A Quantitative Trait Locus on 13q14.2 for Trunk Strength

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Previous findings show strong evidence for the role of retinoblastoma (Rb) in myoblast proliferation and differentiation. However, it is not known whether variation in the retinoblastoma gene (*RB1*) is responsible for normal variation in human muscle strength. Therefore, a linkage analysis for quantitative traits was performed on 329 young male siblings from 146 families with muscle strength, using a polymorphic marker in *RB1* (D13S153 on 13q14.2). Trunk strength, a general strength indicator that requires activation of large muscle groups, was measured on a Cybex TEF isokinetic dynamometer. We found evidence for linkage between locus D13S153 at 13q14.2 and several measurements of trunk flexion with LOD scores between 1.62 and 2.78 ($.002 < p < .0002$). No evidence for linkage was found with trunk extension. This first exploration of the relationship between *RB1* and human muscle strength through linkage analysis warrants efforts for further fine mapping of this region.

At all ages, an adequate muscular fitness is essential for activities of daily living (Lamoureux et al., 2002). The inability to perform domestic tasks, the increasing number of falls and (hip) injuries with ageing, the lower back problems in sedentary Western society and the insufficient strength to perform well in sports are just a few examples demonstrating the importance of strong and healthy muscles. It is well established that a large genetic component is involved in interindividual variation in muscle strength (30%–90%; Huygens et al., 2004; Loos et al., 1997; Pérusse et al., 1987; Thomis et al., 1998). However, the 'Human gene map for performance and health-related fitness phenotypes' (Pérusse et al., 2003) indicates that only a few studies have reported significant association of allelic variants in potent quantitative trait loci (QTL) with muscle strength characteristics, but hardly any of these findings have been replicated. Moreover, a biological or physiological explanation of how the QTL might influence the strength trait is often lacking or unclear (e.g., vitamin D receptor [*VDR*; Geusens et al., 1997] and angiotensin converting enzyme [*ACE;* Folland et al., 2000]). In contrast, the insulin-like growth factor 1 (*IGF1*) gene, is one of the few identified QTLs for fat-free mass with a clear biological rationale, proven by both linkage and association approaches (Sun et al., 1999).

Retinoblastoma (*RB1*) has a threefold function in myogenesis: the phosphorylation status of Rb regulates myoblast proliferation, mediation of terminal cell cycle exit and differentiation, and control of cell survival (DeCaprio et al., 1992; Gu et al., 1993; Langley et al., 2002; Rosenthal & Cheng, 1995; Thomas et al., 2000). Furthermore, the importance of *RB1* for muscle mass can be deducted from its central role in three pathways or interactions with muscle regulatory proteins: first, the myostatin pathway, known for its dramatic effect on muscle mass development in animals (Bogdanovich et al., 2002; Grobet et al., 1997; Langley et al., 2002; McPherron et al., 1997; Thomas et al., 2000), second, the interaction of Rb with MyoD and subsequent regulation of myoblast differentiation (Gu et al., 1993), and third, opposing early and late effects of *IGF1* on the phosphorylation status of Rb (Rosenthal & Cheng, 1995).

Considering the importance of an adequate muscular fitness for public health and the lack of in-vivo studies about the role of Rb in human skeletal muscle, a linkage analysis between a marker located in the retinoblastoma gene (D13S153 on 13q14.2) and a general muscle strength indicator (trunk strength) was performed within the Leuven Genes for Muscular Strength Study. Written informed consent was given by each participant.

Trunk strength is a general strength measure that involves activation of large muscle groups. Maximal concentric trunk flexion and extension tests were taken from 329 young (17 to 36 years) male Caucasian volunteers from 146 families on an isokinetic dynamometer (Cybex TEF). Peak torque (Nm), work (J) and average power (W) were measured at

Received 14 July, 2004; accepted 23 August, 2004.

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Table 1

Linkage Results of Trunk Flexion in 329 Male Siblings with Marker D13S153 (RB1)

Trait	LOD score	*p*-value
Torque at 30° at 60°/s	2.78	.0002
Torque at 30° at 75°/s	0.98	.02
Torque at 30° at 120°/s	0.18	.2
Total work at 60°/s	1.06	.013
Total work at 75°/s	1.9	.002
Total work at 120°/s	0.56	.05
Average power at 60°/s	1.62	.003
Average power at 75°/s	0.79	.03
Average power at 120°/s	0.25	.14

60°/s (3 repetitions), 120°/s (5 repetitions) and 75°/s (25 repetitions). Peak torque was also measured at 30° during the flexion and extension movements and tests were taken following the guidelines of the manufacturer. Skinfolds, circumferences, lengths and widths were taken to determine body dimensions and composition (Huygens et al., 2004).

DNA was extracted from EDTA whole blood samples and microsatellite marker D13S153 (CA repeats) was genotyped using the following primers: 5' – FAM – CATGTTGGTGTACGTCTATACAG – 3' (forward), and 5' –CAGCAGTGAAGGTCTAAGCC – 3' (reverse). Thermal cycling conditions were: 94°C for 5 min and 30 cycles of 94°C × 30 sec, 60°C × 45 sec and 72°C × 1 min, followed by a final extension of 7 min at 72°C. Amplified PCR fragments were loaded on an ABI 3100 Genetic Analyzer (Applied Biosystems) and size calling was performed by the Genescan software (v.3.7).

Linkage analysis was performed with the Merlin software package (Abecasis et al., 2002) using a non-parametric quantitative trait statistic, implemented in the general framework of Whittemore and Halpern (1994) and Kong and Cox (1997; –qtl option). The maximum LOD scores of 2.78 (p = .0002) and 1.9 (p = .002) were obtained for trunk flexion torque in 30° at 60°/s and total work at 75°/s trunk flexion respectively with D13S153 (Table 1). In addition, average power at trunk flexion 60°/s also showed suggestive evidence for linkage (LOD = 1.62; p = .003) with the same marker.

Significance of a within-family component of association effect of the D13S153 marker was tested based on the orthogonal decomposition of between- and within-family components of association effects in a variance-components framework using all available sibling data in QTDT (Abecasis et al., 2000). An empirical global *p*-value is reported for all alleles at the microsattelite marker (Table 2), using 1000 Monte-Carlo permutations. The chi-square test for the within-family component of association was not significant at the alpha level of .05 level for any of the trunk flexion strength variables, although a trend might be observed for the maximal torque, total work and average power in truck flexion strength at 75%.

These results strongly suggest that variants in or near the candidate gene *RB1* can explain part of the interindividual variance of trunk strength in young male adults, although no strong evidence is found that alleles at the D13S153 marker would act as the functional QTL itself. Such variants may act by affecting the phosphorylation status of the tumor suppressor Rb: phosphorylated Rb promotes myoblast proliferation and hence muscle growth, whereas hypophosphorylated Rb promotes cell cycle exit and differentiation.

Trunk extension strength did not reveal any signal of linkage (p > .3 for all measurements). The trunk extensor muscles are predominantly postural muscles (to keep the back straight) — and differences in daily use of the trunk flexor musculature might induce larger interindividual differences. Therefore the effect of the *RB1* genotype might be larger for the flexors and hence easier to detect by linkage with these phenotypes. The coefficients of variation confirm the larger variability in trunk flexion strength (average CV = 25.2) than in trunk extension strength (average CV = 18.5).

Since this was the first explorative study to examine the role of *RB1* in skeletal muscle strength in humans, only a single-point linkage analysis was performed. The current results require confirmation by a linkage study using a denser marker map in a 10 cM region surrounding *RB1*. At this point, no guarantee can be given that the *RB1* gene itself is indeed a causal QTL. The standard error of a chromosomal location estimate based on a linkage analysis of complex traits is large (Roberts et al., 1999) and further association tests, or possibly functional analysis, would be required to prove causality. In addition, retinoblastoma has a direct effect on muscle mass, but accurate measures of regional muscle mass of the trunk flexor and extensor musculature could not be

Table 2

Test for Within-Family Association Effect* of Marker D13S153 (RB1) on Trunk Flexion Strength in 329 Male Siblings

Trait	Chi² (7 *df*)	*p*-value
Torque at 30° at 60°/s	7.96	.2
Torque at 30° at 75°/s	9.8	.1
Torque at 30° at 120°/s	8.53	.2
Total work at 60°/s	7.86	.2
Total work at 75°/s	11.14	.09
Total work at 120°/s	9.34	.2
Average power at 60°/s	8.17	.2
Average power at 75°/s	12.77	.06
Average power at 120°/s	9.13	.2

Note: *Orthogonal decomposition as described by Abecasis et al., 2000. Model for the variances is V = Ve + Vg + Va (Ve = nonshared environmental effects, Vg = polygenic effects and shared environmenal effects, Va = additive major gene effect). Empirical *p*-values calculated using 1000 Monte-Carlo permutations.

https://doi.org/10.1375/twin.7.6.603 Published online by Cambridge University Press

derived based on anthropometric measurements. Therefore, it would be interesting to study CT-scan based or MRI measures of back and abdominal muscle cross-sectional areas in relation to *RB1*.

In conclusion, animal and in-vitro studies show that retinoblastoma is an important regulator of the myoblast cycle. When Rb is hyperphosphorylated, it activates other cell cycle genes for DNA synthesis and cell proliferation, hence, muscle can grow and strength may increase. However, no in-vivo studies have been reported that examined the relationship between *RB1* and human muscle strength. Our results suggest that the chromosomal region of *RB1* (13q14.2) contains a QTL for human muscle strength; however, alleles at the D13S153 locus do not seem to be significantly associated with trunk flexor or extensor strength.

Acknowledgments

This study and W. H. were supported by grant OT/98/39 of the Research Fund of the Katholieke Universiteit Leuven. J. Aerssens is presently affiliated with the Drug Discovery Unit, Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium.

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https://doi.org/10.1375/twin.7.6.603 Published online by Cambridge University Press

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