeveloped area in the Upper East Region in Ghana in West Africa. The vast majority of the inhabitants are involved in non-commercial agriculture performed by manual labour. Infectious diseases are the main causes of death.[18] The prevalence of human immunodeficiency virus (HIV) is low (< 4%) compared with other sub-Saharan regions.[18] Since 2002, we have followed a horticultural population in the Garu-Tempane District in the Upper East Region, comprising approximately 25 000 inhabitants living in 32 villages. For each household, we determined the household property value and the socioeconomic status in 2007 according to the Demographic and Health Survey method.[19] Elaborate descriptions of the research population have been given elsewhere.[12,19-21]

Ethical approval was given by the Ethical Review Committee of Ghana Health Services, the Committee Medical Ethics of Leiden University Medical Center, and by the local chiefs and elders. Because of illiteracy, informed consent was obtained orally from the participants in the local language. A consent form was read out to each participant with an explanation on the purpose and conduction of this research project.

DNA collection and genotyping

We collected buccal swabs between 2002 and 2006 of 4336 individuals.[12] Common genetic variation (minor allele frequency

≥ 5%) in the *IL10* gene region was determined by genotyping 20 SNPs, selected from the Yoruba population in the HapMap database (release #21, r^2 = 0.8) and genotyped using mass spectrometry (Seqeunom, San Diego, CA, USA). All SNPs were in Hardy-Weinberg equilibrium, with one exception where a minor deviation was observed.[12] We have recently reported that population stratification is unlikely to influence associations with genetic variation in autosomal genes, as analysis of autosomal DNA, mtDNA and y-chromosomal genetic variation patterns in the research area revealed that women-mediated gene flow is nearly fully random, whereas men-mediated gene flow is highly reduced.[22] This genetic substructure is an immediate result of the patrilocal society. We addressed residual population stratification by adjusting all analyses for tribe. Familial relatedness among individuals was addressed by adjusting all analyses for household.

Handgrip strength and BMI

Handgrip strength and body mass index (BMI) were measured in 2009 and 2010 in 923 individuals aged 50 years and older, recruited independently from the genetic samples. Data on the *IL10* gene were available for 554 of them. We consecutively visited all villages in the research area, in which we set up a mobile fieldwork station. We approached all individuals aged 50 years and older and brought less mobile participants by car. Inclusion was limited by the duration of both field visits. Individuals did not participate if they were unable to leave the house, were absent from the research area for a longer period or refused participation. Handgrip strength was measured using a calibrated Jamar hand dynamometer (Sammons Preston, Bolingbrook, IL, USA) with the participant in an upright position and the arm of the measured hand unsupported parallel to the body. The width of the dynamometer's handle was adjusted to each participant's hand size so that the middle phalanges rested on the inner handle. The participants were instructed to exert maximal force once by each hand. We used the highest measurement of both hands in our analyses. Body height and weight were measured by a calibrated length scale and weighing scale. A BMI of 18.5 kg/m^2 or lower was defined as underweight and a BMI above 18.5 kg/m^2 as a normal BMI, according to the classification of the Food and Agriculture Organization and World Health Organization.[23,24]

Cytokine production capacity

The production capacities of IL10 and TNFα were measured in blood samples that were taken in 2005, 2006, or 2008. Previous publications, studying measurements from these years separately, have reported the procedure by which the blood samples were processed.[15,16,25] Venous blood was locally incubated with *Escherichia coli* LPS, the supernatants frozen and shipped for measurement of cytokine concentrations in the Netherlands by enzyme-linked immunosorbent assay (ELISA). The procedure has been reported to have a small intraindividual compared with the interindividual variation[9] and to be replicable with an interval of two years in this research

area.[16,25] We combined the measurements from all three years of 1177 individuals of whom data on the *IL10* gene were also available.

Analyses

The program Haploview (Broad, Cambridge, MA, USA)[26] was used to test for Hardy-Weinberg equilibrium. Statistical analyses were performed with SPSS Statistics 20 (IBM, Armonk, NY, USA) and Stata/SE 12.0 (StataCorp, College Station, TX, USA). The relations between *IL10* gene SNPs and haplotype copies, cytokine production capacities, and handgrip strength were assessed by linear mixed models. These analyses were adjusted for age, sex, and tribe as fixed factors and household as a random factor. Analyses with handgrip strength were additionally adjusted for height as a fixed factor. Cytokine concentrations were natural-logarithmically transformed due to skewedness and standardised as *z* scores per sex[27] within each year of measurement. The *z* scores of 2005, 2006, and 2008 were averaged into one *z* score per individual. Haplotypes were defined as proinflammatory if associated with lower levels of IL10 and higher levels of TNFα upon stimulation with LPS. Haplotypes were defined as anti-inflammatory if associated with higher levels of IL10 and lower levels of TNFα upon stimulation with LPS.[12] In all haplotype analyses, the posterior probabilities of pairs of haplotypes per individual, as estimated by PHASE,[28] were used as weights.

Table 7.1 • General characteristics of the Ghanaian study population

	Men	Women
Number of individuals	196	358
Age, years	73.0 (9.2)	63.4 (9.2)
Tribe, *n* (%)		
Bimoba	142 (72.4)	252 (70.4)
Kusasi	44 (22.4)	84 (23.5)
Mamprusi	2 (1.0)	12 (3.4)
Busanga	2 (1.0)	8 (2.2)
Fulani	2 (1.0)	1 (0.3)
other	4 (2.0)	1 (0.3)
Number of households	190	299
Household property value, median (iqr) US$	1028 (580–1782)	1183 (585–2055)
Height, cm	166.0 (6.8)	157.9 (6.7)
Weight, kg	49.4 (7.8)	45.4 (7.5)
Body mass index, kg/m^2	17.9 (2.3)	18.2 (2.5)
Body mass index ≤ 18.5 kg/m^2, *n* (%)	113 (57.6)	204 (57.0)
Handgrip strength, kg	29.2 (8.1)	23.4 (5.9)

Data are presented as means with standard deviations unless specified otherwise. Iqr: interquartile range.

Results

Table 7.1 displays the characteristics of 554 individuals aged 50 years and older of whom *IL10* gene variants and handgrip strength were known. Their characteristics were similar as compared with all 4336 individuals of whom *IL10* gene variants were measured and with all 923 individuals of whom handgrip strength was measured (data not shown). Approximately half of them had a BMI of 18.5 kg/m^2 or lower, which is regarded as underweight.[23,24] Mean handgrip strength (with standard deviation) was 27.3 (7.6) kg for those with a normal BMI and 24.1 (6.7) kg for those with underweight. Table 7.2 shows that handgrip strength did not differ between tribes.

Table 7.2 • Associations between tribe and handgrip strength

	n	Handgrip strength, kg
Bimoba	394	26.6 (0.3)
Kusasi	128	26.8 (0.6)
Mamprusi	14	26.3 (1.7)
Busanga	10	30.7 (2.0)
Fulani	3	21.9 (3.6)
Other	3	27.7 (2.8)
p value		0.30

Handgrip strength is presented as means with standard errors, adjusted for age and sex. Differences in handgrip strength between tribes were tested with ANCOVA.

IL10 gene variants and cytokine production capacity

It has been previously shown that several SNPs in the *IL10* gene region influence the production capacities of IL10 and TNFα measured in two independent groups in 2006 and 2008 in this research area.[12,16] First, we confirmed that these relations were present in 1177 individuals of whom *IL10* gene variants were known and of whom measurements of cytokine production capacities were combined from 2005, 2006, and 2008 (Figure 7.1A). When restricting this group to the individuals of whom handgrip strength was also known (n = 457), a similar pattern was present (Figure 7.1B).

It has been previously shown that the SNPs in the *IL10* gene region constitute two haplotypes that influence the production capacities of IL10 and TNFα: a proinflammatory haplotype 1 and an anti-inflammatory haplotype 3.[12,16] We reanalysed the relations between the haplotypes of the *IL10* gene and cytokine production capacities in the 1177 individuals of whom *IL10* gene variants were known and of whom measurements of cytokine production capacities were combined from 2005, 2006, and 2008. We confirmed an additive genetic effect for both haplotypes. With each additional copy of the proinflammatory haplotype 1, z scores of IL10 production capacity were 0.08 lower (standard error (SE) = 0.04; p = 0.028) and z scores of TNFα production capacity were 0.11 higher (SE = 0.04; p = 0.001). With each additional copy of the anti-inflammatory haplotype 3, z scores of IL10 production capacity were 0.19 higher (SE = 0.07; p = 0.005) and z scores of TNFα were 0.10 lower (SE = 0.07; p = 0.167). When restricting this group to the individuals of whom handgrip strength was also known (n = 457), simi-

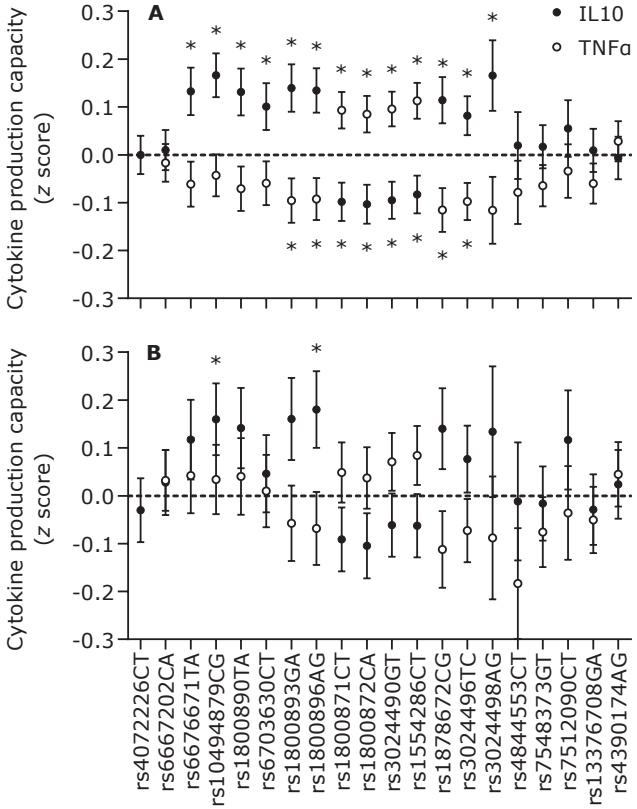

Figure 7.1 • Associations of *IL10* gene SNPs with cytokine production capacities. The relations are shown between the minor allele of each *IL10* gene SNP and the production capacities of IL10 and TNFα for (A) individuals of whom *IL10* gene variants and cytokine production capacities were known (n = 1177) and (B) individuals of whom *IL10* gene variants, cytokine production capacities, and handgrip strength were known (n = 457). Cytokine production capacities are expressed as z scores with standard errors for carriers of at least one copy of the minor allele, adjusted for age, sex, tribe, and household. * $p < 0.05$.

lar patterns were found (data not shown). Haplotype 2 was not related with the production capacities of IL10 (p = 0.813) or TNFα (p = 0.364) and was used in this study as a negative control. Results were not different between men and women.

IL10 gene variants and handgrip strength

Figure 7.2A shows the 20 genotyped SNPs in the *IL10* gene region. Most of the SNPs tag a single linkage disequilibrium (LD) block. The haplotype structure and the frequencies of these haplotypes were previously calculated for all individuals of whom *IL10* gene variants were measured (n = 4336).[12] Haplotype frequencies and Hardy-Weinberg equilibria of the SNPs were not materially different when restricting this group to the individuals of whom handgrip strength was also known (Tables 7.3 and 7.4). Furthermore, allelic frequencies were not different between tribes, with a few exceptions between small and large tribes (Table 7.5).

Figure 7.2B shows that in the individuals of whom *IL10* gene variants and handgrip strength were known, carriers of distinct SNPs had higher or lower handgrip strength when compared with non-carriers. The pattern followed the predefined

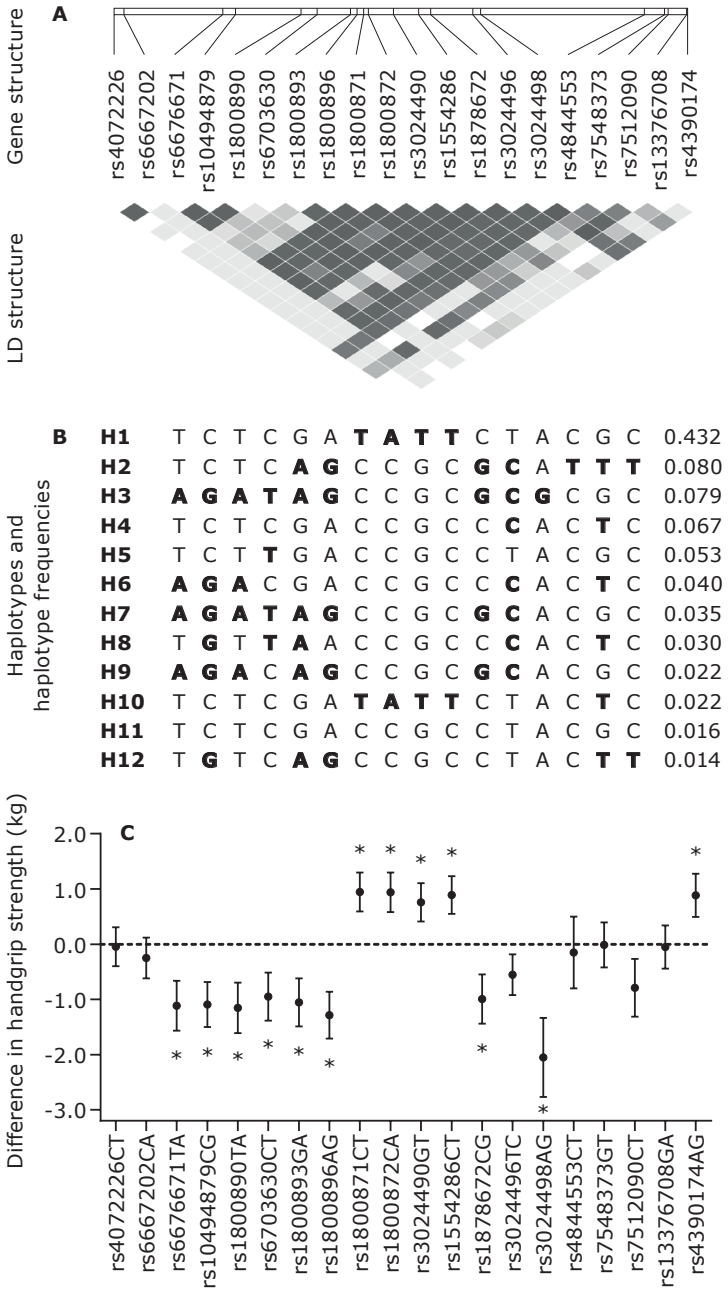

Figure 7.2 • Associations of *IL10* gene SNPs with handgrip strength. Schematic overview of the *IL10* gene region with the locations of the genotyped SNPs indicated by vertical lines (A). Pairwise linkage disequilibrium (LD) as observed in the entire genotyped population (n = 4336) is depicted in greyscale. Population frequencies of the different haplotypes (if > 1%) are presented with the minor alleles of each SNP indicated in bold (B).[12] The relation between the minor allele of each *IL10* gene SNP and handgrip strength for individuals of whom *IL10* gene variants and handgrip were known (C; n = 554). Handgrip strength is expressed as the deviance from the population's mean in kilograms (kg) with standard errors for carriers of at least one copy of the minor allele, adjusted for age, sex, tribe, household, and height. * $p < 0.05$.

Table 7.3 • Haplotype frequencies of the *IL10* gene in the Ghanaian study population and in the entire Ghanaian genotyped population

Haplotype	Study population	Entire genotyped population
n	554	4336
H1	0.457	0.432
H2	0.066	0.080
H3	0.063	0.079
H4	0.069	0.067
H5	0.048	0.053
H6	0.040	0.040
H7	0.048	0.035
H8	0.021	0.030
H9	0.026	0.022
H10	0.021	0.022
H11	0.012	0.016
H12	0.015	0.014
p value	0.79	

Differences in haplotype frequencies between both populations were tested with the Pearson chi-squared test.

anti-inflammatory and proinflammatory haplotype structures shown in Figure 7.2A and Figure 7.1. Results were not different between men and women.

Figure 7.3 shows handgrip strength for carriers and non-carriers of the proinflammatory haplotype 1 and the anti-inflammatory haplotype 3. We observed an additive genetic effect on handgrip strength, which increased with each additional copy of the proinflammatory haplotype 1 and decreased with each additional copy of the anti-inflammatory haplotype 3. Among individuals with a normal BMI, the positive association between the proinflammatory haplotype 1 and handgrip strength was equally strong (p for interaction = 0.398) and the negative association between the anti-inflammatory haplotype 3 and handgrip strength was stronger (p for interaction = 0.009). Both haplotypes were not associated with BMI, neither in all individuals nor in those with BMI above 18.5 kg/m^2 ($p > 0.200$). Contrary to haplotypes 1 and 3, haplotype 2 was not associated with handgrip strength ($p = 0.922$). Results were not different between men and women.

Cytokine production capacity and handgrip strength

Figure 7.4 shows that IL10 and TNFα production capacities were not related to handgrip strength, although an increase in IL10 production capacity concurred with a declining trend in handgrip strength among individuals with a normal BMI. When stratifying by sex, IL10 production capacity was not associated with handgrip strength in either men or women ($p > 0.800$), while TNFα production capacity was positively associated with handgrip strength in men ($n = 128$; $p = 0.007$) but not in women ($n = 329$; $p = 0.819$).

Discussion

We found that distinct haplotypes of the *IL10* gene, associated with variation in the cytokine production capacities of immune cells, were related to handgrip strength in rural Ghana. A proinflammatory haplotype with a population frequency of 43.2% was associated with higher hand-

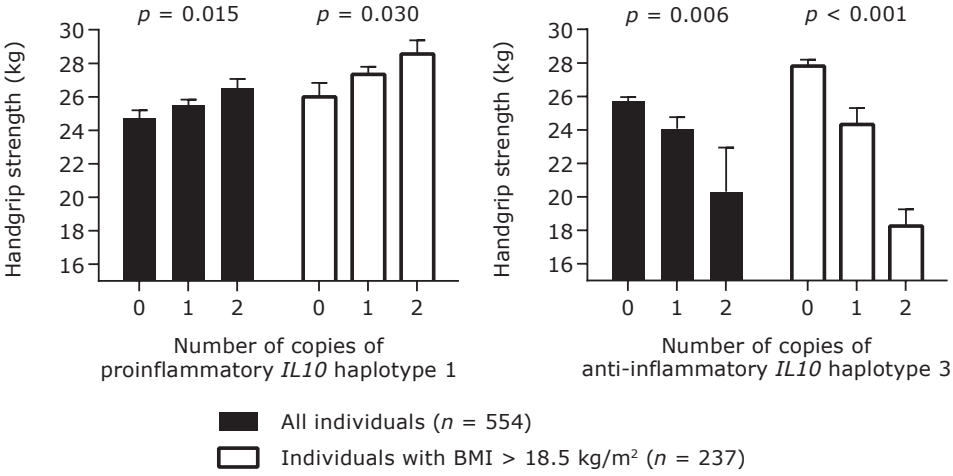

Figure 7.3 • Associations of *IL10* gene haplotypes with handgrip strength in all individuals and in individuals with a normal BMI. Handgrip strength for individuals of whom *IL10* gene variants and handgrip were known (n = 554) presented as means with standard errors, adjusted for age, sex, tribe, household, and height (p values for trends). A BMI of 18.5 kg/m^2 or lower is regarded as underweight.[23,24] For the haplotype structures and frequencies, see Figure 7.2A.

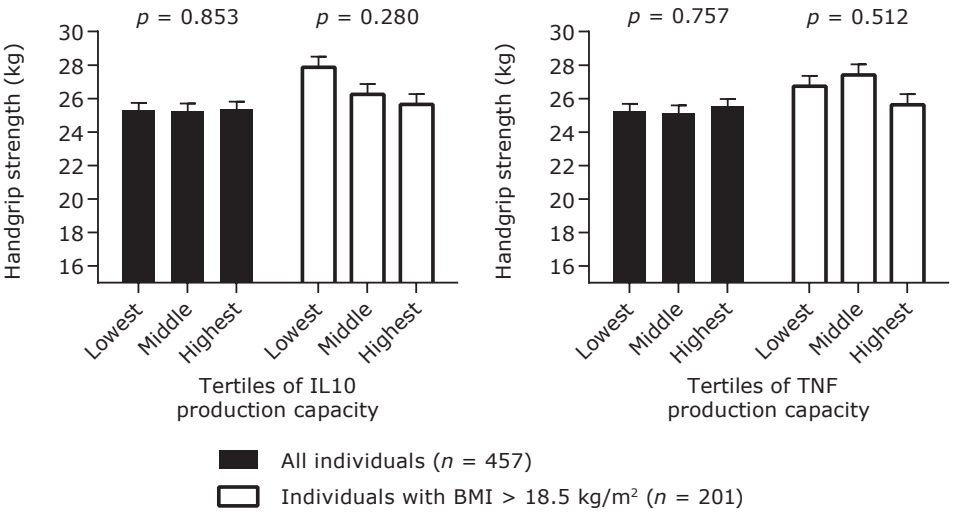

Figure 7.4 • Association of cytokine production capacities with handgrip strength in all individuals and in individuals with a normal BMI. Handgrip strength for individuals of whom *IL10* gene variants, cytokine production capacities, and handgrip strength were known (n = 457) presented as means with standard errors, adjusted for age, sex, tribe, household, and height (p values for trends). A BMI of 18.5 kg/m^2 or lower is regarded as underweight.[23,24]

Table 7.4 • Locations and minor allele frequencies of the *IL10* gene SNPs in the Ghanaian study population and in the entire genotyped population

IL10 SNP	Alleles	Location	Study population		Entire genotyped population	
			MAF	HWE	MAF	HWE
rs4072226	C/T	promotor	0.463	0.936	0.456	0.865
rs6667202	C/A	promotor	0.499	0.851	0.484	0.602
rs6676671	T/A	promotor	0.195	0.870	0.200	0.832
rs10494879	C/G	promotor	0.269	0.823	0.284	0.025
rs1800890	T/A	promotor	0.192	0.822	0.201	0.865
rs6703630	C/T	promotor	0.206	0.579	0.220	0.066
rs1800893	G/A	promotor	0.256	0.005	0.284	0.196
rs1800896	A/G	promotor	0.260	0.064	0.284	0.401
rs1800871	C/T	promotor	0.509	0.984	0.470	0.302
rs1800872	C/A	promotor	0.506	0.811	0.472	0.034
rs3024490	G/T	intron	0.523	0.695	0.484	0.013
rs1554286	C/T	e/i boundary	0.500	0.433	0.468	0.157
rs1878672	C/G	intron	0.228	0.072	0.244	0.612
rs3024496	T/C	exon	0.393	0.928	0.425	0.043
rs3024498	A/G	exon	0.066	0.904	0.083	0.129
rs4844553	C/T	3′ UTR	0.080	0.963	0.096	0.084
rs7548373	G/T	3′ UTR	0.282	0.790	0.297	0.190
rs7512090	C/T	3′ UTR	0.126	0.681	0.132	0.084
rs13376708	G/A	3′ UTR	0.320	0.784	0.327	0.273
rs4390174	A/G	3′ UTR	0.290	0.981	0.282	0.630

Alleles indicate major/minor alleles. MAF: minor allele frequency. HWE: *p* values for Hardy-Weinberg equilibrium. E/i boundary: boundary between an exon and an intron. 3′ UTR: three prime untranslated region.

Table 7.5 • Minor allele frequencies of the *IL10* gene SNPs per tribe

***IL10* SNP**	**Bimoba**	**Kusasi**	**Mamprusi**	**Busanga**	**Fulani**	**Other**	***p* value**
n	394	128	14	10	3	3	
rs4072226	0.443	0.516	0.536	0.400	0.500	0.500	0.29
rs6667202	0.524	0.429	0.464	0.550	0.250	0.400	0.10
rs6676671	0.186	0.213	0.250	0.300	0.000	0.200	0.25
rs10494879	0.367	0.276	0.286	0.350	0.167	0.300	0.48
rs1800890	0.183	0.211	0.250	0.300	0.000	0.125	0.33
rs6703630	0.194	0.238	0.250	0.300	0.000	0.300	0.12
rs1800893	0.261	0.262	0.154	0.222	0.167	0.000	0.20
rs1800896	0.260	0.264	0.231	0.300	0.177	0.200	0.87
rs1800871	0.509	0.508	0.429	0.550	0.833	0.500	0.79
rs1800872	0.506	0.504	0.429	0.550	0.833	0.500	0.77
rs3024490	0.527	0.512	0.429	0.556	0.833	0.500	0.92
rs1554286	0.503	0.500	0.364	0.500	0.833	0.333	0.71
rs1878672	0.227	0.238	0.154	0.462	0.167	0.100	0.80
rs3024496	0.398	0.399	0.423	0.350	0.177	0.250	0.34
rs3024498	0.059	0.089	0.071	0.001	0.000	0.000	0.56
rs4844553	0.087	0.075	0.036	0.000	0.000	0.000	0.044
rs7548373	0.300	0.258	0.250	0.056	0.000	0.200	0.009
rs7512090	0.141	0.105	0.105	0.000	0.038	0.000	0.003
rs13376708	0.308	0.352	0.357	0.250	0.167	0.600	0.23
rs4390174	0.275	0.321	0.321	0.350	0.667	0.200	0.19

Differences in minor allele frequencies between tribes were tested with the linear-by-linear association test.

grip strength, while an anti-inflammatory haplotype with a population frequency of 7.9% was associated with lower handgrip strength. These associations were most outspoken after exclusion of individuals with underweight.

We investigated the effect of *IL10* gene variants as genetic determinants of the *ex vivo* production capacities of IL10 and TNFα by various immune cells in blood.[12,16] The impact of genetic variants on the cytokine production capacity by specific immune cells is not precisely known, but whole blood stimulation with LPS has been shown to particularly reflect the variance in cytokine production by monocytes.[9] Using genetic determinants, the analyses of the influence of cytokine production capacity on muscle strength are largely free from confounding and reverse causality.[17] This is an advantage compared with earlier research in which cytokine production capacity was only measured on the phenotypic level.[6]

Mice studies have shown that repair and maintenance of skeletal muscle tissue are dependent on the innate immune system.[2-5] Critical for this role of the innate immune system are monocytes infiltrating muscle tissue after injury[29,30] and producing proinflammatory cytokines, of which most notably TNFα.[31,32] As a counterbalance, the anti-inflammatory cytokine IL10 downregulates the proinflammatory functioning of monocytes and the production of proinflammatory cytokines such as TNFα[33] and is associated with deceleration of skeletal muscle regeneration.[5] In humans, we have recently reported that a higher capacity to produce proinflammatory cytokines, including TNFα, coexists with higher muscle strength and muscle mass.[6] We now show that the same associations exist between genetic determinants of the cytokine production capacity and handgrip strength. These findings support that cytokine production capacity might be important for human muscle repair and maintenance as well.

As an alternative explanation of the association between the *IL10* gene variants and muscle strength, proinflammatory *IL10* gene variants might yield a better resistance to infectious diseases and thereby a better resistance to muscle wasting due to disease. Cytokine production capacity is related to the incidence and severity of infectious diseases.[34] In rural Ghana, infectious diseases are the main causes of death[18] and we have earlier observed in this research area that carriers of a proinflammatory *IL10* gene haplotype have a survival advantage when drinking from pathogen-rich sources like open wells and rivers.[12] Others have found another anti-inflammatory genetic variant associated with a higher IL10 production capacity to be more prevalent among tuberculosis patients compared with healthy controls in Gambia.[35] Such a mechanism could explain why no relation between a SNP on the *IL10* gene and handgrip strength was found in a European population living in an environment where the pathogenic burden is relatively low.[36]

Infectious diseases and malnutrition, which are common in this research area,[18,21,37-39] are closely associated with

underweight.[23] In an attempt to account for the possible effects of ill health on handgrip strength, we repeated the analyses after exclusion of individuals with underweight. Among individuals with a normal BMI, we found an equal relation between the proinflammatory haplotype and a stronger relation between the anti-inflammatory haplotype and muscle strength. Moreover, we found that the haplotypes that were associated with handgrip strength were not associated with BMI. These findings indicate that the relation between *IL10* gene variants and handgrip strength is unlikely to be largely shaped by differences in health.

Although *IL10* gene variants were related to handgrip strength, the production capacity of IL10 was not associated with handgrip strength. As a possible explanation, monocytes activated by a proinflammatory stimulus like LPS migrate into injured muscle tissue and change only after two days into macrophages with an anti-inflammatory phenotype.[2] We measured the cytokine production capacity of IL10 24 hours after stimulation with LPS, which could have been too early to measure the maximum IL10 production capacity. In addition, IL10 is known to have autoregulatory effects, as it strongly inhibits IL10 mRNA synthesis in LPS-activated monocytes.[40] This could have diluted the IL10 production capacity measurement. Another explanation is that the relation might be confounded. Firstly, depletion of muscle tissue by malnutrition or disease could have disrupted the beneficial role of IL10 production capacity in muscle repair and maintenance. As BMI is likely to reflect muscle mass in this lean population, the stronger relation between *IL10* gene variants and handgrip strength in the higher BMI stratum points at this possibility. Secondly, infectious diseases might have interfered with our measurements of IL10 cytokine production capacity. Earlier, we have shown that infectious diseases are highly endemic in the research area and induce a proinflammatory immune response.[39] The relation between the *IL10* gene variants and cytokine production capacity was less outspoken in the smaller group with available data on handgrip strength, possibly due to such environmental factors.[25] Thirdly, physical activity attenuates the production capacity of monocytes[41] but meanwhile improves muscle strength.[42] In this population, physical activity is of vital importance due to the manual labour in farming and housekeeping that is necessary for subsistence up to the highest ages. Fourthly, while we measured cytokine production in whole blood, muscle tissue is recognised to be a cytokine producing organ itself. Although little has been reported about the muscle-specific production of IL10, the *IL10* gene might exert its effects in muscle tissue in an autocrine and paracrine manner.[43] Such a mechanism would not be revealed by the analysis of cytokine production capacity in whole-blood samples.

While we observed no relation between IL10 production capacity and handgrip strength, we observed a positive relation between TNFα production capacity and handgrip strength in men, but not in women. This finding is in agreement with previous research in Europeans.[6] A men-specific positive relation has been found between

TNFa production capacity upon stimulation with LPS, a toll-like receptor 4 (TLR4) agonist, and muscle mass and strength. A women-specific positive relation has been found between TNFα production capacity upon stimulation with Pam3Cys-SK4, a TLR2/1 receptor agonist, and muscle mass. These findings indicate that in skeletal muscle tissue, the TLR4 pathway is predominant in men and the TLR2/1 pathway is predominant in women.

Our study has some limitations. Firstly, as in all genetic association studies, we cannot exclude that the *IL10* gene variants are in linkage disequilibrium with variants of other genes that affect handgrip strength. However, we have previously described that this is unlikely, because resequencing of the *IL10* gene region and its surroundings did not result in any variants additional to the SNPs that were genotyped in the *IL10* gene.[12] Secondly, we did not document possible epigenetic variation in the *IL10* gene. There is growing evidence that caloric intake and dietary composition modify epigenetic marks,[44,45] which can influence transcription of the *IL10* gene in immune cells.[46] Malnutrition, being common in the research area,[18,21,37,38] could thereby affect the relation between the *IL10* gene and muscle strength. Lastly, this is a cross-sectional study, while it would be valuable to associate *IL10* gene variants with longitudinal decline in handgrip strength with age.

In conclusion, this study shows that *IL10* gene variants associate with the production capacities of IL10 and TNFα and strongly relate to handgrip strength in rural Africa. A haplotype reflecting a proinflammatory immune response associates with higher muscle strength, while a haplotype reflecting an anti-inflammatory immune response associates with lower muscle strength, especially after exclusion of individuals with underweight. Future studies are needed to elucidate whether variants of the *IL10* gene determine handgrip strength through their role in the repair of skeletal muscle tissue directly or indirectly through their role in the defence against infectious diseases.

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