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Association between MCT1 T1470A polymorphism and climbing status in Polish and Japanese climbers

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ABSTRACT: Sport climbing will become an official event at the 2020 Tokyo Olympics; it is a popular wilderness sport among athletes and amateurs. Our previous study suggested that the T1470A polymorphism (rs1049434) of the monocarboxylate transporter 1 (MCT1) gene is associated with athletic performance and physiological phenotypes. The purpose of this study was to investigate the frequency of MCT1 T1470A polymorphism in Polish and Japanese climbers using a case-control study. Our sample consisted of 226 climbers (Japanese: n = 100, 64 male and 36 female; Polish: n = 126, 97 male and 29 female) and 1028 non-athletic controls (Japanese, n = 407; Polish = 621) who were genotyped for the MCT1 T1470A polymorphism (rs1049434) using the TaqMan SNP genotyping assay or restriction enzyme. The frequency of the TT genotype and T allele was significantly higher in climbers than in controls among the Polish subjects (genotype: p = 0.030, allele: p = 0.010); however, there were no significant differences in the genotype and allelic frequencies between the Japanese climbers and controls (genotype: p = 0.968; allele: p = 0.803). Our results suggested that the frequency of the T allele (TT+TA genotype) in the MCT1 T1470A polymorphism is over-represented in Polish climbers but not in Japanese climbers. In addition, the frequency of the T allele and TT genotype in Polish lead climbers is higher than that in controls.

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INTRODUCTION

Sport climbing will become an official event at the 2020 Tokyo Olympics; it is a popular wilderness sport among athletes and amateurs, alike. Competitive climbing is divided into three disciplines: bouldering, lead climbing, and speed climbing. Bouldering and lead climbing have been widely popular. Bouldering consists of short technical routes (called 'problems') on a 4-m high wall; bouldering with shorter bouts of activity is more 'explosive' than lead climbing (30 s vs. 2–7 min) [1, 2]. A previous study suggests that boulder climbers possess greater finger-flexor maximal muscle strength and rapid force capacity compared to lead climbers [3]. Another study showed that the time to fatigue during an intermittent test for failure, using an open crimp at 40% maximal voluntary contraction (MVC), was significantly higher in lead climbers compared to that in boulderers [4]. This physiological response suggests that bouldering requires higher strength and rapid force capacity [3, 5, 6]. Previous research also demonstrated that increasing the difficulty of these sporting activities also increases the levels of blood lactate [7, 8]. Gajewski et al. [9] reported that the estimated lactate clearance in the blood during pre-climbing and at 3 min and 30 min post-climbing correlated significantly with the self-reported climbing ability. From these previous studies, it is clear that various factors determine the performance of competitive climbers, such as strength and endurance capacity (recovery from fatigue).

Athletic performance involves a complex phenotype which is determined by numerous environmental factors such as diet, nutrition, and physical training [10]. However, genetic factors also affect athletic performance. The relationship between genetic polymorphisms and athletic performance has been previously reviewed [11]; ACTN3 R577X is a widely known genetic polymorphism associated with athletic performance [12, 13]. Studies have reported that elite sprint/power athletes have a higher frequency of the RR+RX genotype than the controls. However, our research is the first to study the association between genetic polymorphisms and the climbing status of athletes [14]. Our previous study investigated the frequency of ACTN3 R577X between boulderers, lead climbers, and controls; we concluded that the frequency of the ACTN3 RR genotype is significantly

higher in boulderers than in lead climbers and controls [14]. Ethnic differences can also influence the genetic effects on athletic performance and other phenotypes. Previous studies have suggested that such effects may come into play for some ethnicities, but not for others [15, 16].

Monocarboxylate transporter 1 (MCT1) is a sarcolemmal lactate/proton cotransporter which transports blood lactate into type I muscle fibres and is mainly present in oxidative muscle fibres. The MCT1 T1470A polymorphism (rs1049434) is associated with the rate of blood lactate transport; carriers of the minor T allele have a 35%–40% slower lactate transport rate than non-carriers [17]. Previous studies have suggested that the T1470A polymorphism of MCT1 is also associated with physiological phenotypes [18]. Fedotovskaya et al. [19] suggested that the frequency of the A allele and AA genotype was significantly higher in endurance-oriented athletes. We have reported that the AA genotype is associated with athletic status of Japanese wrestlers [20]. In addition, our previous study suggested that athletes with the AA genotype had lower blood lactate concentrations than those with the T allele during/after the Cycling Repeat Sprint Test and 10 min after the Wingate Test [20]. Cupeiro et al. [21] demonstrated the same trend for high intensity circuit training. Carriers of the A allele are considered to have a faster lactate transport rate in such as type I muscles, allowing the utilization of lactate for energy production.

Thus, we hypothesised that (1) lactate recovery and utilization are the key for analysing climbing performance and (2) the MCT1 AA genotype is associated with climbing performance in the two ethnicities (Polish and Japanese). The purpose of this study was to investigate the frequency of MCT1 T1470A polymorphism in Polish and Japanese climbers using a case-control study.

MATERIALS AND METHODS

Subjects

The present study consisted of 226 climbers (Japanese: n = 100: 64 male and 36 female; Polish: n = 126, 97 male and 29 female) and 1028 non-athletic controls (Japanese: n = 407; Polish: n = 621). A self-reported climbing score in each event (Bouldering and Lead climbing) was submitted by the climbers via a questionnaire. These scores were converted to the IRCRA Reporting Scale (IRS) scores in accordance with the high IRCRA score standards of the International Rock Climbing Association [22]. Based on the higher IRCRA score between Bouldering and Lead climbing, climbers were segregated into three groups: Higher elite (male: more than 28, female: more than 27), Elite (male: 24 to 27, female: 21 to 26), and Advanced (male: 18 to 23, female: 15 to 20). The suggested climbing levels are shown in Table 2. The classification of the event (Bouldering or Lead climbing) was used for IRCRA scoring; if the IRCRA score for boulder climbing was higher than that for lead climbing, the score was classified as Boulder and vice versa. The study protocol was approved by the ethics committees of the Nippon Sport Science University and Medical University of Lublin.

Genotyping

Japanese climbers and controls

The total DNA was extracted and isolated from the saliva of the participants using an Oragene-DNA Kit (DNA Genotek, Ontario, Canada). The MCT1 T1470A polymorphism (rs1049434) was genotyped using TaqMan SNP Genotyping Assay (Assay ID: C___2017662_30) with the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). The genotyping mixture (total volume: 5 μL) contained 2.5 μL of GTXpress Master Mix, 0.125 μL of assay mix (40X), and 1.375 μL of distilled water with 1 μL of genomic DNA (10 ng/μL) per reaction. The thermal cycling conditions included an initial denaturation at 95°C for 20 s, followed by 40 cycles of denaturation at 95°C for 3 s and annealing/extension at 60°C for 20 s. Genotype calls were made based on analysis of the TaqMan assay results using StepOne Software v2.1 (Applied Biosystems).

Polish climbers and controls

Polish climbers were genotyped with the same method as for Japanese climbers and controls. DNA of Polish controls were extracted from the buccal cells using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the manufacturer's instructions. The samples were genotyped for the MCT1 T1470A polymorphism by PCR and restriction enzyme digestion. Forward primer: 5'-AGCAAACGAGCAGAAAAAGG-3', reverse primer: 5'-CTGGGTCATGAACTGCTCAA-3'. Using the primers and the missense polymorphism (rs1049434) described by Cupeiro et al. [9] we searched the sequence against GenBank. Using the Neb Cutter tool (http://tools.neb.com/NEBcutter2/index.php), we indicated the restriction enzyme BccI as the one that allows one to evaluate the presence or absence of missense polymorphism T1470A. The sequence 3′-GGTAG-5′ is recognized by the enzyme that creates three fragments in the mutant sequence (TT: 14, 171, and 202 bp), whereas only two fragments (AA: 14 and 373 bp) are developed when the wild sequence is considered. Four fragments – 14, 171, 202, and 373 bp – were characteristic in the case of TA heterozygotes.

Statistical analyses

χ^2 analysis was used to confirm the observed genotypic frequencies in the Hardy–Weinberg equilibrium and to test whether the rs1049434 genotype frequencies (AA, TA and TT) differ between climbers and controls. In addition, genotype frequencies between the males and females of both ethnicities, and the frequency of the athletic status (Higher elite, Elite, and Advanced), were compared between Polish and Japanese climbers using χ^2 analysis. An unpaired t-test was used to confirm the differences in the characteristics, athletic scores (IRCRA), and athletic experiences between Polish and Japanese climbers. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate the degree of contribution of the MCT1 gene T1470A polymorphism (dominant, recessive and additive models). Significance was set at $p < 0.05$.

RESULTS

Polish climbers had a significantly greater height, weight, and BMI than Japanese climbers (Table 1). Furthermore, the climbing experience, IRCRA score, and athletic status were significantly higher in Polish than in Japanese climbers ($p = 8.47^{-5}$, $p = 3.66^{-14}$, $p = 1.46^{-13}$, respectively; Table 2). The MCT1 T1470A genotypic frequencies among climbers and control participants were in Hardy–Weinberg equilibrium (Polish control: p = 0.96, Polish climbers: p = 0.38; Japanese control: p = 0.98, Japanese climbers: p = 0.97). Demographic characteristics including age, height, weight, and experience among MCT1 T1470A genotypes were not significantly different between Polish and Japanese climbers (data are not shown). In Polish subjects, the frequency distributions of the AA, TA and TT genotypes in MCT1 were 41, 46 and 13% in the control group and 29, 53 and 18% in climbers, respectively (Table 3). The frequency of the TT genotype and T allele was significantly higher in climbers than in controls among the Polish subjects (genotype: p = 0.030, allele: p = 0.010). Similarly, the frequency of the TT genotype and T allele was higher in Polish lead climbers than in controls (genotype: p = 0.012, allele: p = 0.003). In the Japanese group, the frequency distributions of the AA, TA, and TT genotypes in MCT1 were 48%, 43%, and 9% in the controls and 47%, 43%, and 10% in climbers, respectively (Table 3). There were no significant differences in the genotypic and allelic frequencies between Japanese climbers and controls. Additionally, there was no significant difference between the sexes in both ethnic groups (data not shown). The odds ratio of each genetic model is shown in Table 4 (Dominant model: Polish: 1.71 (1.13–2.59), p = 0.0096; Japanese: 1.05 (0.68–1.62), p = 0.84. Recessive model: Polish: 1.47 (0.88–2.47), p = 0.15; Japanese: 1.08 (0.52–2.25), p = 0.84, p = 0.84. Additive model: Polish: 1.44 (1.09–1.90), p = 0.011; Japanese: 1.04 (0.75–1.46), p = 0.80).

TABLE 1. Characteristics of Polish climbers and Japanese climbers.

	Polish	Japanese	P value
Male	(n = 97)	(n = 64)	
Height (cm)	178.1 ± 5.8	169.0 ± 5.9	**9.77×10^{-18}**
Weight (kg)	69.1 ± 11.4	59.0 ± 5.7	**1.04×10^{-9}**
BMI	21.7 ± 2.7	20.6 ± 1.5	**0.003**
Female	(n = 29)	(n = 36)	
Height (cm)	163.8 ± 4.8	158.7 ± 6.1	**0.0006**
Weight (kg)	54.0 ± 4.1	49.5 ± 5.9	**0.001**
BMI	20.1 ± 1.0	19.6 ± 1.5	0.15

TABLE 2. Athletic experience and athletic status in Polish climbers and Japanese climbers.

	Polish n = 126	Japanese n = 100	P value
Experience (year)	12.2 ± 5.3	9.0 ± 6.8	**8.47×10^{-5}**
IRCRA score	25.7 ± 2.1	22.8 ± 3.3	**3.66×10^{-14}**
Athletic status			
High Elite, n (%)	24(19)	2(2)	
Elite, n (%)	102(81)	64(64)	**1.46×10^{-13}**
Advanced, n (%)	0(0)	34(34)	

TABLE 3. Frequency of genotype and allele of MCT1 T1470A in Polish and Japanese climbers and controls.

	genotype			p value*	Allele		p value*
	AA	TA	TT		A	T	
Polish cohort							
All Climbers (n = 126)	37(29)	67(53)	22(18)	0.030	141(56)	111(44)	0.010
Lead climbers (n = 52)	13(25)	26(50)	13(25)	0.012	52(50)	52(50)	0.003
Boulderers (n = 74)	24(33)	41(55)	9(12)	0.268	89(60)	59(40)	0.296
Controls (n = 621)	258(41)	285(46)	78(13)		801(64)	441(36)	
Japanese cohort							
All Climbers (n = 100)	47(47)	43(43)	10(10)	0.968	137(69)	63(31)	0.803
Lead climbers (n = 20)	7(35)	11(55)	2(10)	0.498	25(62)	15(38)	0.356
Boulderers (n = 80)	40(50)	32(40)	8(10)	0.915	112(70)	48(30)	0.882
Controls (n = 407)	196(48)	173(43)	38(9)		565(69)	249(31)	

* compared with control. The frequency of TT genotype and T allele were no significant difference between lead climbers and boulderers in Polish and Japanese respectively (Polish: genotype, p = 0.163, allele, p = 0.111, Japanese: genotype, p = 0.449, allele, p = 0.361).

TABLE 4. Odds ratio of each genetic model (dominant, recessive and additive) in Polish and Japanese climbers

	Polish			Japanese		
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Dominant						
AA	1.00	1.13–2.59		1.00	0.68–1.62	
TA+TT	1.71		0.0096	1.05		0.84
Recessive						
AA+TA	1.00	0.88–2.47		1.00	0.52–2.25	
TT	1.47		0.15	1.08		0.84
Additive	1.44	1.09–1.90	0.011	1.04	0.75–1.46	0.80

OR: Odds Ratio, CI: Confidence interval

DISCUSSION

The present study investigated the association between the MCT1 T1470A polymorphism and the climbing status of Japanese and Polish using a case-control study. We found that the frequency of the T allele in the MCT1 T1470A polymorphism is over-represented in climbers (44%) than controls (36%) in Polish, but not in Japanese climbers (31%) and controls (31%, $p = 0.803$). We hypothesised that the allelic frequency of MCT1 T1470A AA is higher in climbers with better blood lactate clearance. Competitive climbing has the characteristics of a high-intensity intermittent and endurance exercise: repeated climbing and rest periods in bouldering, with climbers attempting to climb as high as they can on a wall higher than 15 m in lead climbing. In addition, climbing on steeper walls requires the use of anaerobic energetic pathways [8]. In contrast to our hypothesis, the T allele frequency in Polish climbers was higher than that in controls. In addition, our results were observed only in Polish subjects; therefore, we concluded that the association between a genetic variant and athletic performance might be dependent on the ethnic background of the population.

Several case-control studies have reported an association between the MCT1 T1470A polymorphism and the athletic status [19, 20, 23, 24]. Guilherme et al. [23] suggested that the AA genotype in MCT1 T1470A polymorphism (homozygous for major allele) is associated with sprint/power performance. Our previous study suggested that wrestlers who required high-intensity intermittent bouts have higher frequencies of AA genotypes as compared to controls [20]. In addition, we have reported that the T-allele carriers have a higher blood lactate concentration following the 5th set and final set of repeated cycling sprints, 10 sets of 10 s sprints (50 s recovery), than those of AA genotype (5th set: 11.4 ± 2.6 vs. 13.7 ± 4.2, final set: 13.6 ± 3.3 vs. 16.2 ± 3.5, $p < 0.05$). However, the opposite result was observed in power-oriented athletes [24]. Furthermore, frequencies of the A allele and AA genotype have been found to be significantly higher in endurance-oriented athletes than in the control group [19]. The superior lactate clearance ability of MCT1 T1470A AA genotype carriers relative to T-allele carriers may impact athletic performance.

Several studies have measured blood lactate concentrations after and during climbing exercises. Studies have reported the blood lactate concentration to be about 5 mmol/L after the lead climbing exercise [1, 25–27]. Torre et al. [28] investigated blood lactate concentration before and at 2, 4, 6, 8 min after bouldering in national competitions. Their results showed that the peak blood lactate accumulation after the competition was 6.6 ± 1.1 mmol/L, which was in line with a previous study in lead climbers [25]. The lactate concentration after lead climbing and bouldering was lower than that after previously reported intermittent exercises and resistance training [20, 21]. Thus, we can assume that climbing performance cannot be affected by blood lactate clearance.

Fryer et al. [4] determined a climbing specific measure of strength in the finger flexor maximal volitional contraction. The apparatus has a climbing specific handhold attached to a load cell which, once calibrated, can determine the specific strength and endurance in the finger and wrist flexors while climbing. The MVC was found to be significantly greater in lead climbers and boulderers as compared to that in controls. In addition, a study on the morphological characteristics suggested that climbers had better developed muscles in the forearm and lower leg as compared to controls [29]. Furthermore, Massidda et al. [30] suggested that the percentage of fat-free mass was significantly higher in soccer players with the TT genotype and in the T-allele compared to the AA genotype. Several previous studies concluded that metabolites can lead to stimulation of the muscle hypertrophy system [31, 32]. The metabolites produced by resistance training, such as blood lactate, lead to acidification and ultimately activation of chemoreceptors stimulating the release of growth hormone (GH) in the hypothalamic pituitary system [31]. Goto et al. [32] examined the effect of exercise-induced metabolic stress on hormonal responses and chronic muscular adaptations. They compared

the acute and long-term effects of a no-rest regimen (NR: 3–5 sets of 10 repetitions at 10-repetition maximum (RM) with an inter-set rest period of 1 min) and those of a regimen with a rest period within a set (WR: completed the same protocol as the NR regimen, but took a 30 s rest period at the midpoint of each set of exercises). In the acute response, the NR regimen, but not the WR regimen, induced strong lactate and caused a marked increase in muscle cross-sectional area. This suggests that metabolites such as blood lactate can cause a much larger increase in muscle cross-sectional area. We might conclude that since the MCT1 T allele showed high levels of lactate accumulation during intermittent exercise including resistance training [20, 21], it is associated with a muscle hypertrophy response. However, the association between MCT1 T1470A polymorphism and blood lactate concentration during or after climbing has not been clarified. In addition, the effect of lactic acid on hypertrophy in humans also remains unknown.

Herein, the frequency of the MCT1 T1470A polymorphism was significantly different between Polish climbers and non-athletic controls. With respect to athletic performance, the athletic status was strongly associated with genetic effects. Furthermore, the IRS score was higher among Polish climbers than among Japanese climbers, and the frequency of the classified athletic status (High Elite, Elite, and Advanced) displayed the same trend. Several studies have reported significant linear correlations between athletic status and candidate genetic variants [12, 13]. Herein, Polish climbers had a significantly greater height, weight, IRCRA score and BMI than Japanese climbers, probably because the requirements regarding muscle function depend on the body weight of professional climbers (Table 1), thus potentially explaining the differences between these two ethnicities in accordance with morphological characteristics, athletic status, and climbing styles such as main climbing goal (rock climbing or competition). In addition, the limitations of this study include the fact that we did not use the same method of genotyping in the two ethnic groups. However, the frequency of MCT1 T1470A polymorphism in this study is in line with European controls in 1000 Genomes Selection Browser 1.0 [33].

CONCLUSIONS

In conclusion, although our results suggest that compared with the controls, the T allele of the MCT1 T1470A polymorphism is over-represented in Polish climbers, we could not replicate these results in the Japanese group. Further research using case-control studies with larger and more diverse climber cohorts are required to investigate the association between genetic factors and climbing performance.

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Conflict of interest

The authors have no conflict of interest to declare.

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