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A comparison between cohorts of different activity level.

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List of abbreviations

1RM	One repetition maximum
A	Adenine
ACE	Angiotensin-converting enzyme
ACTN1, 2, 3, 4	α -actinin-1, -2, -3, -4
BMI	Body mass index = body weight [kg] / (body height [m]) ²
bp	Base pairs
C	Cytosine
EDTA	Ethylenediaminetetraacetic acid
FFM	Fat free mass
G	Guanine
HERITAGE Family Study	HEalth, Risk factors, exercise Training and GEnetics - a huge family study that analyses the role of the genotype in the cardiovascular, metabolic, and hormonal responses to aerobic exercise training realized in the United States and Canada
IGF	Insulin like growth factor
I/D	Insertion/deletion polymorphism
INS	Insulin
LBM	Lean body mass
MS	Microsatellite repeat polymorphism
MSTN	Myostatin
NTC	Negative control

List of abbreviations

PAFQ	Physical Activity Frequency Questionnaire
PAL	Physical Activity Level
SD	Standard deviation
SNP	Single nucleotide polymorphism
T	Thymidine
TGF	Transforming growth factor
TGS	Total genotype score
UTR	Untranslated region
VO ₂	Oxygen uptake
VO ₂ max	Maximal oxygen uptake

1 Aims of the study

In recent years, molecular genetic methods have been improved and analyses of the human genome have become possible. Although humans share over 99.9% of their DNA sequence, 0.1% difference is enough to make every individual unique. It is evident that genetic factors influence our phenotypes. Genetic factors are slight variations in the DNA sequence, often only in the extent of the exchange of one nucleic base. Whereas mutations in the human genome are rare and occur in less than 1% of the world's population, the term "polymorphism" signifies variations in the DNA sequence that are common in more than 1% of the world's population. Polymorphisms appear all 100 to 1000 letters. These loci are called "alleles". The combination of alleles at any variant is known as genotype, which is determined by the genetics of one's parents. A genotype can be homozygote or heterozygote, whereupon homozygote means two copies of the same allele, independently if it is the ancestral or the variant allele. An individual is categorized "heterozygote" for a certain gene locus when he or she carries one allele of each copy (Roth, 2007).

Much work focused on different polymorphisms that might determine physical activity and success in competition (Rankinen, Bray, Hagberg, Perusse, Roth, Wolfarth & Bouchard, 2006a). Huge analyses were carried out in endurance athletes (e.g. the Gen-Athlete Study, Wolfarth, 2002). Comparisons between endurance athletes and sprinters or other power athletes were also performed (Yang, MacArthur, Wolde, Onywera, Boit, Lau, Wilson, Scott, Pitsiladis & North, 2007 and others more) as well as training studies with sedentary individuals (e.g. the HERITAGE Family Study, Bouchard, Leon, Rao, Skinner, Wilmore & Gagnon, 1995). Although even in athletes that can be easily assigned to either endurance or strength dominated sports, the influence of certain gene polymorphisms is discussed due to contradictory data published (Rankinen et al., 2006a). In complex sports as skiing the influence of genetic determinants on success in competition is even more difficult to prove. Nevertheless, it is supposed that there are heritable factors that have an impact on skiing performance.

We speculated that there are gene variants or gene variant constellations that favour success in competition. Therefore, we aimed to screen for some selected gene variants in alpine skiers and sedentary controls to assess demonstrative differences in the frequency of performance-related genotypes between study groups. Five polymorphisms were planned to be analysed, whereof two had been well described in literature and three of them had been underrepresented in published data. As we could

unfortunately not get a representative sample of alpine skiers, the new approach was to compare performance-related genotypes between sedentaries, active people and endurance athletes. For polygenic effects, the concept of the Totale Genotype Score (TGS) originally developed by Williams and Folland (2008) should be used. To optimize and ease sample collection and DNA isolation, different methods were compared in order to find a preferably cheap, fast and for the probands as easy as possible one.

In fact, the work is part of a huge genotype project, which aimed to compare genotype frequencies of over 20 performance-related polymorphisms between sedentaries and athletes of endurance-, strength-dominated, and complex sport disciplines, respectively.

2 Literature review

Until now, genetic variants among excellent alpine skiers have not been described in published data. Only Druzhevskaya, Ahmetov, Astratenkova & Rogozkin (2008) included alpine skiers in their analyses as a subgroup of power athletes. On the one hand, it can be assumed that there are certain heritable factors that favour success in skiing competitions and that the frequency of these favouring gene variants is higher in elite skiers than in sedentary controls. On the other hand skiing is a complex sport and it is not completely clear which skills are crucial for a high skiing performance. A genetic profile can be helpful identifying such performance-determining parameters in skiers.

As already mentioned there exist a huge number of genetic variants throughout the human genome and a part of them is discussed as co-determining factors for physical performance. In fact, there are different types of genetic variants known and the location of the polymorphism is important to its possible impact (Roth, 2007). The most common type of genetic variants is a single nucleotide polymorphism (SNP). In this case, only one nucleic base is changed, for example an adenine (A) is replaced by a guanine (G). Another type is an insertion/deletion (I/D) polymorphism. The insertion allele includes a stretch of specific DNA nucleotides, whereas this part is not present at the deletion allele. The third group of polymorphisms are microsatellite repeats (MS). Short DNA stretches of two or three nucleic bases are repeated at different frequencies. The number of repeats in the DNA stretch identifies the allele. Whereas SNPs and I/Ds have only two different possible alleles, the length of the repeat polymorphism is highly variable and different repeat frequencies are grouped together (Roth, 2007). For example, the IGF1 gene carries a MS polymorphism with 11 to 24 repeats reported, but alleles are identified as 19 microsatellite repeats or repeat numbers different from 19 (see 2.1.2.1).

2.1 Selected polymorphisms related to physical activity and performance

Since the 1990s sport sciences have begun to focus on the impact of gene variants on performance and fitness parameters (Rankinen, Perusse, Rauramaa, Rivera, Wolfarth & Bouchard, 2001). The magnitude of genetic determination for success in different sports remains unknown but heritability for VO_2max is estimated 25-40% (Wolfarth, Boulay, Perusse, Rankinen, Rauramaa, Rivera & Bouchard, 2001). The human gene map for performance and health-related fitness phenotypes annually summarizes candidate genes, which may be partly responsible for performance and fitness parameters (Rankinen et al., 2001; Rankinen, Perusse, Rauramaa, Rivera, Wolfarth & Bouchard, 2002; Perusse, Rankinen, Rauramaa, Rivera, Wolfarth & Bouchard, 2003; Rankinen,

Perusse, Rauramaa, Rivera, Wolfarth & Bouchard, 2004; Wolfarth, Bray, Hagberg, Perusse, Rauramaa, Rivera, Roth, Rankinen & Bouchard, 2005; Rankinen et al., 2006a). The human obesity gene map contains genes that might be responsible for body weight and health parameters related to adiposity and nutritional status (Rankinen, Zuberi, Chagnon, Weisnagel, Argyropoulos, Walts, Perusse & Bouchard, 2006b).

Only few analyses dealt with the interaction of a couple of candidate genes and their impact on performance. The comparison of genetic variants of nine world-class cross-country runners and the cross-country world champion 2007 in seven candidate genes highlighted preferable variants concerning endurance performance (Gonzalez-Freire, Santiago, Verde, Lao, Oivan, Gomez-Gallego & Lucia, 2008). Among others the gene coding for α -actinin-3 (ACTN3), the muscle-specific creatine kinase gene (CKMM) and the myostatin gene (MSTN) were selected and the theoretically optimal genotype for endurance performance was determined (Gonzalez-Freire et al., 2008).

The comparison of the genotype of rowers, runners, road cyclists, and controls in the seven gene variants (ACTN3, angiotensin-converting enzyme (ACE), peroxisome proliferators-activated receptor γ coactivator 1 α , adenosine-monophosphate deaminase, CKMM, MSTN) revealed no significant differences between groups except for the ACE gene variant (Muniesa, Gonzalez-Freire, Santiago, Lao, Buxens, Rubio, Martin, Arenas, Gomez-Gallego & Lucia, 2008).

In the Genathlete Study (Wolfarth, 2002) endurance trained athletes were compared with sedentary controls concerning gene variants in the ACE, the erythropoietin receptor, the CKMM, the endothelial nitrooxid synthetase, and the α -2A-adrenergic receptor gene and others more. Results differed between the selected candidate genes. For example, variants in the erythropoietin receptor gene seemed to have an impact on VO_2max in the study population whereas for the polymorphism in the CKMM gene no differences between study groups were observed (Wolfarth, 2002).

The following selected genes and their described variants were repeatedly discussed in their involvement in endurance or strength performance, carbohydrate metabolism, and physical activity but most published data is controverse and does not allow to draw clear conclusions.

2.1.1 Alpha-actinin-3

One of the best-described polymorphism is the R577X variant (rs1815739) in the gene coding for α -actinin-3 (ACTN3) (Macarthur & North, 2005). Its impact on physical performance is evident according to an emerging number of analyses (Yang et al., 2007;

Ahmetov, Druzhevskaya, Astratenkova, Popov, Vinogradova & Rogozkin, 2008; Druzhevskaya et al., 2008, and others more).

ACTN3 is one of four α -actinin isoforms present in the human organism. Whereas ACTN1 and ACTN4 are non-muscle cytoskeletal, calcium-sensitive isoforms, ACTN2 and ACTN3 are present in muscle tissue and act calcium independently. Both muscle α -actinin isoforms are coded by different genes and they have been highly conserved through evolution. The two muscle-specific isoforms are 80% identical and 90% similar, but whereas ACTN2 is present in all muscle fibers, ACTN3 is exclusively expressed in fast twitch (type FT 2b) muscle fibers. ACTN3 is a predominant component of the Z-discs in type-II-muscle fibers where it anchors actin filaments of the muscle contractile apparatus (North, Yang, Wattanasirichaigoon, Mills, Easteal & Beggs, 1999; Mills, Yang, Weinberger, Vander Woude, Beggs, Easteal & North, 2001; MacArthur & North, 2004).

Two mutations were highlighted in the ACTN3 gene during research on muscle diseases. The Q523R variant (rs1671064) is an A to G transition in exon 15 and is in strong linkage disequilibrium to the R577X polymorphism (rs1815739), which is a C to T transition in exon 16 at residue 577. The R577X variant results in a conversion from an arginine to a stop codon. The presence of the stop codon causes complete ACTN3 deficiency. Nowadays it is clear that the null allele variant (X-allele) does not result in any pathological phenotype but that it has an impact under certain conditions. About 16% of people worldwide carry the XX null genotype and are therefore ACTN3 deficient. It is speculated that in case of ACTN3 deficiency, ACTN2 can compensate for the missing ACTN3 (North et al., 1999; Mills et al., 2001; MacArthur & North, 2004). The frequency of the XX null genotype strongly differs among ethnic groups (North et al., 1999; Yang et al., 2007).

2.1.1.1 ACTN3 R577X (rs1815739) variant and elite athlete status

An Australian cohort consisting of 436 controls, 194 elite endurance athletes as well as 107 sprint and power athletes was screened for their genotype in the ACTN3 gene (Yang, MacArthur, Gulbin, Hahn, Beggs, Easteal & North, 2003). The RR genotype and the R-allele were significantly higher in female and male sprint athletes than in controls and endurance athletes. The XX genotype was found to be slightly higher in endurance athletes than in controls. As there were no differences between the control and the whole athlete group, it seems as the genotype in the sprint/power and endurance group deviated in opposite directions and cancelled each other out. The high frequency of the RR genotype among sprinters and power athletes could be due to an advantageous effect of this genotype on power performance (Yang et al., 2003).

A Finnish study cohort (Niemi & Majamaa, 2005) consisted of 141 elite athletes, among them 40 endurance specialists, 68 sprint athletes and 120 athletes that could not be clearly related to either sprint or endurance dominated sportsmen. Over 1,000 subjects served as control population. The XX genotype was significantly higher in the endurance and the control subgroups than in sprinters indicating an inverse correlation between sprinting performance and the XX genotype. Among the top-level sprinters (participants of European or World Track and Field Championships), no one carried the XX genotype.

Controversely, there were no differences in the ACTN3 genotype found between world-class runners, cyclists, lightweight rowers and a Spain male control group (Muniesa et al., 2008). In a qualitative comparison of seven top endurance runners, the RR or the RX genotype turned out as the theoretically favourable genotype for success. Among nine top-level cross-country runners (finished at least under the best twenty participants of the Cross-Country World Championship for more than one time on the long distance), eight of them carried the RR or RX genotype including the world champion 2007 (Gonzalez-Freire et al., 2008).

In a Greek cohort of 181 control subjects and 101 track and field athletes no significant differences between the groups could be detected, not even when the athlete group was divided into power and endurance oriented athletes (Papadimitriou, Papadopoulos, Kouvatsi & Triantaphyllidis, 2008). Interestingly, when the power athlete group was further divided, a significant higher frequency of the RR genotype as well as a significant lower frequency of the XX genotype was found in the subgroup of only sprint athletes (Papadimitriou et al., 2008). These results were confirmed by the outcomes of Moran, Yang, Bailey, Tsiokanos, Jamurtas, MacArthur, North, Pitsiladis and Wilson (2007). They screened adolescent Greeks for their genotype in the ACTN3 gene as well as for body composition and power/strength related phenotypes. An association with the genotype was only found in male participants for the 40-meter sprint time. Carriers of the XX genotype had significantly lower sprint times and therefore ran faster. As there were no associations found for endurance tests and other power-related tests (e.g. vertical jump, sitting basket throw, handgrip strength) except the 40-meter sprint with the ACTN3 genotype, the researches speculated that the ACTN3 RR genotype favours power generation by repeated cycles of muscle contraction and not force caused by a single muscle contraction (Moran et al., 2007). However, in white and black elite body builders and power lifters the XX genotype was significantly underrepresented in white and not present in black strength athletes (Roth, Walsh, Liu, Metter, Ferrucci & Hurley, 2008).

Studying a Nigerian cohort of control subjects and power athletes revealed no differences between both groups (Yang et al., 2007). As there was neither a control individual carrying

the XX genotype nor any of the power athletes, this genotype distribution could mask any genotype effects between the two cohorts. Results confirm different genotype distributions in different ethnic groups (Yang et al., 2007).

In 468 Caucasian athletes of different power-oriented sports the XX genotype and the X-allele were found in a significantly lower frequency than in about 1,200 Russian control subjects (Druzhevskaya et al., 2008)

Until today, it is not completely clear why ACTN3 deficiency does impair sprint performance, because the null variant does not have any implications in activities of normal living (North et al., 1999). In mouse models, it seems as ACTN3 deficiency results in a more efficient muscle metabolism that may favour endurance performance (North, 2008). However, also the high congenital fiber type distribution could be influenced by the ACTN3 genotype and would explain the strong association with power/sprint performance (Vincent, De Bock, Ramaekers, Van den Eede, Van Leemputte, Hespel & Thomis, 2007). Fortyfour of 90 ACTN3 genotyped and strength tested healthy men underwent a muscle biopsy of the *m. vastus lateralis*. RR homozygotes had significantly higher dynamic quadriceps' torques and greater type IIx fibers than carriers of two nonsense alleles (XX). Furthermore, it was found that the proportion of ACTN3 was higher in type IIx fibers than in type IIa fibers (Vincent et al., 2007). Controversely, North et al. (1999) did not find any impact of the ACTN3 polymorphism on fiber distribution in their initial sample of 125 genotyped and muscle-biopsied subjects. However, it is documented that ACTN2 does not completely overlap ACTN3 expression and that the ACTN2 to ACTN3 ratio varies between different muscle fiber types in mice (Mills et al., 2001).

As some study results indicated that there could be a favourable effect of the ACTN3 XX genotype on endurance performance (Yang et al., 2003; Niemi & Majamaa, 2005), further research was applied on this subject. ACNT3 R577X genotype was determined in 50 professional Spain cyclists, who had finished at least one of the three 3-week stage races Tour de France, Giro d'Italia and Vuelta a Espana, in 52 middle-distance runners who participated in Olympic games and in 123 Caucasian male controls (Lucia, Gomez-Gallego, Santiago, Bandres, Earnest, Rabadan, Alonso, Hoyos, Cordova, Villa & Foster, 2006). Additionally, the gas exchange ration was measured using open circuit spirometry during an all-out test to determine endurance performance. The three study groups did not differ from each other concerning their genotype contribution indicating that the XX genotype does not have any advantageous effect on endurance performance (Lucia et al., 2006).

Since modern middle distance and marathon running is dominated by East Africans, Yang et al. (2007) genotyped 350 Ethiopian and Kenyan elite runners as well as 356 corresponding controls for the R577X polymorphism. Ethiopians and Kenyans both athletes and control subjects differed in their genotype contribution. Whereas the XX genotype was present in 11% of Ethiopians, only 1% of the Kenyans carried the ACTN3 null genotype. In both study populations, the frequency of the XX genotype was lower than in European samples. No differences between the athlete and control subjects were found; neither in Ethiopians nor in Kenyans. After splitting the cohorts into male and female subjects, an genotype effect was neither found (Yang et al., 2007).

Genotype frequency of elite Italian rowers did not significantly differ from controls either concerning the R577X polymorphism or the Q523R variant (rs1671064) in the ACTN3 gene (Paparini, Ripani, Giordano, Santoni, Pigozzi & Romano-Spica, 2007). Therefore, authors concluded that both polymorphisms did not influence endurance performance in men (Paparini et al., 2007). The same results were obtained in 457 Caucasian male triathletes who competed in an Ironman Triathlon. Their ACTN3 R577X genotype distribution did not significantly vary from 143 healthy Caucasian men (Saunders, September, Xenophontos, Cariolou, Anastassiades, Noakes & Collins, 2007).

In 556 Russian athletes competing in endurance-oriented sports ACTN3 R577X genotype frequencies were determined as well as in about 1,200 healthy controls (Ahmetov et al., 2008). Whereas the control study group did not differ from the calculated genotype frequency in Hardy-Weinberg equilibrium¹, the athletes' genotype distribution was significantly different from the predicted one. The XX genotype and the X-allele were significantly underrepresented in endurance athletes compared with Hardy-Weinberg equilibrium as well as with controls. It is speculated that even if the ACTN3 577X genotype may confer an advantageous effect for the aerobic metabolism (Ahmetov et al., 2008; North, 2008), in modern endurance sport at the international top level, also forthful muscle contractions at high velocities are necessary for success (Lucia et al., 2006; Ahmetov et al., 2008).

2.1.1.2 ACTN3 R577X variant (rs1815739) and response to training

As the anteceded chapter focused on the ACTN3 R577X genotype (rs1815739) and elite athlete status, the genotype effect on physical performance was also highlighted in untrained individuals, often in combination with a training intervention:

¹ The Hardy-Weinberg Equilibrium theorem was assessed to calculate the behaviour of two alleles at a single gene locus in a study population. Nowadays it is used in association studies to detect genotyping errors and to disease susceptibility (Ryckman & Williams, 2008, p. 1)

Walsh, Liu, Metter, Ferrucci and Roth (2008) screened 848 subjects aged from 22 to 90 years for their genotype of the ACTN3 R557X polymorphism and strength in the knee extensor muscle. Lower eccentric and concentric strength levels were found in XX homozygotic women as well as lower FFM. No differences between genotype groups and strength values were documented in men (Walsh et al., 2008).

In a training study, initial strength was evaluated in about 600 genotyped subjects (Clarkson, Devaney, Gordish-Dressman, Thompson, Hubal, Urso, Price, Angelopoulos, Gordon, Moyna, Pescatello, Visich, Zoeller, Seip & Hoffman, 2005). After a strength-training period of 12 weeks, strength measurements were repeated. In the male subgroup, no differences between baseline strength and response to training were found between genotype groups, whereas in females ACTN3 577X homozygotes had the lowest baseline strength levels. Interestingly, the highest values were not found in RR individuals but in heterozygotes. After the training period females carrying the XX genotype had the highest increases in 1RM absolute strength as well as relative to their body weight (Clarkson et al., 2005).

In contrast, in 102 genotyped elderly no differences in baseline strength could be detected concerning the ACTN3 R577X genotype either in men or in women (Roth, Delmonico, Rabon-Stith, Karma, Walsh & Hurley, 2005). In response to a 10-week strength-training program, XX homozygotic and heterozygotic men increased their relative and absolute peak power significantly less than RR homozygotes. No differences were observed in females (Roth et al., 2005). Whereas Roth et al. (2005) trained the knee extensor muscle, Clarkson et al. (2005) focused on the elbow flexor.

In 120 trained individuals, no association between the ACTN3 R577X genotype with 30-s Wingate cycling as well as with knee extensor strength and fatigability during isokinetic exercise was found (Norman, Esbjornsson, Rundqvist, Osterlund, von Walden & Tesch, 2009). As there were differences between RR and XX homozygotes in the peak torque after repeated bouts of exercise found, it was speculated that the polymorphism would influence training response. Additional muscle biopsies revealed an association between the polymorphism and the ACTN2 expression indicating that ACTN2 may compensate for the lack of ACTN3 in carriers of the stop codon (X). No differences in the muscle fiber composition was observed between genotype groups (Norman et al., 2009).

2.1.2 Insulin like growth factors

Insulin like growth factors (IGFs) are polypeptides that share structural and functional homologies with insulin. Whereas the insulin like growth factor 1 (IGF1) is regulated by the growth hormone, the insulin like growth factor 2 (IGF2) is not affected by the growth

hormone. In fact, the primary regulator of IGF2 expression remains unknown. Several other hormones, as for example estrogens and adrenocorticotrophic hormone, have an impact on IGF levels as well as nutrition (Yu & Rohan, 2000).

IGF1 has an anabolic effect via an increased amino acid and glucose uptake into the cell. IGF1 further regulates cell proliferation, cell differentiation and cell apoptosis. IGF1 levels increase until puberty and decline with age thereafter (Yu & Rohan, 2000; Allen, Davey, Key, Zhang & Narod, 2002; Canzian, McKay, Cleveland, Dossus, Biessy, Rinaldi, Landi, Boillot, Monnier, Chajes, Clavel-Chapelon, Tehard, Chang-Claude, Linseisen, Lahmann, Pischon, Trichopoulos, Trichopoulou, Zilis, Palli, Tumino, Vineis, Berrino, Bueno-de-Mesquita, van Gils, Peeters, Pera, Ardanaz, Chirlaque, Quiros, Larranaga, Martinez-Garcia, Allen, Key, Bingham, Khaw, Slimani, Norat, Riboli & Kaaks, 2006). Since IGF1 enhances mitosis, it is discussed as parameter for fat free mass (FFM) and muscle strength (Sun, Gagnon, Chagnon, Perusse, Despres, Leon, Wilmore, Skinner, Borecki, Rao & Bouchard, 1999; Kostek, Delmonico, Reichel, Roth, Douglass, Ferrell & Hurley, 2005; Lopez-Alarcon, Hunter, Gower & Fernandez, 2007) and therefore it is an interesting parameter concerning physical activity and performance.

Like IGF1, IGF2 has an impact on cell proliferation and differentiation and a mitogenic and antiapoptotic effect but it is supposed that IGF2 plays a major role in prenatal development and growth. Both IGF1 and IGF2 mediate their effects through binding to the same receptor, the IGF-1 receptor (Yu & Rohan, 2000). Several studies focused on the hypothesis that variation in the IGF2 gene is associated with interindividuell differences in body mass and muscle strength across the adult life span (Schrager, Roth, Ferrell, Metter, Russek-Cohen, Lynch, Lindle & Hurley, 2004; Devaney, Hoffman, Gordish-Dressman, Kearns, Zambraski & Clarkson, 2007). In this context, IGF2 gene variants may become important for the determination of physical performance.

2.1.2.1 Genetic variants in the IGF1 gene

Interindividual differences in serum IGF1 levels are high and are considered to be determined by 60% by genetic factors (Hong, Pedersen, Brismar, Hall & de Faire, 1996). However, there are controversial results published dealing with genetic variants in the IGF1 gene and their effects on different phenotypes (Table 1).

Table 1: Genetic variants in the IGF1 gene and their effects on different phenotypes

CA dinucleotide repeat (MS): 19>#19 tandem repeats in the promoter	
Allen et al. (2002)	No impact on circulation IGF1 plasma levels in Caucasian men
Jernstrom et al. (2001)	Higher IGF1 serum levels in noncarriers of the 19 allele but only in women using oral contraceptive

Kostek et al. (2005)	Greater increases in muscle strength and muscle volume after training in carriers of the 19 CA allele in older adults
Morimoto et al. (2005)	No impact on circulation IGF1 plasma levels in Caucasians
Rosen et al. (1998)	Significant lower IGF1 serum levels in homozygotes of the 19 CA allele
Vaessen et al. (2001)	Significant higher body height and IGF1 serum levels in homozygotes of the 19 CA allele
Van Heemst et al. (2005)	Trend (not significant) for lower body height and relative mortality risk in female inhabitants of The Netherlands carrying the non-dominant (≠19) allele (lower IGF1 levels)

CT dinucleotide repeat (MS): 189bp>≠189bp tandem repeats in the 5' region

Lopez-Alarcon et al. (2007)	European and African-American female noncarriers (≠189bp) seem to be predisposed to higher leg muscle mass but to worse endurance and energy economy
Sun et al. (1999)	Lower baseline levels of BMI, fat mass, percent body fat, and FFM and lower FFM gains in response to aerobic training in 189bp-homozygous Caucasians
Van Heemst et al. (2005)	No impact on insulin/IGF1 signalling pathways or longevity in elderly Dutch

rs2162679 (SNP): A>G in the intron

Canzian et al. (2006)	No impact on circulating IGF1 plasma levels in Caucasian women
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rs35765 (SNP): C>A in the promoter region

Canzian et al. (2006)	No impact on circulating IGF1 plasma levels in Caucasian women
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rs35767 (SNP): C>T in the promoter region

Canzian et al. (2006)	No impact on circulating IGF1 plasma levels in Caucasian women
Devaney et al. (2007)	No impact on muscle regeneration after bouts of eccentric strength training in Caucasians

rs6214 (SNP): C>T in on Exon 4, 3'UTR

Canzian et al. (2006)	No impact on circulating IGF1 plasma levels in Caucasian women
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rs6220 (SNP): T>C in on Exon 4, 3'UTR

Canzian et al. (2006)	No impact on circulating IGF1 plasma levels in Caucasian women
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bp – base pairs, MS - microsatellite polymorphism, SNP - single nucleotide polymorphism, UTR - untranslated region

In a huge study breast cancer risk was correlated with genetic factors including variants in the IGF1 gene and their effects on IGF1 plasma levels (Canzian et al., 2006). The population studied included about 2,400 women with the majority of Caucasian origin.

None of the five examined SNPs (rs35765, rs35767, rs2162679, rs6220, rs6214) revealed a significant association with serum IGF1 levels (Canzian et al., 2006).

Effects of eccentric strength burden on muscle repair mechanisms were compared between 156 individuals (half of them women) genotyped for various SNPs in the IGF1, IGF2, and IGF-binding protein genes (Devaney et al., 2007). Nearly three-quarter were of Caucasian origin. In the IGF1 gene, only one SNP (rs35767/-C1245T) in the promoter region was analyzed but no association was found (Devaney et al., 2007).

Another study group hypothesised that higher IGF1 levels are linked to premenopausal breast cancer risk (Jernstrom et al., 2001). The study population consisted of 503 premenopausal women of the ethnic groups white, black, Asian, and Indian-Pakistani. The intake of an oral contraceptive was assessed too due to the possible impact of exogenous estrogens on the IGF1 plasma levels. Genotyping also included a cytosine-adenine (CA) tandem repeat polymorphism (microsatellite polymorphism) in the IGF1 promoter region. Genetic variants of the CA microsatellite polymorphism are usually divided into carriers of 19 tandem repeats and noncarriers of the 19 repeat allele, because 19 tandem repeats are the most common variant, whereas alleles from 11-24 CA repeats are reported (Weber & May, 1989; Allen et al., 2002). Results show an association between noncarriers of the 19 repeat allele and higher IGF1 levels, but only in women using oral contraceptives. No association could be detected in women that do not apply exogenous estrogens.

Contrary to these results Allen et al. (2002) found no association between the CA microsatellite polymorphism and IGF1 serum levels in nearly 700 Caucasian men.

In 129 middle-aged men and 194 women major of Caucasian origin, the CA microsatellite polymorphism was linked neither to IGF1 serum levels nor to physical activity in the study population but there was an association with IGF binding protein 3 levels (Morimoto et al., 2005).

In a sub-study of the Rotterdam study, 900 men and women aged averagely 65.9 years were screened for anthropometric variables and the CA repeat polymorphism in the IGF1 gene (Vaessen et al., 2001). Further, the association between that polymorphism and type 2 diabetes as well as myocardial infarction was examined. Individuals homozygotic for the 19 CA allele had a significant higher body weight and significant higher serum IGF1 levels than noncarriers or heterozygotes. The relative risk (Odds Ratio) for a type 2 diabetes mellitus was 1.7 in noncarriers of the 19 CA repeat allele and 1.4 in heterozygotes. The relative risk for a myocardial infarction was 70% elevated for noncarriers and 20% for heterozygotes (Vaessen et al., 2001).

Serum IGF1 levels were also analysed in 171 Caucasians to highlight an association between the IGF CA microsatellite polymorphism and bone mineral density (Rosen et al., 1998). Contrary to the results of Vaessen et al. (2001), in this study cohort IGF1 serum levels were significantly decreased in 19 CA homozygotes.

67 healthy but inactive Caucasians over 50 years of age were screened for their genotypes in the IGF1 gene as well as for anthropometric parameters (Kostek et al., 2005). The subjects completed strength training for the knee extensor on one leg for 10 weeks and the training response was linked to the genotype. Variants examined were the SNPs rs19779, rs40395, and rs82681 as well as the CA microsatellite polymorphism. None of the four SNPs examined revealed any association with anthropometric measurements, initial strength, or training response. Initial strength (One repetition maximum, 1RM) was not significantly different between genotype groups but the increase of strength was significantly higher in carriers of the 19 CA allele. The presence of one or two alleles of the 19 CA repeats did not affect strength increase. Furthermore, there was a significant higher increase in muscle volume in 19 CA carriers than in individuals missing the 19 CA allele. Improvements in muscle quality, defined as strength in Newtons per muscle volume, were not different between genotype groups (Kostek et al., 2005).

Van Heemst, Beekman, Mooijaart, Heijmans, Brandt, Zwaan, Slagboom & Westendorp (2005) examined the impact of various genetic variants on the mortality risk of humans. In the analysis of 1,245 elderly inhabitants of The Netherlands, two microsatellite polymorphisms in the IGF1 gene were included - the CA repeat polymorphism as well as the cytosine-thymidine (CT) repeat polymorphism in the 5' region (Sun et al., 1999) of the IGF1 gene. There was a significant impact neither of the CT nor of the CA genotype on examined phenotypes (insulin/IGF1 signalling pathway). Nevertheless, a trend for the CA microsatellite polymorphism was found for lower body height and lower relative mortality risk due to a reduced insulin signalling pathway only in females (van Heemst et al., 2005).

The CT dinucleotide repeat polymorphism was also discussed to be responsible for interindividual responses to aerobic exercise training (Sun et al., 1999). The hypothesis was that differences of the IGF1 levels due to genetic variants would influence training induced changes in body composition. The study population consisted of 99 Caucasian families (502 healthy and sedentary subjects; HERITAGE Family Study) who followed an exercise-training program on a cycle ergometer for 20 weeks. Fat mass, percent of body fat, body mass index (BMI), fat free mass (FFM), and abdominal visceral fat were chosen as parameters for body composition. At baseline, 189bp homozygotes had significant lower levels for BMI, fat mass, FFM and percent body fat but in response to the conducted

aerobic training, they gained only half of the amounts of FFM in comparison to 189bp non-homozygotes and heterozygotes (Sun et al., 1999).

Lopez-Alarcon et al. (2007) compared body composition (FM, lean body mass (LBM), arm fat mass, leg fat mass, nonbone arm, leg lean mass), exercise performance (endurance time on the treadmill during a VO_2max test), and exercise economy (submaximal VO_2 measurement during stairclimbing and cycling) in premenopausal sedentary African-American and European-American women genotyped for the IGF1 CT microsatellite polymorphism. Participants' characteristics were comparable between genotypes. However, noncarriers of the 189bp allele had higher values of nonbone arm, leg lean mass and absolute oxygen uptake during cycling. No racial differences were observed. Results indicate a genetic predisposition to better endurance and exercise economy in individuals carrying the 189bp allele in the IGF1 gene. Simultaneously they might have a disposition to lower leg muscle mass (Lopez-Alarcon et al., 2007).

2.1.2.2 IGF1 and its antagonist

As IGF1 is a human growth factor, its expression has to be regulated to avoid excessive tissue growth. In heart myostatin demonstrated to be this regulating factor acting as a *chalone*² (Shyu, Ko, Yang, Wang & Kuan, 2005). Myostatin is a known negative regulator of muscle growth belonging to the transforming growth factor (TGF) β family (see chapter 2.1.3). The expression of myostatin is discussed to be influenced by genetic variance like IGF1 expression and it is directly linked to the IGF1 signalling pathway (Gaussin & Depre, 2005). The interaction of IGF1 and myostatin is well described by Shyu et al. (2005) and Gaussin & Depre (2005). Whereas IGF1 is important for cardiac cell growth, it simultaneously activates the expression of myostatin, which constitutes as negative feedback mechanism. When IGF1 levels exceed physiological levels of hypertrophy, myostatin expression is upregulated and reduces muscle growth (Gaussin & Depre, 2005). The dependent expression regulation of myostatin and IGF1 is important for the balance of muscle growth as shown in Figure 1.

In fact, the process of regulation is much more complex than it is shown in the above model. Training studies did not always find elevated IGF1 levels after resistance training that aimed to enhance muscle hypertrophy (Walker, Kambadur, Sharma & Smith, 2004). Myostatin levels were decreased after strength training in the same study population but there were no differences between strength training limited to the elbow flexor muscle and a whole body workout. These results indicate that effects of IGF1 and myostatin may not only be mediated through central regulation mechanisms but also or perhaps basically

² A "*chalone*" is an "*agent, that acts as tissue-specific mitotic inhibitor in the manner of a negative feedback mechanism*" (Bullough, 1965, p. 1692).

through autocrine-paracrine actions (Walker et al., 2004).

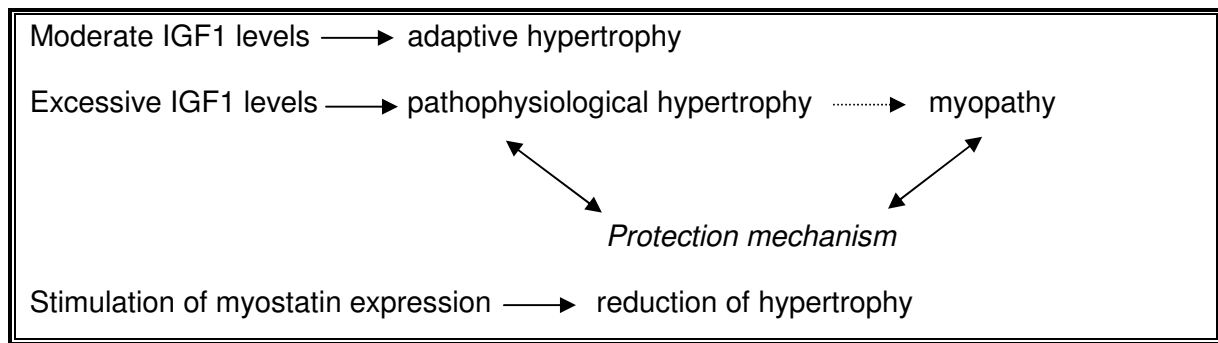


Figure 1: IGF1 and its chalone myostatin (based on Gaussin & Depre, 2005)

Analysing effects of polymorphisms within the insulin-signalling pathway, an interaction of the CA microsatellite polymorphism in the IGF1 gene and a novel MSTN polymorphism concerning the development of an athlete's heart was found (Karlowitz, Scharhag, Rahnenfuhrer, Schneider, Jakob, Kindermann & Zang, 2009). In 75 male endurance athletes, homozygotes of the 19 CA allele in the IGF1 gene had significant lower left ventricular masses than heterozygotes or homozygotes of the variant alleles. The IVS+88_90delA polymorphism in the MSTN gene, which was described for the first time, also showed an impact: athletes carrying the one deletion allele (A/-) had significant lower left ventricular masses than homozygotes of the ancestral allele (A/A). Lower levels in homozygotes of the deletion allele (-/-) in comparison to AA carriers were not significant. Individuals who carried the IGF1 CA 19/19 polymorphism and the MSTN A/- polymorphism had significant lower left ventricular masses than non-carriers of the combination did and differences were greater than effects of the single polymorphisms. Further, a role of a polymorphism in the IGF1 receptor gene was reported. No impact of polymorphisms was described in female endurance athletes nor in male control subjects but sample sizes were with 35 and 27 subjects, respectively, small (Karlowitz et al., 2009).

2.1.2.3 IGF2

Genetic variants in the IGF2 gene are discussed to be linked to body weight, adiposity and glucose tolerance (O'Dell, Miller, Cooper, Hindmarsh, Pringle, Ford, Humphries & Day, 1997) as well as to muscle mass and strength (Schrager et al., 2004). The most commonly analyzed polymorphism in this context is the Apal polymorphism in the 3'-UTR of the IGF2 gene with the ancestral G-allele and the A-allele as variant (Gaunt, Cooper, Miller, Day & O'Dell, 2001; Schrager et al., 2004). The polymorphism is also described as SNP 820G/A (Gaunt et al., 2001), SNP G17200A, and rs680 (Devaney et al., 2007). Table 2 gives an overview on different used nomenclatures and on effects of various polymorphisms in the IGF2 gene.

Table 2: Genetic variants in the IGF2 gene and their effects on different phenotypes

Apal polymorphism (rs680; SNP): G>A in the 3'-UTR; synonyms: 820G/A, G17200A	
Devaney et al. (2007)	Higher extend of parameters of muscle damage in men homozygotic for the A-allele
Gaunt et al. (2001)	Significant lower BMI values in AA homozygotes
O'Dell et al. (1997)	Significant lower body weight and higher serum IGF2 in Caucasian AA homozygotic men
Sayer, Syddall, O'Dell, Chen, Briggs, Briggs, Day and Cooper (2002)	Higher IGF2 serum levels in AA homozygotes; by trend (not significant) higher body height and weight in GG homozygotic men and in AA homozygotic women; no impact on adult grip strength
Schrager et al. (2004)	Higher grip strength levels in GG women; Lower FFM in AA women; no genotype effects were found in men
Alul polymorphism (SNP): T>C on exon 3; synonyms: 1252T/C	
Gaunt et al. (2001)	No effects on BMI
rs2230949 (SNP): C>T at position 16646 in the 3'-UTR	
Devaney et al. (2007)	Higher extend of parameters of muscle damage in men homozygotic for the C-allele
rs3213216 (SNP): G>A at position 12655 in the 1st intron	
Devaney et al. (2007)	No impact on the response after strength training
rs3213220 (SNP): T>C at position 13705 in the 1st intron	
Devaney et al. (2007)	Higher extend of parameters of muscle damage in heterozygotic women
rs3213221 (SNP): C>G at position 13790 in the 1st intron	
Devaney et al. (2007)	Higher extend of parameters of muscle damage in men homozygotic for the G-allele
rs4244808 (SNP): A>C at position 1364 in the 1st intron	
Devaney et al. (2007)	Higher extend of parameters of muscle damage in men homozygotic for the G-allele
rs7924316(SNP): G>T at position 11711 in the 3-Downstream region	
Devaney et al. (2007)	Highest extend of parameters of muscle damage in AA men and heterozygotic women in comparison to the other gender and genotype groups
SNP on nucleotide 1156: T>C in exon 3 of the IGF2 gene (GenBank accession no.: Y13633)	
Gaunt et al. (2001)	Significant higher BMI values in CC homozygotes

SNP on nucleotide 1926: C>G in exon 7 of the IGF2 gene (GenBank accession no.: X07868)

Gaunt et al. (2001)	Significant lower BMI values in GG homozygotes
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SNP on nucleotide 6815: A>T on the 5' end of the IGF2 gene (GenBank accession no.: L15440)

Gaunt et al. (2001)	Significant lower BMI values in TT homozygotes
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SNP - single nucleotide polymorphism, UTR - untranslated region

In a sample of more than 1,000 Caucasian men, AA homozygotes of the IGF2 Apal (rs689) polymorphism had a significant lower body weight as well as significant higher serum IGF2 levels (O'Dell et al., 1997). The conclusion drawn by the authors favours a better glucose utilisation by AA homozygotes of the IGF2 Apal (rs689) polymorphism that explains the lower body weight (O'Dell et al., 1997).

The same results were assessed by Sayer, Syddall, O'Dell, Chen, Briggs, Briggs, Day & Cooper (2002) who studied more than 700 men and women from Hertfordshire. Higher IGF2 serum levels were found in individuals homozygotic for the A-allele of the IGF2 Apal (rs689) polymorphism. However, only a non-significant association could be found for genotype, body height and weight to be higher in GG homozygotic men of the IGF2 Apal (rs689) polymorphism. In women, the trend was vice versa. No impact of the Apal genotype (rs680) was found on adult grip strength.

Gaunt et al. (2001) searched for gene variants in the IGF2 gene locus that may influence the BMI of more than 2,700 men of the Northwick Park Heart Study II. Eleven SNPs on different loci of the IGF2 gene were selected for analysis but only four of them had an impact on the BMI. Homozygotes of the rare Apal A-allele (rs680) had significant lower BMI values than heterozygotes and GG homozygotes. Lower BMI values were also observed in carriers of the variant allele (TT and GG, respectively) of SNPs 6815A/T and 1926C/G. The 1156T/C polymorphism had an inverse effect: carriers of the variant allele (CC) had higher BMI values than homozygotes of the ancestral T-allele (Gaunt et al., 2001).

Higher grip strength levels following different measurement protocols were documented in women homozygotic for the G-allele of the IGF2 Apal (rs689) polymorphism in comparison to AA women (Schrager et al., 2004). Homozygotic women for the A-allele had also significant lower FFM than heterozygotes or GG homozygotes. Contrary, no significant genotype effects were found in men (Schrager et al., 2004).

In 156 men and women of three-quarter Caucasian origin, genotype effects on strength loss, muscle soreness, and increases in circulating levels of creatine kinase and myoglobin concentration after muscle-damaging eccentric exercise were examined (Devaney et al., 2007). All assessed parameters described muscle damage after bounds

of eccentric exercise. Seven SNPs of the IGF2 gene were selected for comparison: rs2230949 (C16646T in the 3'-UTR), rs3213221 (C13790G in the 1st intron), rs3213220 (T13705C in the 1st intron), rs3213216 (G12655A in the 1st intron), rs4244808 (A1364C in the 1st intron), rs7924316 (G11711T in the 3-Downstream region), and rs680 (G17200A, Apal, in the 3'-UTR). All analyzed SNPs in the IGF2 gene except the IGF2 rs3213216 polymorphism had an impact on muscle damage at least in a subgroup. In GG homozygotic men for the C13790G SNP (rs3213221) immediate postexercise strength losses, soreness 3 days postexercise, and serum creatine kinase activity 7 days postexercise were greater than in individuals carrying other genotypes. The same was observed in men homozygotic for the A-allele of the Apal SNP (rs680). For men heterozygotic for the C16646T polymorphism (rs2230949) muscle soreness 4 days after exercise was significantly lower than in men homozygotic for the C-allele. Only one subject (0,6%) was homozygotic for the rare T-allele (rs2230949). The same effects could not be determined in women whereas the impact of the T13705C polymorphism (rs3213221) on study parameter was significant only in women: Baseline strength was higher in heterozygotes; homozygotes of the rare C-allele were not found in the study population. Further, myoglobin and creatine kinase activity were significantly higher 4 days postexercise in heterozygotic women than in TT homozygotes. Homozygotic men (GG and TT) for the G11711T polymorphism (rs7924316) showed greater strength loss and greater muscle soreness after exercise than heterozygotes. In men carrying the wild-type genotype (GG) of the G11711T polymorphism (rs7924316) postexercise myoglobin and creatine kinase activity was higher than in heterozygotes. Summing up, results affirm the thesis, that people may be differently predisposed to susceptibility to excessive exercise. Variants in the IGF2 gene seem to be of major impact (Devaney et al., 2007).

2.1.3 Myostatin

Myostatin is a negative growth factor belonging to the TGF- β family. Its synonym is growth and differentiation factor 8 (GDF8). It regulates muscle growth from embryogenesis to the development of mature adult tissue and it is nearly exclusively expressed in muscle tissue. An inhibition of the myostatin signalling transduction pathway leads to an increase of muscle mass (McPherron, Lawler & Lee, 1997; McNally, 2004). Interestingly, a loss of adipose tissue was found concomitant to increased muscle growth due to myostatin inhibition (McPherron et al., 1997; McNally, 2004; Rodgers & Garikipati, 2008).

Myostatin is synthesized and secreted in its inactive preform. After dimerisation myostatin is re-cleaved and binds to the TGF- β receptor activating the Smad signalling pathway that is regulatory in gene expression. Follistatin can inhibit myostatin by binding the latent myostatin form as there are also other myostatin binding proteins known (Rodgers &

Garikipati, 2008). The overexpression of myostatin evidently causes loss of muscle mass and function (McPherron et al., 1997; McNally, 2004). The role of myostatin in muscle mass regulation should always be considered together with other regulatory factors of muscle growth like IGF1 (McNally, 2004; Rodgers & Garikipati, 2008). Insuline like growth factors are considered to be antagonists of myostatin (see chapter 2.1.2.2) and muscle fiber size is maintained due to the balance of these positive and negative growth regulators (Lalani, Bhasin, Byhower, Tarnuzzer, Grant, Shen, Asa, Ezzat & Gonzalez-Cadavid, 2000).

Analyses of the myostatin mRNA levels of the soleus and gastrocnemius muscles of weight-bearing and hindlimb unloading mice indicate actions of myostatin different to that of a negative regulator of muscle growth (Carlson, Booth & Gordon, 1999). Higher myostatin mRNA levels were found only in the gastrocnemius before significant muscle atrophy occurred. The gastrocnemius muscle is predominately composed of type II muscle fibers. No elevated myostatin mRNA levels could be detected in the soleus, which has a high percentage of type I muscle fibers and showed greater muscle atrophy due to hindlimb unloading. Furthermore, analyses revealed the highest myostatin levels in muscles with the greatest fiber cross sectional area contradicting the theory that myostatin is a negative regulator of muscle growth. The authors conclude that under normal physiological conditions myostatin does not regulate fiber size because of its presence in muscles with the highest fiber cross sectional areas (Carlson et al., 1999), whereas in myostatin knockout mice muscle hypertrophy is seen (McNally, 2004; Rodgers & Garikipati, 2008). Myostatin is discussed to be involved in the reinforcement of a fast muscle phenotype because it may be able to downregulate the expression of slow type I fiber proteins and therefore is indirectly responsible for muscle fiber size. Further, myostatin may act as a negative regulator of satellite cell proliferation in fast skeletal muscles, which could explain high amounts of myostatin mRNA in muscles with high fiber cross sectional areas (Carlson et al., 1999). However, Gonzalez-Cadavid, Taylor, Yarasheski, Sinha-Hikim, Ma, Ezzat, Shen, Lalani, Asa, Mamita, Nair, Arver & Bhasin (1998) did not find any differences concerning myostatin immunostaining neither in type I nor type II muscle fibers.

Myostatin inhibition due to genetic variants, drugs, or specific inhibitors are considered to promote performance due to higher muscle mass and therefore better strength performance (McNally, 2004; Matsakas & Diel, 2005; Walsh, Metter, Ferrucci & Roth, 2007). In a study examining long-term effects of myostatin inhibition, myostatin null mice had enhanced markers of regeneration and were more resistant to acute and chronic injuries than controls (Wagner, Liu, Chang & Allen, 2005). In HIV-patients with a decrease

in muscle mass higher myostatin levels were documented (Gonzalez-Cadavid et al., 1998). In persons with decreased pituitary activity, growth hormone treatment increased lean body mass. Concomitant a down-regulation of myostatin was observed as well as a better aerobic performance, which could be due to the higher muscle mass. According to these results, anabolic effects of growth hormone might be at least partly mediated by decreased myostatin levels (Liu, Thomas, Asa, Gonzalez-Cadavid, Bhasin & Ezzat, 2003).

2.1.3.1 Single nucleotide polymorphism K153R (rs1805086)

A loss of function mutation in the myostatin gene is considered to cause increased muscle mass. Summing up all described mutations in the myostatin gene, eight polymorphisms (Saunders, Good, Lawrence, Ferrell, Li & Nachman, 2006) have been detected in the first two of the three exons of the myostatin gene. Most of them do not cause any amino acid exchange or are not common in the screened populations (Ferrell, Conte, Lawrence, Roth, Hagberg & Hurley, 1999; Seibert, Xue, Fried & Walston, 2001). The most promising polymorphism (rs1805086) is a switch from the nucleotide adenine to guanine in position 153 on exon 2 causing the exchange of lysine by arginine in the myostatin protein. The K153R polymorphism in the myostatin gene was found more often in African-Americans than in Caucasians (Ferrell et al., 1999; Seibert et al., 2001). A comparison of the genetic variants in the myostatin gene between high responders and non-responders of different athlete status did not reveal any significant relationship between muscle mass increase after strength training and heritability. However, none of the responders was homozygotic for the less frequent R153 allele with the amino acid arginine present, whereas three of the nonresponders were R153 homozygotes (100% of RR homozygotes) of the African-Americans (Ferrell et al., 1999).

The influence of the K153R polymorphism on strength was aimed to be assessed in a training study with young men (Thomis, Huygens, Heuninckx, Chagnon, Maes, Claessens, Vlietinck, Bouchard & Beunen, 2004). The hypothesis was that with the presence of the R153 allele, initial strength levels were lower but strength gain due to heavy resistance strength training was higher. The hypothesis could not be approved because only one of the 57 volunteers was homozygotic for the R153 variant. The frequency of the K153R polymorphism was lower in the used population than described elsewhere (Ferrell et al., 1999). All subjects were twin pairs (Thomis et al., 2004).

Participants of the Women's Health and Aging Study II were genotyped for the K153R polymorphism and results were correlated with strength measurements of the grip, hip, and knee flexors (Seibert et al., 2001). Higher strength levels were seen in participants

carrying the KK genotype in comparison to RR homozygotes and heterozygotes. The rare RR genotype was only found in African-Americans but not in Caucasians (Seibert et al., 2001). A comparison of the impact of the myostatin genotype on the flexor strength in different joints revealed that the hip flexion was affected the most. As the major hip flexor is the iliopsoas which is primarily composed of type I muscle fibers, authors conclude an influence of the myostatin gene variant predominantly on type I muscle fibers (Seibert et al., 2001). This is contradictory to the results of myostatin mRNA levels in different muscle types described in mice by Carlson et al. (1999).

32 volunteers of different age and sex participated in a 9 week one-leg strength training program for the knee extensor and agreed in genotyping their K153R gene variant (Ivey, Roth, Ferrell, Tracy, Lemmer, Hurlbut, Martel, Siegel, Fozard, Jeffrey Metter, Fleg & Hurley, 2000). Among these participants, no man carrying the rare R-allele was present, whereas five of the 14 women were heterozygous. Differences in muscle volume increase after strength training were not statistically significant, but there was a tendency that heterozygotes had a greater pretraining muscle mass and a higher increase in muscle mass due to strength training. The trend in the muscle mass increase after training was maintained when the baseline muscle volume was covaried in the analysis (Ivey et al., 2000). Results indicate an influence of the K153R myostatin polymorphism on muscle mass response to training but suggestions are limited due to the small sample size. Besides these findings gender differences concerning the frequency of the rare R-allele were noted with a higher incidence in women (Ivey et al., 2000). Outcomes disagree with those of Seibert et al. (2001) and Carlson et al. (1999) because in their analyses the R-allele was linked to weaker phenotypes or to less training response. On the other hand results affirm the hypothesis of Thomis et al. (2004).

Roth, Martel, Ferrell, Metter, Hurley and Rogers (2003) found a decreasing myostatin expression in 15 subjects completing the same heavy resistance strength training as previously described. The higher initial myostatin levels (2 weeks before strength training) were the greater was myostatin expression decrease (48 to 72 hours after strength training). Neither gender nor age differences could be observed. No genotypes of the participants were available (Roth et al., 2003).

In comparison of the selected gene variants linked to endurance performance in nine excellent cross-country runners including one world champion, the KK genotype was interpreted as the more favouring one. Six of the nine runners were homozygotic for this variant (Gonzalez-Freire et al., 2008). Authors did not discuss to very low frequency of the R-allele, which may cover advantageous effects of it on endurance performance.

Between excellent lightweight rowers, professional road cyclists, Olympic-class runners, and non-athletic controls no differences were found in the frequency of the myostatin gene variant (Muniesa et al., 2008).

Summing up published data there is no clear evidence whether the KK or the RR genotype is more favourable for performance. Training studies are even contradicting concerning whether the expression of myostatin increases or decreases after resistance training (Willoughby, 2004; Raue, Slivka, Jemiolo, Hollon & Trappe, 2006).

On the other hand, there are further links that the myostatin as a negative growth regulator has an impact on performance because performance phenotypes are affected by other polymorphisms, even in other species, especially when the variant causes a loss-of-function mutation: Lee (2007) showed that the increase in muscle mass results in better running speed and sprint performance in dogs. Furthermore, there is a positive correlation between racing grade and myostatin deficiency mutation in dogs (Mosher, Quignon, Bustamante, Sutter, Mellersh, Parker & Ostrander, 2007). There is evidence that myostatin inhibition correlates with athletic performance which is also supported by a boy carrying a myostatin nonsense mutation and having unusual strength for his age. His mother, heterozygotic for the same mutation, was a professional sprinter (McNally, 2004; Lee, 2007; Mosher et al., 2007).

2.2 Alpine skiracing

Alpine skiracing is a complex sport and therefore it is difficult to decide, which specific ability is crucial for success in alpine skiing. Certainly, the skiing technique as well as the skis have a high influence on skiing performance at high levels (Jeschke, Lorenz, Filadoro, Tusker, Grosser & Weinbuch, 1994). Nevertheless, physiological and metabolic aspects play at least a minor role in success in skiing competition (Axtell, Rinehardt, Finn, Stofan, Martens, Kenefick & Pier, 1997; Bosco, 1997). During 1990 and 2000 researchers and sport scientists tried to highlight the importance of different physiological and metabolic conditions concerning skiing performance, whereas afterwards studies focused increasingly on technical and biomechanical aspects.

In 1983, Brown and Wilkinson (1983) compared VO_2max of national, divisional and club skiers. They could not find any differences in the VO_2max that would allow distinguishing between the different skiing classes but strength, power and anaerobic fitness outcomes were higher in national skiers. Results indicate that aerobic fitness is not determining for success in skiing competition but it is supposed that anaerobic fitness, strength and power measurements are adequate for distinguishing between different classes of ski-racers (Brown & Wilkinson, 1983).

Following Andersen and Montgomery (1988), the aerobic demands in alpine skiracing account for up to 90-95% but the high VO_2max levels in elite skiers may rather reflect their training program than the physiological demands of the competition. In fact, a high glycogen utilisation seems to be necessary due to the impeded blood flow and the therefore impaired oxygen delivery during the race (Andersen & Montgomery, 1988).

Assessed VO_2max levels in competitive alpine skiracers were 52.9 ± 6.8 ml/kg body weight/min (Stanek, von Duvillard & LeMura, 1997), and 55 ± 3.5 ml/kg body weight/min (females) and 60 ± 4.7 ml/kg body weight/min (males) (Neumayr, Hoernagl, Pfister, Koller, Eibl & Raas, 2003). Whereas athletes of other complex sport disciplines as for example martial arts, ball games, and heptathlon, have comparable VO_2max values (about 50 - 60 ml/kg body weight/min), endurance athletes (e.g. runners, rowers, cyclists, cross-country skiers) averagely have higher ones (about 65 - 70 ml/kg body weight/min) (de Marees, 2002). Unfortunately, assessed VO_2max values in alpine skiers were not divided into the subcategories slalom, giant slalom and downhill (Stanek et al., 1997; Neumayr et al., 2003).

Due to this described impeded blood flow during the skiing competition, Axtell et al. (1997) support the thesis that aerobic fitness is co-determining for success. Analysing cardiorespiratory and metabolic characteristics of junior athletes of the United States' ski team it was found that aerobic as well as anaerobic capacities were highly developed. Leg strength and power are equally important because highly developed leg strength allows the muscle to perform at submaximal effort during downhill passages. The submaximal function results in an increased blood flow indicating a higher percentage of aerobic energy supply and avoids the accumulation of anaerobic by-products and strenuous fatigue. In summary, this thesis indicates that although anaerobic capacities are crucial for success in competition, a highly developed aerobic system is likewise necessary for rapid regeneration when lactic acid accumulates. Both metabolic pathways therefore seem to act symbiotically (Axtell et al., 1997).

Steadman, Swanson, Atkins and Hagerman (1987) even state, that aerobic fitness is more important than anaerobic conditioning in alpine skiers because strength and power during the race can only be maintained with a high functional aerobic system. Findings of decreasing oxygen uptake during the alpine skiing season are contrary (Haymes & Dickinson, 1980). Decreasing VO_2max levels assessed on a cycle ergometer test may reflect the importance for muscular endurance capacity in alpine skiracing. As muscular endurance capacity is co-responsible for competition performance, it may develop during the skiing season whereas aerobic capacity is higher at the beginning of the season due to pre-seasoning aerobic fitness training (Haymes & Dickinson, 1980).

Using cycling ergometer protocols in alpine skiers for testing physiological characteristics are discussed contradictory because they determine general fitness levels but not specific motor demands (White & Johnson, 1991; Baum, Hoy, Leyk & Essfeld, 1997). Although heart rates, VO_2max and lactate measurements can be helpful in the training process and validation of alpine skiers, in laboratory tests incremental exercise protocols should be used because they better reflect race demands (White & Johnson, 1991; Hartmann, Mader, Babel, Spitzenpfeil & Mester, 1997; Reid, Johnson, Kipp, Albert & White, 1997). The use of incremental protocols instead of long duration all-out tests is supported by results of Baum et al. (1997). The comparison of sport students not familiar with alpine skiing and D-kader alpine skiers revealed no differences. As ski races last between 45 seconds and 2 minutes, tests for correlation analysis with performance in competition should also lie within this period (Bacharach & von Duvillard, 1995). Even the use of the Wingate test, which is an exercise protocol of maximal cycling against a given resistance calculated on the basis of body weight for 30, 60, 90, or 120 seconds could not highlight any correlation between test outputs and the national USSA points representing skiing performance (Petelin von Duvillard & Knowles, 1997). However, the Wingate test caused a better correlation than the box jump test or the vertical jump test did (Petelin von Duvillard & Knowles, 1997). Similar outcomes are described by Reid et al. (1997) who correlated the results of a test battery including High Box Jumping, Hexagonal Obstacle, Single Leg Lateral Vault and Two Leg Lateral Vault test with skiing classes. Although there were partly significant different test outcomes between four different skiing classes, the test battery was not able to distinguish between all four levels (Reid et al., 1997).

The same test battery was used by Klika and Malina (1997) in combination with antropometric measurements to predict skiing performance in youth. In females, weight, vertical jump performance and high box jumping performance predicted national skiing points (similar to FIS points) with a determination coefficient of 0.52. In males, age, high box jumping performance and skinfold thickness turned out to be relevant for success with a determination coefficient of 0.31 (Klika & Malina, 1997).

Although some tests were able to partly predict skiing performance (Klika & Malina, 1997), none of them could include skiing technique which is undisputable a main factor for success in alpine ski races. Nevertheless, physiological properties are likewise important because they allow realising technical demands (Bosco, 1997). A crucial physiological skill for success in competition is speed endurance. As during ski races the leg extensor acts in stretch-shortening-cycles, the muscular activity is similar as in jumps. Improvements of speed endurance (continuous jumping) also improved characteristics of explosive power outputs suggesting that especially fast twitch muscle fibers represent the

physiological background of success in skiing competition. Subjects rich in fast twitch fibers are also favoured in their anaerobic capacity.

As speed endurance was proposed as the most predictable parameter for success in skiing competition (White & Johnson, 1991; Bosco, 1997), two training theories emerged (Steadman et al., 1987): either strength training and jumping enhances speed endurance or speed endurance is improved on the basis of a highly developed aerobic metabolic pathway. Bosco et al. (1997) favored strength and jumping training to improve speed endurance because speed endurance turned out to depend on explosive power and relative explosive strength and not on maximal dynamic strength. As there exists a positive relationship between explosive strength and testosterone levels, strength training is an important training tool to increase testosterone levels whereas aerobic endurance training can even inhibit testosterone production due to a stimulated release of beta-endorphine. Testosterone can even favour the switch of fast twitch muscle fibers to a more glycolytic profile (Bosco, 1997). Concordantly, Frick, Schmidtbleicher, Raschner and Müller (1997) recommend continuous jumps for slalom training because they stimulate the sport-specific stretch-shortening cycles in knee and hip extensors.

Whereas most analyses did not differ between various skiing disciplines, a comparison of German ski racers in 1990 highlighted differences between specialists (Jeschke et al., 1994). Whereas for slalom and giant slalom strength endurance turned out to be the most determining physiological skill, in downhill and super G races the percentage of fast twitch muscle fibers determines performance. In slalom and giant slalom dynamic strength and aerobic endurance as parts of strength endurance are important because of the high frequency of rhythmic changes. In downhill and super G the ability to build and endure high levels of lactate is crucial which is a characteristic of fast twitch glycolytic fibers. Nevertheless, aerobic endurance also has an impact on success but not in the same extent as it has in slalom races. Authors conclude, that endurance capacity is more important to ski racing competition performance than strength parameters are (Jeschke et al., 1994). Similar outcomes are assessed in longitudinal studies of the Austrian Ski Team between 1997 and 2000 (Neumayr et al., 2003). Success in skiing competition is based on many physiological variables with aerobic power and muscle strength as the two main determining factors (Neumayr et al., 2003).

Apart from all these theories, alpine skiers were assigned to the power athlete group concerning the ACTN3 R577X genotype in an genetic study of Russian athletes and controls (Druzhevskaya et al., 2008).

3 Materials and methods

3.1 Subjects

The study was approved by the ethic committee of the University of Medicine of Vienna and of the general hospital of Vienna (AKH). All participants gave written informed consent for saliva sample collection, the DNA extraction and the allelic discrimination in over 20 gene loci (see Appendix) before analysis. Only five polymorphisms are part of this analysis, whereas the remaining ones are assessed within other parts of the whole gene project. Participants were informed advised of their rights and risks and all samples were coded and not registered with the names of the participants. Anonymous sample guaranteed the highest data security for participants.

Table 3: Exclusion criteria for athletes

- | |
|---|
| <ul style="list-style-type: none"> – Proven intake of performance-enhancing substances (WADA) – Existence of coronary heart diseases – Existence of chronic diseases – Existence of a manifest diabetes mellitus type I (insulin-dependent) or type II. A manifest type II diabetes mellitus is given if fasting blood glucose levels are higher than 126 mg/dl and there appear typical symptoms as polyurie and thirst at two different days or fasting blood glucose levels are higher than 126 mg/dl and not-fasting blood glucose exceeds 200 mg/dl at two different days. |
|---|

Table 4: Inclusion criteria for sedentary controls

- | |
|--|
| <ul style="list-style-type: none"> – Good health status – No existence of a manifest diabetes mellitus type I (insulin-dependent) or type II. – No known coronary heart diseases or other chronical disorders – A maximum of three hours of exercise or exhausting leisure time per week (for sedentary controls) – no professional sport engagement but more than three hours of exercise or exhausting leisure time per week (for active controls) – At least 18 years old |
|--|

Alltogether 244 volunteers donated saliva samples and were genotyped for the five selected polymorphisms. 40 of them met inclusion criteria for one of the athlete groups

(see Appendix, Table 3). Control subjects had to meet the inclusion criteria listed in Table 4. They were recruited via a public notice on the Austrian Institute for Sports Medicine (Österreichisches Institut für Sportmedizin) in Vienna, Auf der Schmelz 6 (see Appendix). The group consisted of 204 sedentary and moderate active volunteers (Table 5).

Originally, the high-performance athlete group should consist of the national team as well as the A, B, and C-kader of the Austrian ski federation. Because Austria is one of the top nations in the international ski circuit, it can be assumed that selected athletes are of the world's best. Unfortunately, the Austrian national ski team has not been available yet, but athletes of other sports (athlete group inclusion criteria for athlete groups are listed in the Appendix - Written informed consent), mostly runners, agreed in collaboration (Table 5). Runners and road cyclists were group together as endurance athletes. Athletes of other disciplines (not endurance-dominated) are analysed in other parts of the whole gene project. Runners (800 meters to marathon running) had to have a personal best in at least one discipline that corresponds more than 800 points following the IAAF Scoring Table³. Road cyclists had to be participants of international competitions of the Union Cyclist Internationale and to be a member of a professional road cycling team.

Table 5: Study population subcategories

Sedentary (<3 hours exercise/week)	87 subjects
Active (>3 hours exercise/week)	117 subjects
Athletics - middle and long distance run	38 subjects
Road cycling	2 subjects

Characteristics of the study cohorts (sedentary, active, athletes) are shown in Table 6. 26 of the 40 endurance athletes had already finished their career when they donated their saliva sample, whereas the remaining 14 were still active. Retired athletes filled out the athlete's questionnaire (chapter 3.2.2) and referred the answers to their active period.

³ The IAAF (International Association of Athletics Federation) Scoring Table was developed in 1982 and is yearly updated. According to statistical methods, each performance is assigned a certain score. The IAAF Scoring Table allows interdisciplinary comparisons because the same score corresponds to the same performance even in different disciplines.

Table 6: Characteristics of study cohorts

N = 244	Sedentary (<3 hours exercise/week) n = 87	Active (>3 hours exercise/week) n = 117	Athletes (endurance) n = 40
% female (n)	61% (53)	47% (55)	3% (12)
Age [years]	39.6 ± 15.38 (20-77)	34.2 ± 15.96 (19-70)	32.13 ± 11.58 (18-67)
BMI [kg/m ²]	24.3 ± 5.25 (17.9-45.7)	22.9 ± 2.69 (17.3-31.3)	20.98 ± 2.18 (17.1-25.6)
PAL	1.68 ± 0.30 (1.27-2.77)	1.87 ± 0.37 (1.13-2.9)	2.28 ± 0.21 (1.14-2.88)

Values are expressed as mean ± standard deviation, minimum and maximum is given in braces

3.2 Physical activity questionnaires

Both, athletes and sedentaries were asked to fill out a questionnaire assessing personal data, the amount of training and other training details and weekly physical activity, respectively.

3.2.1 Physical activity questionnaire for sedentary controls

Sedentaries were asked to fill out the Physical Activity Frequency Questionnaire (PAFQ; see Appendix) to assess their weekly physical activity including work, leisure time and mileage.

The evaluation of PAFQ offers the energy expenditure per day, which also reflects physical activity when it is corrected for age, sex and body height and weight.

The questionnaire was validated previously using a heart rate monitor over 3 days (Bernstein, Sloutskis, Kumanyika, Sparti, Schutz & Morabia, 1998); group activities were chosen on the basis of 24 hours recalls⁴ and calculated referring to the Joint FAO/WHO/UNU Expert Committee on Energy and Protein Requirements (1985).

⁴ 24 hours recall: A person is asked about his or her eating habits in the last 24 hours without announcement. The nutrient intake is calculated with the use of the nutritional information panel (Elmadfa, 2004, p. 52).

3.2.1.1 PAFQ computing

With the PAFQ, the overall activities of one week can be assessed. If the total amount of hours is different from 168, a two step correction has to be made (Bernstein et al., 1998). First, the total sleep time is set to 45:30 hours if it is less or to 70 hours if it is more. Second, the total duration for non-sleep activities is calculated and proportionately adjusted so that the sum of sleep and non-sleep time yields 168 hours. All corrections were calculated using Microsoft Excel® 2003.

The energy expenditure is computed by multiplication of the time spent for activities with multiples of the basal metabolic rate. Multiplication factors were used according to Ainsworth, Haskell, Whitt, Irwin, Swartz, Strath, O'Brien, Bassett, Schmitz, Emplaincourt, Jacobs & Leon (2000) except activity levels for the leisure time activities, and sitting which refer to the the Joint FAO/WHO/UNU Expert Committee on Energy and Protein Requirements (1985). The basal metabolic rate is calculated with the help of personal data as follows: men: basal metabolic rate = 1 kcal/hour x body weight; women: 0,9 kcal/hour x body weight (Elmadfa, 2004). The energy expenditure depends on sex, age, body height, and other factors whereas the Physical Activity Level (PAL) is independent from these variables. The PAL can be calculated by dividing the energy expenditure per day by the basal metabolic rate (Elmadfa, 2004).

The part “sports – detailed information” is not included in the original validated questionnaire, but it was included to collect some qualitative information on sport activities carried out by controls.

3.2.2 Physical activity questionnaire for athletes

The PAFQ was not used for athletes because it does not reflect an athlete's lifestyle. To allow a comparison of the PAL of sedentaries and athletes, the PAL of athletes was set at 2.3 which reflects routine training and can be sustained for extended periods of time (Shetty, 2005).

The questionnaire for athletes assessed besides personal data training details as main disciplines, amounts of training in hours and kilometers per week, age of access into competition and the personal appreciation of the importance of either strength or endurance for success in competition (see Appendix).

3.3 Determination of genetic variants

3.3.1 Pretests to compare DNA sample collection and isolation protocols

Table 7: Overview of compared methods for DNA isolation

Sample collection protocol	Medium	Isolation protocol
Take blood sample	Venous blood (EDTA)	PeqLab Blood DNA Isolation Kit
Biozym Buccal Brushes	Saliva	Epicentre® Buccal Amp™ DNA Extraction Kit
Biozym Buccal Brushes	Saliva	Gentra® Purgene® Buccal Cell Kit
Mouthwash (Listerine®)	Saliva	Gentra® Purgene® Buccal Cell Kit
Oragene™ Self Collection Kit	Saliva	Ethanol extraction
Oragene™ Self Collection Kit	Saliva	Gentra® Purgene® Buccal Cell Kit

To find a preferably cheap, fast and for the probands as easy as possible method for DNA collection as well as an efficient protocol to isolate DNA of sufficient amount and pureness, different methods were compared prior to the start of sample collection (Table 7).

3.3.1.1 DNA Isolation using PeqLab Blood DNA Isolation Kit

3.3.1.1.1 Sample collection

Venous blood was collected by a physician in a tube containing EDTA as anticoagulant. Three participants volunteered as donors. Two of them were male; one was female.

3.3.1.1.2 DNA isolation

For DNA isolation, the peqGOLD Blood DNA mini Kit (Safety-Line, article number 12-3482-xx) from PeqLab, Biotechnology GmbH, Erlangen, Germany, was used.

Lysis: 500 µl blood, 50 µl OB Protease, 500 µl BL Buffer and 5 µl RNase were mixed, afterwards incubated for 10 minutes at 70° C.

Ligation: Lysate was mixed with 520 µl isopropanol and 650 µl of the mixture were transferred to the HiBind DNA-column in a 2 ml centrifuge tube. Tube was centrifuged for 1 minute at 8000 xg and flow-through was wasted. Procedure was repeated three times to load and ligate the whole lysate-isopropanol mixture on the column.

Wash: Column was transferred to a new 2 ml centrifuge tube und loaded with 600 µl DNA-Wash Buffer, afterwards centrifuged for 1 minute at 8000 xg. Flow-through was discarded. This step was repeated twice without changing the tube. After the wash, the column was centrifuged at maximum speed for 2 minutes to ensure complete dryness.

Elution: Column was transferred to a new 2 ml centrifuge tube and loaded with 100 µl Elution Buffer that had previously been heated to 70° C. Column was incubated at room temperature for 2 minutes, afterwards centrifuged for 1 minute at 8000 xg. Elution step was repeated a second time, afterwards column was wasted.

Quantification: Absorption of the undiluted eluat was measured at 230, 260, 280 and 320 nm using a Biophotometer. Isolated amount of DNA was calculated by multiplication the absorption at 260 nm with 50 (extinction for DNA); quality was evaluated using the ratio of the absorption at 260 and 280 nm, which should be higher than 1.7. Furthermore, there should be a distinct peak to see at the absorption of 260 nm.

Results: The obtained amount of DNA out of 500µl blood was somewhat lower than the expected one given by the supplier (5.6 – 8.4 µg). The quality of the obtained DNA indicated by the absorption ratio at 260 and 280 nm and a distinct peak at 260nm was satisfactory; see Table 8: and Figure 2.

Table 8: Absorption, concentration and amount of isolated DNA from venous blood using peqGOLD Blood DNA mini Kit

Sample	Absorption at λ [nm]:				DNA [ng/µl]	Ratio 260/280	Isolated amount [µg]
	230	260	280	320			
1	0.055	0.355	0.165	0.029	17.75	2.2	3.55
2	0.095	0.443	0.218	0.029	22.15	2.0	4.43
3	0.013	0.211	0.09	0.019	10.55	2.3	2.11

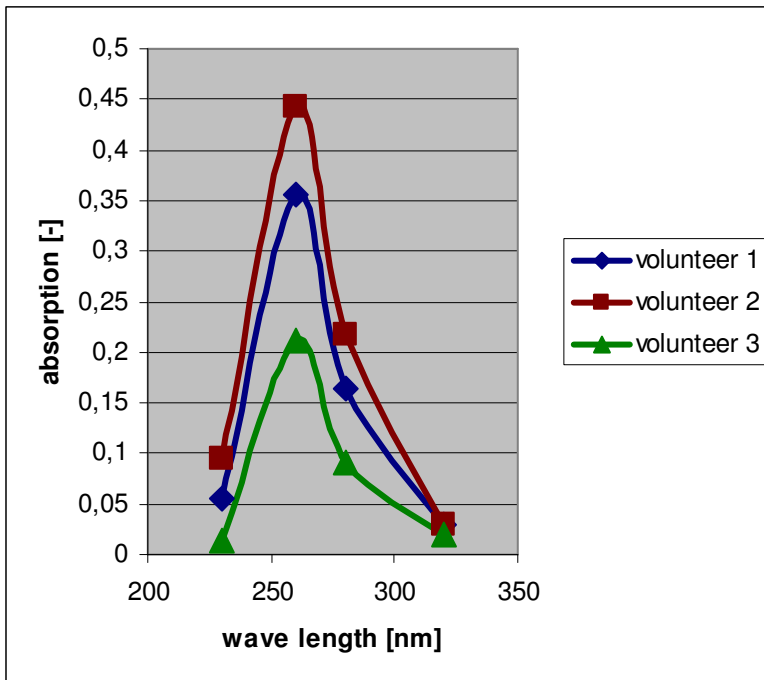


Figure 2: Spectra of absorption with maximum peak at 260 nm

3.3.1.2 Saliva sample collection using sterile buccal brushes

3.3.1.2.1 Sample collection

Saliva samples were collected from three volunteers using sterile buccal brushes (Catch-All™ Sample Collection Swabs, article number QEC091H) from Epicentre® Bio Technologies, Biocym Scientific GmbH, Biocym Biotech Trading GmbH, Vienna, Austria. Brushes were rotated against the left, the right, or both cheeks for 20 times, airdried, afterwards closed with the original hard-pack plastic carrier. Volunteers were asked not to drink, eat or smoke one hour before sample collection.

3.3.1.2.2 DNA Isolation using Buccal Amp™ DNA Extraction Kit

DNA was isolated using QuickExtract™ DNA Extraction Solution from Epicentre® Bio Technologies, Biocym Scientific GmbH, Biocym Biotech Trading GmbH, Vienna, Austria (article number QE09050).

The kit contained a single tube-system. Buccal brushes were rotated in the precast extraction solution for 10 times and pressed against the side while removing them. Solution was mixed using a vortex and incubated for 1 minute at 65° C, followed by a period of 2 minutes and 98° C.

Quantification: Quality evaluation and quantification was measured using a Jenway 6305 Spectrophotometer. Unfortunately, absorption measurements were not

successful, probably due to a very low DNA concentration or poor DNA quality. The supplier does not recommend absorption measurement to ascertain DNA concentration but because alternative methods had not been exercised in our laboratory, absorption measurement was the method of choice.

3.3.1.2.3 DNA Isolation using Gentra® Puregene® Buccal Cell Kit

DNA was isolated from saliva samples collected by sterile buccal brushes (see 3.3.1.1.1) using Gentra® Puregene® Buccal Cell Kit from Qiagen, Hilden, Germany (article number 158845).

Cell Lysis: Collection brush was removed from the handle using sterile scissors and was placed in a 1.5 ml centrifuge tube containing 300 µl Cell Lysis Solution. After addition of 1.5 µl Proteinase K, sample was incubated for 1 hour at 55° C.

RNase treatment: 1.5 µl RNase A was added to the lysate and sample was incubated for 15 minutes at 37° C.

Protein Precipitation: Sample was cooled on ice for 1 minute. After addition of 100 µl Protein Precipitation Solution, sample was again placed on ice for 5 minutes. Sample was centrifuged for 3 minutes at 13,000 xg and 24° C to form a tight protein pellet.

DNA Precipitation: Supernatant was transferred to a 1.5 ml centrifuge tube containing 300 µl Isopropanol and 0.5 µl Glycogen Solution. Sample was mixed and incubated for at least 5 minutes at room temperature. Sample was centrifuged for 5 minutes at 13,000 xg and 24° C. Supernatant was discharged; tube was dried using absorption paper. After addition of 300 µl ethanol, sample was centrifuged for 1 minute at 13,000 xg and 24° C. Ethanol was discharged and tube was opened in the laminar flow for 10 minutes to air dry.

DNA Hydration: To re-hydrate DNA, 15 µl Hydration Solution were added and sample was allowed to stand overnight at room temperature.

Quantification: Sample was diluted 1:50 using Hydration Solution. Absorption was measured at 230, 260, 280 and 320 nm using a Biophotometer. Isolated amount of DNA was calculated by multiplication the absorption at 260 nm with 50 (factor for DNA); quality was evaluated using the ratio of the absorption at 260 and 280 nm and the characteristics of the absorption spectra.

Results: Obtained DNA ranged from 0.2 µg to 4.12 µg, whereas the obtained amount differed between volunteers and was comparable within controls from the same

donator. DNA quality was unsatisfactory as the ratio of the absorption at 260 and 280 nm, which should be greater than 1.7, was just about one. Additionally, there was no clear peak to observe at 260 nm.

3.3.1.3 Saliva sample collection using Oragene™ Self Collection Kit

3.3.1.3.1 Sample collection

Saliva samples were collected via mouthwash using Oragene™ Self Collection Kit, Vial Format OG 100, DNA Gentotek Inc., Kanata, Ontario, Canada. Oragene™ Self Collection Kit is an all-in-one system for DNA collection, preservation, transportation and purification of DNA from saliva. Volunteers salivated into the prepared collecting tube for saliva until it was filled up to the given mark. Volunteers were asked not to drink, eat or smoke one hour before sample collection. After gentle shaking, sample was aliquoted to 250 µl.

3.3.1.3.2 DNA purification (Ethanol extraction)

Sample incubated for 1 hour and 50° C. After addition of 10 µl DNA Purifier, sample was placed on ice for 10 minutes. Sample was centrifuged at 15,000 xg and 24° C for 5 minutes; supernatant was transferred to a new centrifuge tube. After addition of 2.5 µl glycogen solution and 250 µl 95-100% ethanol, sample was incubated for 10 minutes at room temperature, afterwards centrifuged at 15,000 xg and 24° C for 2 minutes. Supernatant was discharged carefully without losing the pellet and tube was dried using absorbent paper. 125 µl 70% ethanol were added and sample was incubated for 1 minute at room temperature. Afterwards ethanol was discharged and tube was dried again. After addition of 50 µl DNA Buffer, sample was incubated at room temperature for two days to ensure rehydration of DNA.

Addition of glycogen solution and the ethanol wash step were optional and the protocol was done with and without those two steps.

Quantification: Absorption was measured at 230, 260, 280 and 320 nm using a Biophotometer. Isolated amount of DNA was calculated by multiplication of the absorption at 260 nm with 50 (factor for DNA); quality was evaluated using the ratio of the absorption at 260 and 280 nm and the characteristics of the absorption spectra.

Results: DNA was concentrated between 4.0 µg/ml and 8.7 µg/ml, whereas obtained amount differed between volunteers and was comparable within controls from the same donator. Absolute DNA yields ranged from 1.6 up to 3.48 µg, which is distinctly less than the expected amount given by the supplier with 110 µg. DNA quality was

unsatisfactory but problems during the protein precipitation step may be responsible for poor ratios of the absorption at 260 nm and 280 nm (<1.7). There were no appreciable differences in the result whether the optional glycogen addition and the optional ethanol wash were done or not.

3.3.1.3.3 DNA Isolation using Gentra® Purgene® Buccal Cell Kit

After gentle shaking, sample (saliva mixed with Oragene™ Self Collection Kit) was aliquoted to 250 μ l in 1.5 ml centrifuge tubes and incubated for 1 hour and 50° C.

Cell Lysis: 62.5 μ l Cell Lysis Solution and 1.66 μ l RNase A Solution were added and sample was incubated for 10 minutes at room temperature.

Protein Precipitation: After addition of 104.4 μ l Protein Precipitation Solution, sample was centrifuged for 5 minutes at 15,000 xg and 24° C to form a tight protein pellet.

DNA Precipitation: Supernatant was transferred to a 1.5 ml centrifuge tube containing 313 μ l Isopropanol and 2.5 μ l Glycogen Solution. Sample was mixed and centrifuged for 3 minutes at 2,000 xg and 24° C. Supernatant was discharged; tube was dried using absorbent paper. After addition of 312.5 μ l 70% Ethanol, sample was centrifuged for 1 minute at 2,000 xg and 24° C. Ethanol was discharged and tube was opened in the laminar flow for at least 10 minutes to air dry.

DNA Hydration: To re-hydrate DNA, 25 μ l Hydration Solution were added and sample was allowed to stand overnight at room temperature.

Quantification: Sample was diluted 1:50 using Hydration Solution. Absorption was measured at 230, 260, 280 and 320 nm using a Biophotometer. Isolated amount of DNA was calculated by multiplying the absorption at 260 nm with 50 (factor for DNA); quality was evaluated using the ratio of the absorption at 260 and 280 nm and the absorption spectra.

Results: DNA was concentrated between 4.3 μ g/ml and 10.7 μ g/ml, whereas obtained DNA amounts differed between volunteers and they were comparable within controls from the same donor. Absolute DNA yields ranged from 0.86 μ g up to 2.14 μ g, which is distinctly less than the expected amount, and the necessary amount, respectively. DNA quality was slightly impaired because the ratio of the absorption at 260 and 280 nm, which should be greater than 1.7, was somewhat lower (1.4 – 1.6).

3.3.1.4 Sampe collection via mouthwash and DNA isolation using the Gentra® Purgene® test kit

3.3.1.4.1 Sample collection

Saliva was collected using a mouthwash protocol. Participants were asked not to eat, drink or smoke the hour before sample collection. They swished 10ml Listerine® mouthwash for at least 20 seconds throughout the mouth and expectorated into a 50 ml centrifuge tube.

3.3.1.4.2 DNA Isolation using Gentra® Purgene® Buccal Cell Kit

DNA was extracted from saliva samples using Gentra® Purgene® Buccal Cell Core Kit A with the following protocol. Chemicals used are listed in Table 9.

Lysis: Collected samples were centrifuged for 10 minutes with 2,000 xg at 25° C using a Hettich Rotina 450R centrifuge. The step was repeated if the pellet was loose after centrifugation, otherwise, supernatant was wasted and 1.1 ml Cell Lysis Solution were added. After inverting the tube 50 times, samples were incubated at room temperature for 15 minutes during gentle shaking (80 rpm on Elmi shaker DOS-20L).

Table 9: Chemicals necessary for DNA isolation

Reagent	Supplier	Article number
Cell Lysis Solution	Qiagen Sciences	158906
DNA Hydration Solution	Qiagen Sciences	158918
Ethanol for molecular biology 99.8%	Merck	1.08543.0250
Glycogen Solution 20mg/ml	Qiagen Sciences	158930
2-Propanol for molecular biology ≥99%	Sigma	I9516
Protein Precipitation Solution	Qiagen Sciences	158910
Purgene Proteinase K	Qiagen Sciences	158918
RNase A Solution	Qiagen Sciences	158922
Sterile water for irrigation	Laboratoire Aguetant	

Protein Precipitation: From the whole lysate, 500 µl were transfered to 2 ml microcentrifuge tubes in each case. Each sample was extracted in doubles. 5 µl Proteinase K were added, tubes were inverted three times and mixed virgously on a vortex for 20 seconds. After incubation at room temperature for 10 minutes, 5 µl RNase A were added, tubes were inverted 25 times and incubated for 15 minutes at 37° C

using a HLC heating block. 170 µl protein precipitation solution were added, samples were mixed vigorously on a vortex and placed on crushed ice for 10 minutes. Afterwards samples were centrifuged for 10 minutes at 10,000 xg at 4° C using a Hereaus Fresco 17 microcentrifuge.

DNA Precipitation: Supernatant was transferred to a new 2 ml microcentrifuge tube containing 500µl Isopropanol and 2.5 µl glycogen solution. After inverting the tubes for 50 times, they were again centrifuged for 4 minutes at 3,000 xg and 20° C using the Hettich Rotina 450R centrifuge. Supernatant was wasted and tubes were dried using absorbent paper (Kimtech Science- Precision Wipes). The pellet was washed with 70% ethanol (7:3 ethanol: sterile water, v/v) and centrifuged at 300 xg for 4 minutes. Again, supernatant was wasted and tubes were dried using absorbent paper. Samples were allowed to stand open for 1 minute in the laminar flow (Steril Polaris) to make sure that the whole solvent was removed.

DNA Hydration: The DNA pellet was re-hydrated in 200 µl Hydration Solution. After 1 hour at 65° C in the HLC heating block, samples were allowed to stand overnight on a shaker at 80 rpm (Elmi DOS-20L).

Quantification: Afterwards the final DNA concentration and the absorption ratio of 260 nm to 280 nm allowing quality control were measured using the Nanodrop 1000 Spectrophotometer from PeqLab Biotechnologies. Samples were stored in 50 µl aliquots at -20° C.

Results: DNA was concentrated between 10 µg/ml and 300 µg/ml, whereas obtained DNA amounts differed between volunteers and they were comparable within controls from the same donor. Absolute DNA yields ranged from 4 µg up to 120 µg. DNA quality was slightly impaired because the ratio of the absorption at 260 and 280 nm, which should be greater than 1.7, was somewhat lower (1.4 – 1.6).

3.3.1.5 Evaluation of the tested methods for DNA isolation

Sample collection from saliva was preferred, because the obtained DNA quality and amount was satisfactory for further analyses. Further, saliva collection is a non-invasive method and therefore more comfortable for participants and no doctor is needed as he or she were for blood collection. In fact, participants are able to collect the sample on their own with a short description of the collection process.

Table 10 gives an overview on the obtained DNA amounts, the obtained DNA quality and the costs for the analyses of each tested method.

Table 10: Overview on compared methods for DNA collection and isolation

Method: Collection and isolation		DNA amount [absolute; µg]	DNA quality [Absorption: 260/280nm]	Price/sample [€]
Take blood sample (venous EDTA blood)	PeqLab Blood DNA Isolation Kit	2.11 – 4.43	2.0 – 2.3	2.28
Biozym Buccal Brushes (saliva)	Epicentre® Buccal Amp™ DNA Extraction Kit	Absorption measurements were not successful; quantification and quality evaluation not possible		3.79
Biozym Buccal Brushes (saliva)	Gentra® Purgene® Buccal Cell Kit	0.2 – 4.12	<< 1.7	4.15
Mouthwash using Listerine® (saliva)	Gentra® Purgene® Buccal Cell Kit	4.0 – 120.0	1.4 – 1.6	2.23
Oragene™ Self Collection Kit (saliva)	Gentra® Purgene® Buccal Cell Kit	0.86 -2.14	1.4 – 1.6	24.82
Oragene™ Self Collection Kit (saliva)	Ethanol extraction	1.6 – 3.48	<< 1.7 (improveable)	23.88

Although the Oragene™ Self Collection Kit offers a comfortable collection and isolation possibility, the Gentra® Purgene® Buccal Cell Kit in combination with a mouthwash protocol for sample collection was preferred. It can be assumed, that the quality of DNA offered using the Oragene™ Self Collection Kit could be improved eliminating initial problems in the purification step but the kit itself was expensive for all participants regarding the costs per samples. As the isolation protocol was comparable regarding the time and difficulty, the cheaper variant was chosen which was the sample collection using mouthwash combined with DNA isolation using Gentra® Purgene® Buccal Cell Kit. Although the DNA quality was not completely satisfactory, the allelic discrimination could be determined without any problems, therefore absorption ratios (260/280nm) of 1.4 – 1.6 were accepted.

The other methods did not offer a DNA quality that was high enough for further analyses (PCR) or the obtained amount of the DNA was simply too little because in the whole study project, over 20 polymorphisms were planned to be genotyped. That requires a minimum of absolute DNA amount higher than 1.5 µg.

3.3.2 Determination of allelic distribution using polymerase chain reaction

Single nucleotide polymorphisms (SNP) are variants of a single nucleic acid sequence. The allelic discrimination assay is an end-point assay, which means that data is collected at the end of the Polymerase Chain Reaction (PCR) process. It allows the genotyping of two possible variants in a target template sample (the gene locus carrying the polymorphism). The method distinguishes between homozygotes (carriers of two identical alleles, of either a couple of allele 1 or a couple of allele 2) and heterozygotes (carriers of both alleles). The quality of the target DNA sequence cannot be determined with the applied method.

In the allelic discrimination assay for each sample, there is a unique pair of fluorescent dye detectors. Each fluorescent dye detector perfectly matches one of the two alleles (Figure 3). The changes in fluorescence of the dyes associated with the probes are measured at the end of the allelic discrimination run after the signal was amplified due to repeated polymerisation cycles: In an initial step, the DNA double helix (DNA template = sample) is separated. The forward- and reverse primer⁵ (assay components) can bind to each single strand and enable the DNA polymerase⁶ (assay component; yellow symbol in Figure 3) to bind and synthesise the complementary strand. This procedure is repeated and the DNA template proliferates, which at least causes the amplification of the fluorescent signal. Probes⁷ (assay components) carrying one of the two fluorescent dye detectors (V: VIC[®] dye fluorescence; F: FAM[™] dye fluorescence; Figure 3) can bind to the replicated DNA single strands – the VIC[®] dye fluorescence detector only matches allele 1, the FAM[™] dye fluorescence only matches allele 2. Only after perfect match the fluorescent dye detector is quenched (Q: Quencher; assay component; Figure 3) and after repeated polymerisation cycles the changes in the both fluorescence signals are recorded. Increases in the VIC[®] dye fluorescence only indicate homozygosity for allele 1, increases in the FAM[™] dye fluorescence only indicate homozygosity for allele 2, and increases in both fluorescence signals indicate heterozygosity (AppliedBiosystems, 2006).

⁵ Primer: Short RNA strand that enables DNA polymerases to bind to the DNA single strand; “forward” and “reverse” describes the concordance to either one or the other single strand. After cleavage of the DNA double helix two complementary single strands exist (Loeffler, 2001)

⁶ DNA polymerase: Enzyme that synthesises a DNA single strand to a double strand; the generated double strand can serve as new template after a fresh cleavage. (Loeffler, 2001)

⁷ Probe: Short RNA strand that harbours the polymorphism to be analysed (AppliedBiosystems, 2006)

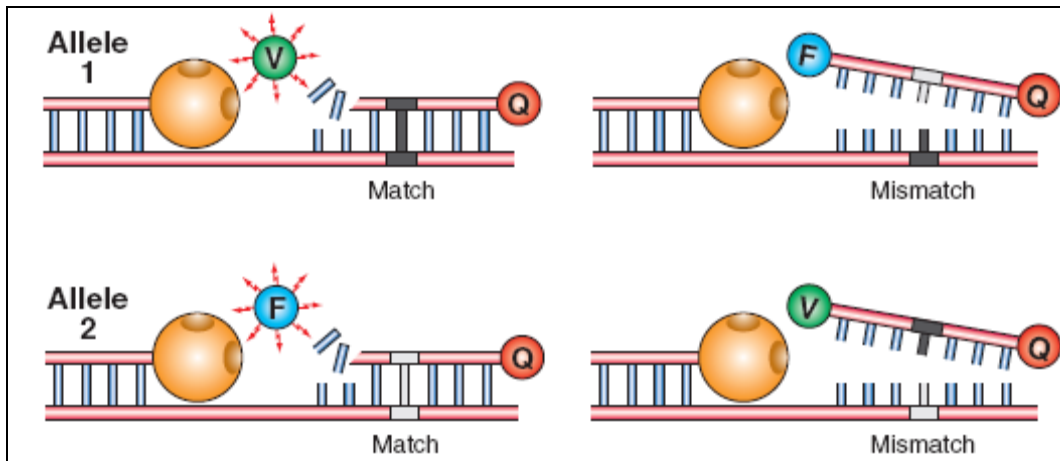


Figure 3: Matches and mismatches between target and probe sequences in the TaqMan® Gene Expression Assays (Livak, Marmaro & Todd, 1995; in AppliedBiosystems, 2006, p. 3)

The analysed gene polymorphisms are listed in Table 11.

Table 11: Overview on analysed gene polymorphisms

Gene variant	Database SNP (rs#)	Base pair exchange/SNP type	Test kit
ACTN3 R577X	rs1815739	C to T transition substitution/ nonsense mutation	Applied Biosystems C_590093_1_
IGF1 AG	rs35767	G to A transition substitution	Applied Biosystems C_799146_10
INS-IGF2 CG/ C13790G	rs3213221	C to G transversion substitution	Applied Biosystems C_3145635_10
INS-IGF2 GT	rs7924316	G to T transversion substitution	Applied Biosystems C_29192859_10
MSTN K153R	rs1805086	C to T transition substitution/ missense mutation	Applied Biosystems C_282184_30

3.3.2.1 Pretest to assess suitable DNA concentration for optimal test conditions

For PCR run conditions, supplier recommend to use 1-20 ng DNA per well. To find out the optimal amount of DNA applied, all kits were tested with the use of 20, 10, 5, and 1 ng DNA per well. The lowest amount of DNA was chosen, which showed a signal that was at least twice as high as the negative control (NTC), or had a comparable high signal as higher concentrations. DNA solutions were prepared as described in chapter 3.3.2.2, PCR run and evaluation of results are described in chapter 3.3.2.3 and 3.3.2.4.

Table 12: Results of a concentration test

Well	Sample Name	Signal Allele X	Signal Allele Y	Call
A1	NTC	0,563	0,619	NTC
A2	NTC	0,548	0,56	NTC
A3	NTC	0,546	0,55	NTC
A4	20ng DNA/well	2,241	2,092	Both
A5	20ng DNA/well	2,201	2,048	Both
A6	10ng DNA/well	2,087	1,962	Both
A7	10ng DNA/well	2,072	1,941	Both
A8	5ng DNA/well	2,018	1,889	Both
A9	5ng DNA/well	2,027	1,878	Both
A10	1ng DNA/well	1,712	1,679	Both
A11	1ng DNA/well	1,829	1,745	Both
B1	20ng DNA/well	2,711	0,654	IGF2 rs3213221C
B2	20ng DNA/well	2,732	0,657	IGF2 rs3213221C
B3	10ng DNA/well	2,687	0,644	IGF2 rs3213221C
B4	10ng DNA/well	2,68	0,648	IGF2 rs3213221C
B5	5ng DNA/well	2,586	0,618	IGF2 rs3213221C
B6	5ng DNA/well	2,602	0,618	IGF2 rs3213221C
B7	1ng DNA/well	2,344	0,598	IGF2 rs3213221C
B8	1ng DNA/well	2,368	0,608	IGF2 rs3213221C
B9	20ng DNA/well	2,237	2,134	Both
B10	20ng DNA/well	2,252	2,104	Both
B11	10ng DNA/well	2,218	2,032	Both
B12	10ng DNA/well	2,238	2,065	Both
C1	5ng DNA/well	2,095	2,08	Both
C2	5ng DNA/well	2,16	2,015	Both
C3	1ng DNA/well	1,891	1,816	Both
C4	1ng DNA/well	1,824	1,812	Both
C5	20ng DNA/well	2,195	1,972	Both
C6	20ng DNA/well	2,178	1,998	Both
C7	10ng DNA/well	2,104	1,986	Both
C8	10ng DNA/well	2,105	2,013	Both
C9	5ng DNA/well	2,104	1,868	Both
C10	5ng DNA/well	2,128	1,868	Both
C11	1ng DNA/well	1,824	1,655	Both
C12	1ng DNA/well	1,692	1,742	Both

Table 12 shows the results of the concentration test with primer for the IGF2 CG polymorphism. 5 ng DNA per well were the chosen DNA amount for this assay because with 1 ng DNA per well the signal was clearly weaker and higher concentrations did not cause much higher signals.

Table 13 shows the used DNA concentrations per well for each applied assay.

Table 13: Suitable amounts of DNA per well for each applied assay

Polymorphism	Used DNA concentration for PCR
ACTN3 R577X (rs1815739)	1 ng DNA per well = 0.09 ng/μl
IGF1 AG (rs35767)	10 ng DNA per well = 0.89 ng/μl
IGF2 CG (rs3213221)	1 ng DNA per well = 0.09 ng/μl
IGF2 GT (rs7924316)	5 ng DNA per well = 0.44 ng/μl
MSTN K153R (rs1805086)	5 ng DNA per well = 0.44 ng/μl

One well contains 11,25μl

3.3.2.2 Test Preparations

The sample concentration was measured using the Nanodrop 1000 Spectrophotometer from PeqLab Biotechnologies and diluted with sterile water to a concentration of 0.89 ng/μl following the calculation shown in Figure 4. If the necessary sample amount to prepare 100μl of the diluted solution was lower than 1 μl, a multiple greater than 1 μl and the corresponding amount of water were used. If the original DNA concentration was higher than 180 ng/μl, an intermediate solution concentrated to 8.9 ng/μl was prepared and diluted 1:10 (v/v). The 0.89 ng/μl concentrate was diluted 1:2 (v/v) to 0.44 ng/μl, which was afterwards diluted 1:5 (v/v) to reach the 0.09 ng/μl solution. Figure 4 also gives three examples to clarify the calculation and dilution steps.

Formula:

$100 \mu\text{l } 0.89\text{ng}/\mu\text{l dilution} = (c [\text{original solution}] * 100) / 0.89 \mu\text{l original solution} + 1 - (c [\text{original solution}] * 100) / 0.89 \mu\text{l sterile water}$

Example 1: $c_{\text{sample}} = 54.2 \mu\text{g}/\text{ml} = 54.2 \text{ ng}/\mu\text{l}$; necessary amount: 100 μl (for PCR and further dilutions)

54.2 ng ... 1 μl

0.89 ng ... x, $x = 0.89 / 54.2 = 0.016$; $0.016 * 100$ (100 μl dilution are necessary) = 1.64 → 1.64 μl sample + 98.36 μl (100-1.64) sterile water → dilution concentrated 0.89 ng/μl

0.44 ng/μl dilution: 50 μl dilution concentrated 0.89 ng/μl + 50 μl sterile water

0.09 ng/μl dilution: 14 μl dilution concentrated 0.44 ng/μl + 56 μl sterile water

Example 2: $c_{\text{sample}} = 95 \mu\text{g}/\text{ml} = 95 \text{ ng}/\mu\text{l}$; necessary amount: 100 μl (for PCR and further dilutions)

95 ng ... 1 μl

0.89 ng ... x, $x = 0.89 / 95 = 0.009$; $0.009 * 100$ (100 μl dilution are necessary) = 0.9 $\rightarrow$ 0.9 μl sample amount is too little for pipetting $\rightarrow$ 150 μl dilution are prepared: 1.41 μl + 148.59 μl sterile water $\rightarrow$ dilution concentrated 0.89 ng/ μl

0.44 ng/ μl dilution: 50 μl dilution concentrated 0.89 ng/ μl + 50 μl sterile water

0.09 ng/ μl dilution: 14 μl dilution concentrated 0.44 ng/ μl + 56 μl sterile water

Example 3: $c_{\text{sample}} = 290.9 \mu\text{g/ml} = 290.9 \text{ ng}/\mu\text{l}$; necessary amount: 100 μl (for PCR and further dilutions)

290.9 ng ... 1 μl

0.89 ng ... x, $x = 0.89 / 290.9 = 0.003$; $0.003 * 100$ (100 μl dilution are necessary) = 0.3 $\rightarrow$ 0.3 μl sample amount is too little for pipetting and the necessary amount of water is with over 150 μl too much for addition $\rightarrow$ an intermediate solution concentrated 8.9 ng/ μl was prepared:

Dilution concentrated 8.9 ng/ μl : 1.22 ($0.3 * 4$, so the amount for pipetting is higher than 1) + 38.78 ng/ μl sterile water (for 40 μl dilution)

0.089 ng/ μl dilution: 10 μl dilution concentrated 8.9 ng/ μl + 90 μl sterile water

0.44 ng/ μl dilution: 50 μl dilution concentrated 0.89 ng/ μl + 50 μl sterile water

0.09 ng/ μl dilution: 14 μl dilution concentrated 0.44 ng/ μl + 56 μl sterile water

Figure 4: Formula for preparing a sample dilution concentrated 0.89 ng/ μl

The assay reagent was prepared light protected. 12.5 μl TaqMan[®] Genotyping Master Mix (Applied Biosystems, P/N: 4371355, L/N: 0811030) was mixed with 0.625 μl TE buffer (Hydration Solution; see Table 9) and 0.625 μl Genotyping Assay 40X (see Table 11) per well. The necessary amount of Master Mix was calculated by multiplying the mentioned amounts of components with the planned number of wells on the reaction plate and adding extra reactions to provide excess volume for the loss that occurs during reagent transfer (AppliedBiosystems, 2006).

3.3.2.3 Polymerase Chain Reaction

For allelic discrimination, a Real-Time PCR System (Applied Biosystems 7500) was used. Samples were pipetted on a 96-well plate, each well containing 13.75 μl assay reagent and 11.25 μl sample. For the negative control (NTC), sterile water was used.

Allelic discrimination of each polymorphism and sample was determined in triplets. After pipetting, the reaction plate was covered with optical adhesive cover and the plate was centrifuged briefly to eliminate air blowing. After loading the reaction plate into the PCR system, the Pre-Read Run was followed by the amplification period. The assay finished with the Post-Read Run (Figure 5 - Figure 7). Assay conditions were assessed

using the corresponding software to the PCR system following the suppliers guidance step by step (AppliedBiosystems, 2006).

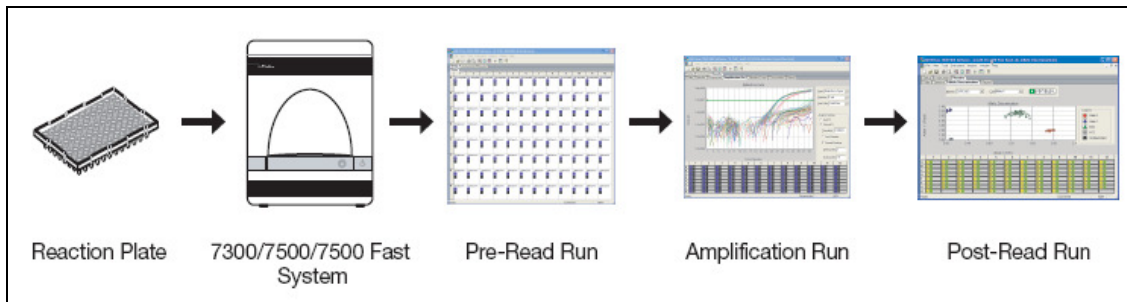


Figure 5: Design of an allelic discrimination assay

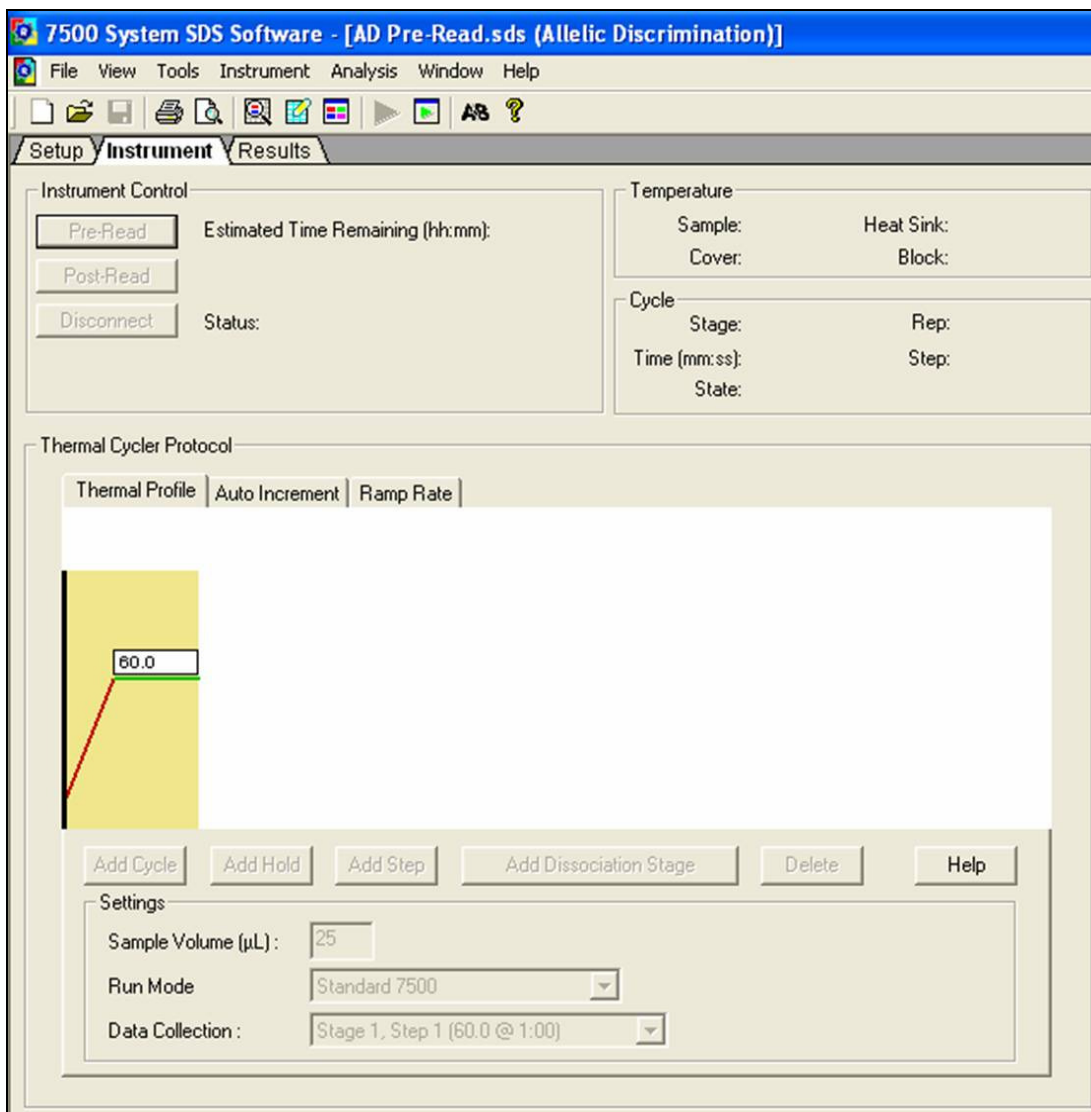


Figure 6: Assay conditions of the PCR Pre-Read and Post-Read Run

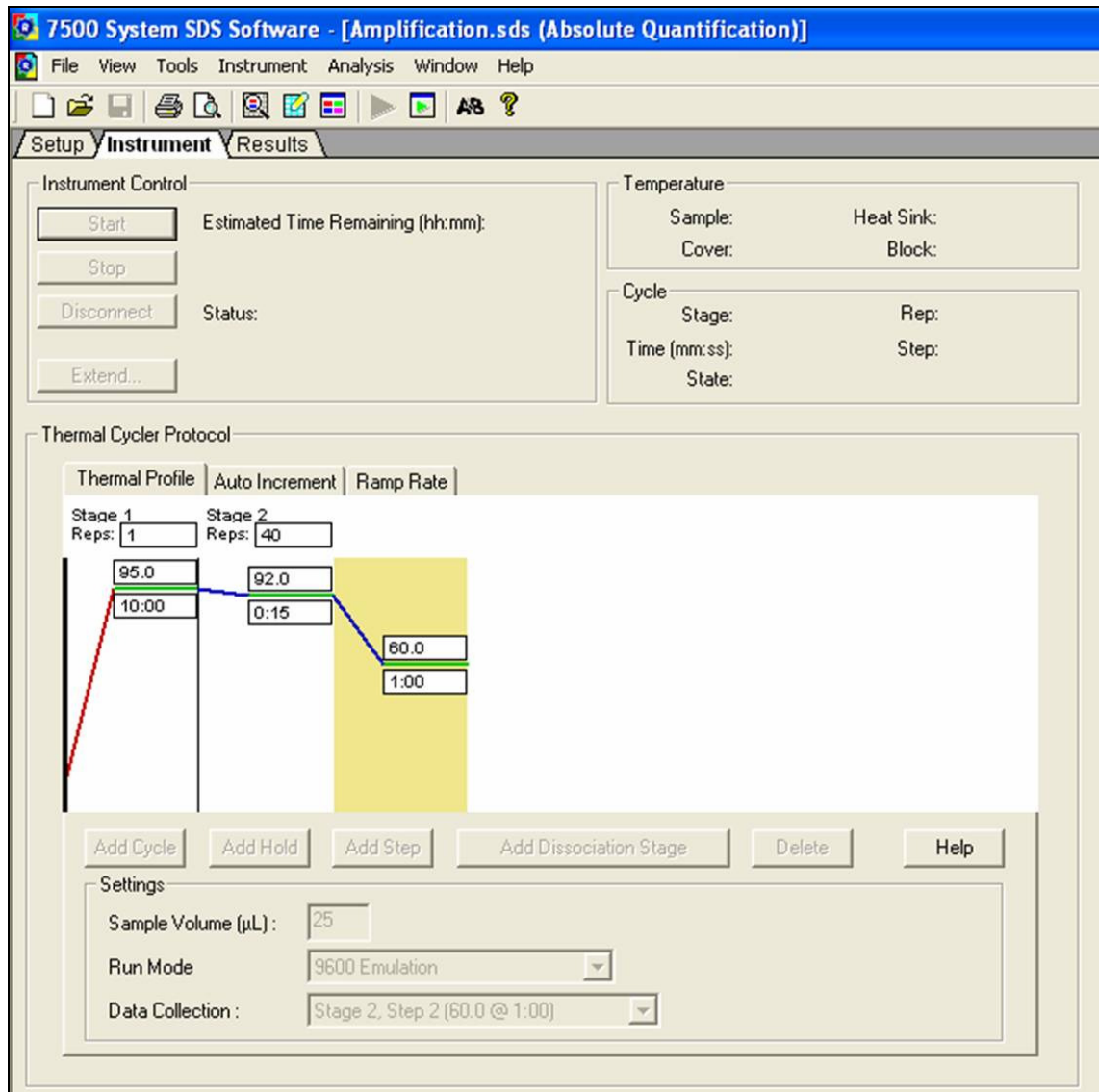


Figure 7: Assay conditions of the PCR amplification run

During the Pre- and Post-Read Run, samples were once heated to 60° C. The amplification run started with a ramp to 95° C that were maintained for 10 minutes. Afterwards 40 repeats of 15 minutes at 92° C followed by one minute at 60° C. Whereas the Pre- and Post-Read Run measured the fluorescence of the two dye detectors (VIC® and FAM™), during the amplification run the DNA stretch that harbors the polymorphism of interest was replicated in repeated cycles. As either the VIC® or the FAM™ detector (homozygotes) or both detectors (heterozygotes) increased due to the quenching process after a successful match on the DNA stretch that harbors the adequate allele variant, the change in fluorescence between the Pre- and Post-Read Run allowed the discrimination of the genotype (AppliedBiosystems, 2006, see also chapter 3.3.2).

3.3.2.4 Evaluation of results

DNA stretches carrying the polymorphism of interest were labeled with two different dyes (VIC[®] and FAM[™]), one for the ancestral allele and one for the variant. Results were interpreted manually according to the given NTC (Figure 8). In samples homozygote for allele 1, only the fluorescent signal of the VIC[®] dye detector increased, resulting in a x-value at least twice as high as the x-value of the NTC. In samples homozygote for allele 2, only the fluorescent signal of the FAM[™] dye detector increased, resulting in a y-value at least twice as high as the y-value of the NTC. Samples that were different from the NTC according to the x- as well as the y-value (fluorescence of the VIC[®] and FAM[™] dye detector increased) were outlined as heterozygotes (AppliedBiosystems, 2006, see Figure 8).

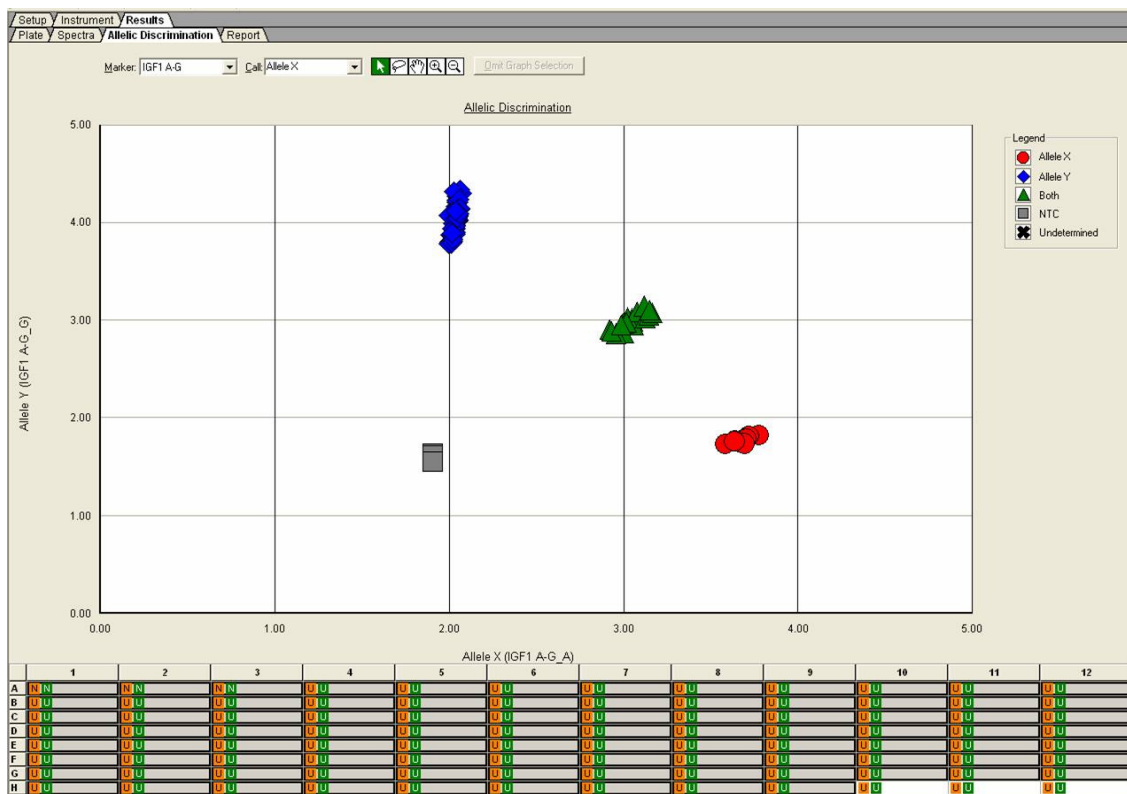


Figure 8: Interpreted results of the PCR Post-Read Run

3.4 Statistical analysis

For all statistical analyses, SPSS for Windows[®] Version 15.0 was used. Normal distribution was tested using the Kolmogorov-Smirnov-Test as well as the histogram and the QQ-Plot. Group differences between the cohorts “controls - athletes” were tested using the Student’s t-test and the Mann-Whitney U-test, respectively. Differences between sedentaries, active people and endurance athletes as well as

gender differences were tested using crosstabulation (Pearson's Chi-Square). Alternatively, the study population was divided into quartiles using the PAL value. The three cohorts of different activity level (first quartile, second and third quartile, fourth quartile) were compared using crosstabulation (Pearson's Chi-Square). The significance level α was set 0.05.

3.4.1 Test for Hardy-Weinberg Equilibrium

Genotype distribution was compared with Hardy-Weinberg equilibrium using Pearson Chi-Square Goodness-of-fit Test and Exact Test (Ryckman & Williams, 2008). Expected genotype distribution was calculated using the formula $p^2 + 2pq + q^2 = 1$, whereas p and q were the expected allele frequencies (Ryckman & Williams, 2008). Expected minor allele frequencies were given by the kit supplier, the dominant allele frequency was calculated using the formula $q = 1 - p$ ($[p+q]^2 = 1$).

The reference population consisted of 45 Caucasians except for the myostatin polymorphism, which refers to 19 Caucasians (AppliedBiosystems, 2009a; b; f; e; c; d). Minor allele frequencies and expected genotype distributions are listed in Table 14.

Table 14: Minor allele frequencies used for the calculation of the Hardy-Weinberg equilibrium

Polymorphism	AB minor allele and minor allele frequency (n=45 Caucasians) *AGI minor allele frequency (n=19 Caucasians)	Calculated genotype distribution $p^2 + 2pq + q^2 = 1$		
		p^2 : frequency of the homozygote variant genotype	$2pq$: frequency of the heterozygote genotype	q^2 : frequency of the homozygote ancestral genotype
ACTN3 R577X (rs1815739)	T: 0.36	0.130	0.461	0.410
IGF1 (rs35767)	A: 0.13	0.017	0.226	0.757
INS-IGF2 (rs3213221)	C: 0.47	0.221	0.498	0.281
INS-IGF2 (rs7924316)	T: 0.46	0.212	0.497	0.292
MSTN K153R (rs1805086)	C: 0.03*	0.001	0.058	0.941

4 Results and discussion

4.1 Study population

Alltogether 244 volunteers were genotyped for the above-described polymorphisms. 40 of them met inclusion criteria for one of the athlete groups (see Appendix, Table 5, p. 27). The residual 204 control subjects were grouped to sedentary and active subcategories (Table 5, p. 27). Gender distribution, age, BMI and PAL of the three study cohorts are described in Table 6 on page 28.

The whole study group was not in Hardy-Weinberg equilibrium for tested polymorphisms ACTN3 R577X (rs1815739), MSTN K153R (rs1805086), IGF2 (rs3213221) and IGF1 (rs35767). For the IGF2 (rs7924316) polymorphism, the population was in Hardy-Weinberg equilibrium. When the study population was divided into control subjects and endurance athletes, results were the same except for the ACTN3 R577X (rs1815739) polymorphism: whereas control subjects were not in Hardy-Weinberg equilibrium, the athlete group was. Results of the Exact Test and the Person Chi-Square Goodness-of-fit Test were identical, although the Exact Test is recommended (Ryckman & Williams, 2008) for small sample sizes as in our analysis.

Several reasons, for example genotyping errors, might cause genotype distributions different from Hardy-Weinberg equilibrium (Ryckman & Williams, 2008). In our analyses, interpreting the absence of Hardy-Weinberg equilibrium must be done with caution because several assumptions must be met for the derivation of the Hardy-Weinberg theorem (Ryckman & Williams, 2008). Some of them might not be fulfilled in our case: firstly, the population size might be not large enough, especially in the athlete cohort. In fact, it is extremely difficult to get an athlete sample, which is large and even “pure” enough. There is hardly a large athlete cohort engaged on a professional and excellent level in one sport discipline, which would be necessary for significant genotype distribution testing. Other analyses are limited by similar problems (Ivey et al., 2000; Thomis et al., 2004; Muniesa et al., 2008).

In the original study protocol, the Austrian national ski team was planned to be genotyped. The idea of analysing the nation ski team was that Austria is one of the most successful countries in the international ski circuit and a rather large sample cohort might have been possible. Unfortunately, the Austrian Ski Association (ÖSV) has not yet agreed in cooperation.

Although our sample size, especially in the athlete group, was small, the reference population for Hardy-Weinberg testing was as well ($n = 45$).

Another assumption for the derivation of the Hardy-Weinberg equilibrium theorem is that there are no fitness differences among genotypes (Ryckman & Williams, 2008). As accurately, fitness differences among genotypes are our hypothesis, significant differences of the genotype distribution to Hardy-Weinberg equilibrium could be a link for the genotype influence on fitness phenotypes, even though we might not be able to detect genotype differences in our sample cohorts of different activity level. Naturally, the deviation from Hardy-Weinberg Equilibrium is an insufficient evidence for the impact of a polymorphism on fitness parameters.

In fact, testing Hardy-Weinberg Equilibrium using Person Chi-Square Goodness-of-fit test has several limitations and deviation from Hardy-Weinberg Equilibrium should be interpreted with caution (Goddard, Ziegler & Wellek, 2009).

4.2 Differences between cohorts of different activity level

Table 15: Genotype distribution in controls and athletes

Polymorphism and ancestral allele	Cohort	Homozygote ancestral genotype (females/n)	Heterozygote genotype (females/n)	Homozygote variant genotype (females/n)
ACTN3 R577X (rs1815739) Ancestral allele: R (C)	Sedentary	28% (15/22)	46% (22/36)	26% (8/20)
	Active	32% (14/35)	44% (24/48)	23% (11/25)
	Athletes	42% (11/15)	39% (9/14)	19% (4/7)
IGF1 (rs35767) Ancestral allele: A	Sedentary	72% (30/56)	26% (13/20)	3% (2/2)
	Active	70% (33/76)	28% (14/30)	2% (2/2)
	Athletes	78% (20/28)	19% (3/7)	3% (1/1)
IGF2 (rs3213221) Ancestral allele: G	Sedentary	36% (15/28)	49% (21/38)	14% (8/11)
	Active	40% (16/43)	42% (22/45)	18% (11/19)
	Athletes	47% (5/17)	47% (6/17)	6% (1/2)
IGF2 (rs7924316) Ancestral allele: T	Sedentary	37% (19/29)	51% (22/40)	12% (4/9)
	Active	32% (14/34)	49% (25/52)	20% (10/21)
	Athletes	31% (3/11)	53% (7/19)	17% (2/6)

MSTN K153R (rs1805086)	Sedentary	96% (44/75)	4% (1/3)	---
Ancestral allele: K (C)	Active	99% (49/107)	---	1% (0/1)
	Athletes	100% (12/36)	---	---

Distributions of the genotypes of analysed polymorphisms are shown in Table 15. Determination of genotype was successful in 78 sedentary people, 108 active people and, 36 athletes, respectively. Group comparisons were performed on the one hand between sedentaries, active people and endurance athletes; on the other hand, the sample was divided into quartiles using the PAL value as parameter of physical activity as there were also some very active people with high PAL levels (>2.0).

It has to be mentioned that in the original study protocol the Austrian ski team was aimed to be genotyped. Unfortunately, the ski team has not been available yet. As the hypothesis was, that polymorphisms linked with strength, power, and body composition phenotypes might influence success in skiing competition, the analysed polymorphisms were selected within that context. Effects of the selected genetic variants on endurance performance have not sufficiently been resolved, especially for the polymorphisms in the IGF1 (rs35767) and IGF2 (rs3213221 and rs7924316) genes.

4.2.1 The α -actinin-3 R577X (rs1815739) polymorphism

The R577X polymorphism is an allele change from CGA to TGA. Whereas CGA codes for arginine, TGA is a stop codon, the exchange results in a nonsense function of the ACTN3 protein (NCBI, 2009b).

No significant differences ($p>0.05$) between cohorts (neither between quartiles nor between athletes and controls) of different activity level could be found concerning the distribution of the ACTN3 R577X genotype (Table 15, p. 49, Figure 9).

Whereas many researches support that the ACTN3 RR genotype favours sprint and power performance (Yang et al., 2003; Druzhevskaya et al., 2008; Papadimitriou et al., 2008), data is not that evident for the XX genotype and endurance performance. A higher frequency of the XX genotype in sample cohorts of endurance athletes in comparison to controls or sport-/power athletes suggested an advantage of the XX genotype for endurance performance (Yang et al., 2003; Niemi & Majamaa, 2005). The thesis was not confirmed in other endurance athlete samples (Lucia et al., 2006; Muniesa et al., 2008), some did even find an also higher occurrence of the RR genotype in elite endurance athletes (Yang et al., 2007; Ahmetov et al., 2008). A

higher presence of the R-allele in endurance athletes might also reflect requirements in modern endurance competitions: due to high running speeds and final sprints, fast power generation is even important in endurance competitions (Lucia et al., 2006; Ahmetov et al., 2008). Additionally, it must be mentioned that only a higher frequency of XX homozygotes in endurance athletes compared with control subjects indicates a favourable role of the XX genotype. If a higher XX genotype was noticed in endurance athletes than in sprint or power athletes, that likely resulted of the higher RR genotype in the sprint/power cohort. The RR genotype is considered advantageous in complex sports, too. In Italian gymnasts the RR genotype was significantly more frequent than in control subjects (Massidda, Vona & Calo, 2009).

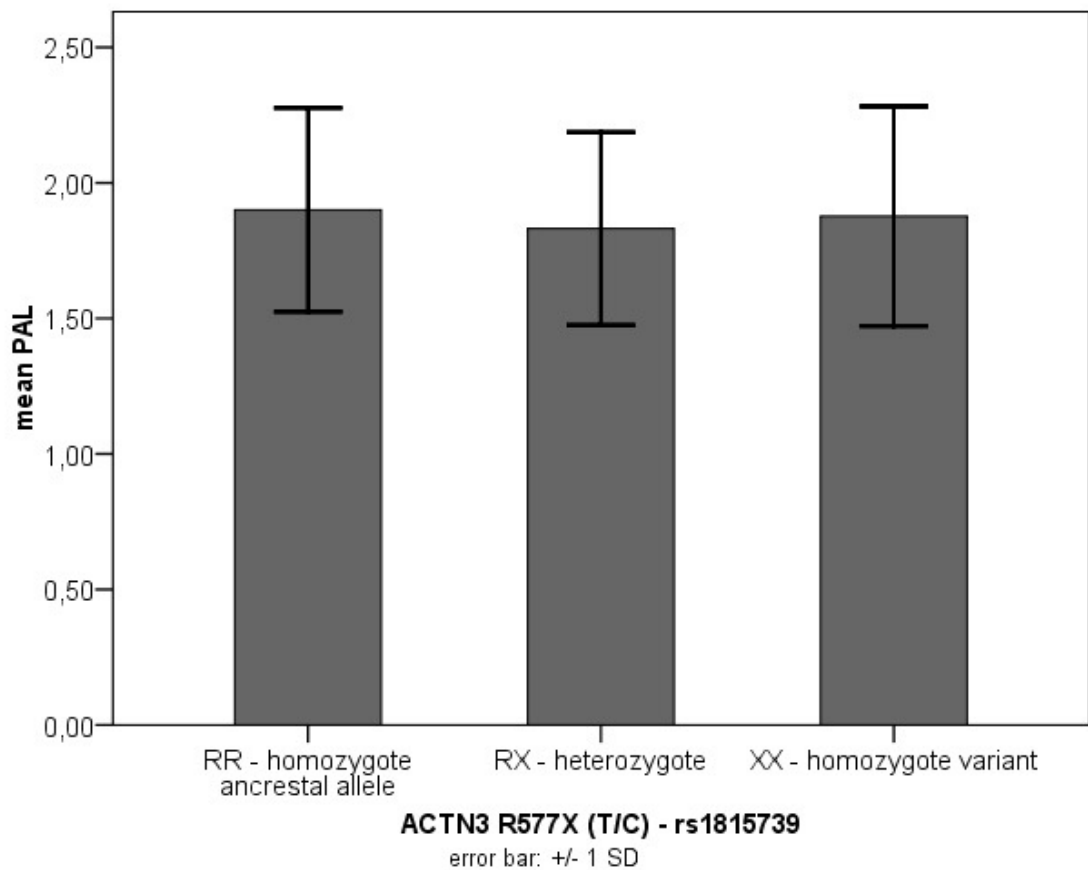


Figure 9: Mean PAL values in the ACTN3 R577X (rs1815739) genotype groups

Results of the present investigation are to be assimilated to the number of negative outcomes. An explanation for diverging results was given by Eynon, Duarte, Oliveira, Sagiv, Yamin, Meckel and Goldhammer (2009). Genotyping of 155 Israeli athletes and 240 sedentaries highlighted a higher frequency of the RR genotype in sprint athletes with a correlation between the RR genotype and success in sprint competition. The XX genotype was more often found in endurance athletes but frequency did not differ

between top-level and national runners. Authors conclude that the XX genotype might be additive to endurance performance but not critical whereas the RR genotype seems to be for sprint performance (Eynon et al., 2009). As the genotyping of East- and West-African athletes (Yang et al., 2007) showed, the RR genotype may also be advantageous, at least not disadvantageous, for endurance performance.

After splitting the control group into sedentary and active individuals, there was neither a difference concerning the ACTN3 R577X genotype distribution. Results agree with the thesis of Yang, Garton and North (2009), who concluded that the polymorphism contributes to the normal variations in muscle strength and sprinting speed, but it was not to be supposed to find differences within the control group. Delmonico, Zmuda, Taylor, Cauley, Harris, Manini, Schwartz, Li, Roth, Hurley, Bauer, Ferrell and Newman (2008) stated that there was an influence of the ACTN3 R577X polymorphism even in a non-athlete cohort, but the effect was only observable in a longitudinal design in older adults: in over 2000 older adults various parameters of muscle function were measured and associated with the ACTN3 polymorphism. Whereas there were no differences between genotype groups at baseline, the increase in the time spent for a 400-meter walk after 5 years was significantly higher in male XX homozygotes. Results indicate that the polymorphism might influence decline in physical performance in older adults but effects on performance were at most weak (Delmonico et al., 2008). No impact of the ACTN3 R577X variant concerning patterns of daily living was found in sedentary individuals (North et al., 1999).

4.2.2 The IGF1 rs35767 polymorphism

The selected polymorphism (rs35767) in the gene coding for IGF1 is a transition substitution of A by G in the nearGene-3 position with an unknown clinical association (AppliedBiosystems, 2009d; NCBI, 2009a).

No significant differences ($p > 0.05$) between cohorts (neither between quartiles nor between athletes and controls) of different activity level could be found concerning the distribution of the IGF1 rs35767 genotype (Table 15, p. 49, Figure 10).

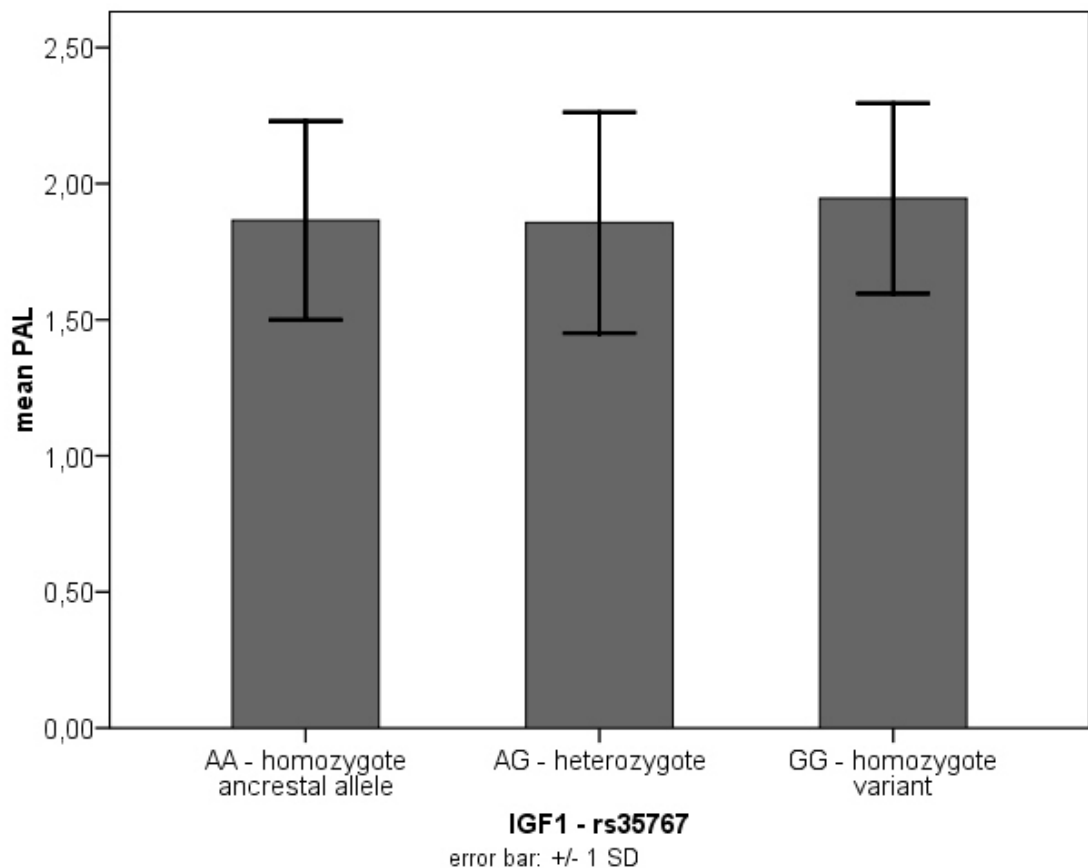


Figure 10: Mean PAL values in the IGF1 (rs35767) genotype groups

As IGF1 is a human growth factor, circulating IGF1 might have an impact on body composition and muscle growth (Sun et al., 1999; Yu & Rohan, 2000; Shyu et al., 2005, and others. See also chapter 2.1.2). Therefore, polymorphisms that influence circulating IGF1 levels might also have an impact on strength phenotypes.

We hypothesised that genotypes that increase IGF1 blood levels might occur in a higher frequency in alpine skiracers because they may support success in competition. Although strength and power-related phenotypes are important in endurance runners and cyclists too (Lucia et al., 2006), excessive muscle growth might be disadvantageous. Therefore, it can be hypothesised that the variant in the IGF1 gene (rs35767) does not distinctly influence endurance performance.

However, published data concerning the IGF1 rs35767 polymorphism is contradictory: The IGF1 rs35767 polymorphism did not show any correlation with circulating IGF1 plasma levels in Caucasian women (Canzian et al., 2006) or with muscle regeneration after eccentric strength exercise in Caucasians (Devaney et al., 2007). Palles, Johnson, Coupland, Taylor, Carvajal, Holly, Fentiman, Silva Idos, Ashworth, Peto and

Fletcher (2008) highlighted an impact of the polymorphism on circulating IGF1 levels in middle-aged women. Lopez-Alarcon et al. (2007) found a moderate impact of the IGF2 CT dinucleotide MS polymorphism on endurance performance (treadmill time and submaximal VO_2 during stairclimbing and cycling) in sedentary premenopausal women of African-American and European-American origin. Carriers of the homozygote 189bp genotype showed higher endurance (time spent on treadmill during $\text{VO}_{2\text{max}}$ test) and better exercise economy (submaximal VO_2 during stairclimbing and cycling). Within the HERITAGE Family Study (Sun et al., 1999), Caucasian sedentaries conducted an aerobic exercise training program. In homozygotes for the 189bp allele of the IGF2 CT dinucleotide MS polymorphism the gain in fat free mass was significantly less than in subjects of the other genotype groups. However, the polymorphism did also influence baseline body composition (Sun et al., 1999). No results on the impact of the IGF1 rs35767 polymorphism on endurance performance could be found.

4.2.3 The IGF2 rs3213221 polymorphism

The selected polymorphism (rs3213221) in the gene coding for IGF2 is a transversion substitution of C by G in the 1st intron of the gene with an unknown clinical association (Devaney et al., 2007; AppliedBiosystems, 2009e; NCBI, 2009d).

No significant differences ($p > 0.05$) between cohorts (neither between quartiles nor between athletes and controls) of different activity level could be found concerning the distribution of the IGF2 rs3213221 genotype (Table 15, p. 49, Figure 11).

The impact of the genotype distribution on endurance performance has not been discussed in published data. Devaney et al. (2007) found differences between genotype groups in the response to eccentric strength training in the elbow flexor muscle only in men: Homozygotes of the rare allele (GG) showed greater strength losses (immediately after exercise), higher muscle soreness (3 days postexercise), and a higher creatine kinase activity as indicator for muscle damage (7 days postexercise) than homozygotes of the ancestral allele (CC). Mean values of the heterozygotes lied in the middle and were not significantly different to those of the homozygotes (Devaney et al., 2007).

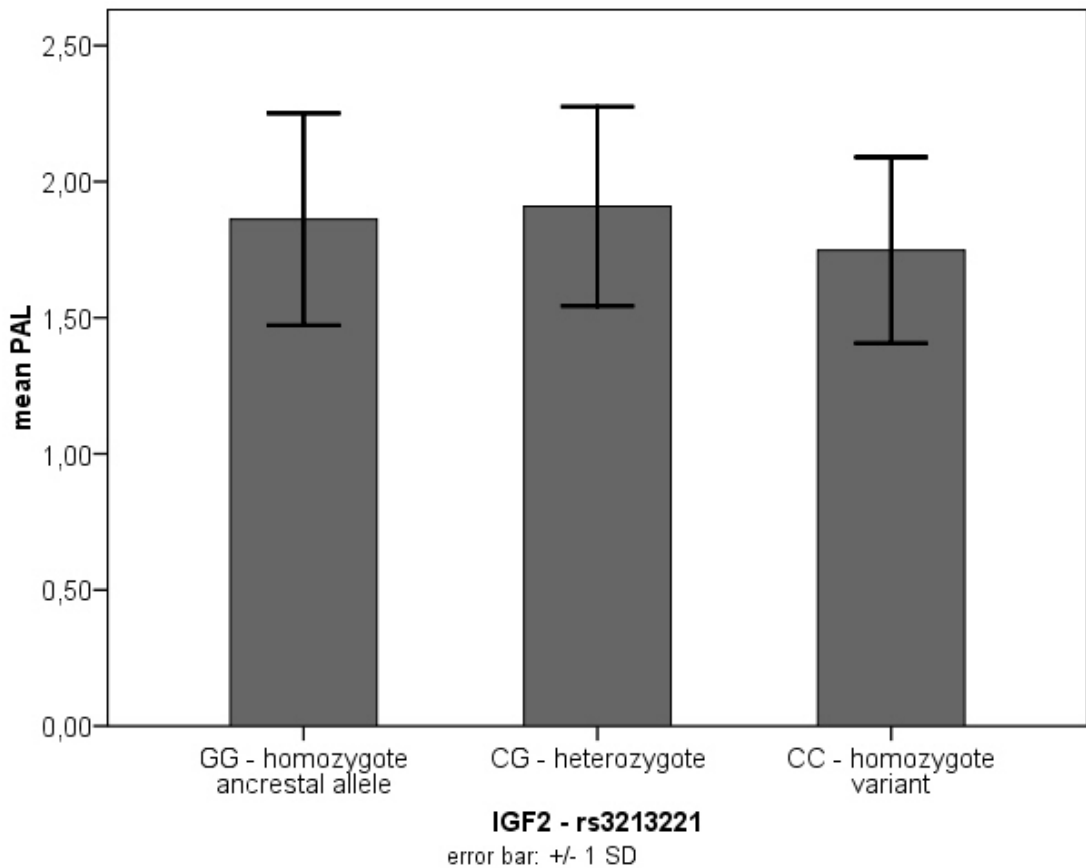


Figure 11: Mean PAL values in the IGF2 (rs3213221) genotype groups

Results of Devaney et al. (2007) indicate an association between the rare G-allele and muscle sensitivity to eccentric strength bounds. Present results did not reveal any differences concerning the IGF2 rs3213221 genotype and overall physical activity. Reasons might be that not the direct impact of a training input was assessed and secondly, the composition of the study cohorts, as only an endurance athlete group was used.

4.2.4 The IGF2 rs7924316 polymorphism

The selected polymorphism (rs7924316) in the gene coding for IGF2 is a transversion substitution of G by T in the intron (3-Downstream region, Devaney et al., 2007) of the gene with an unknown clinical association (AppliedBiosystems, 2009f; NCBI, 2009e).

No significant differences ($p > 0.05$) between cohorts (neither between quartiles nor between athletes and controls) of different activity level could be found concerning the distribution of the IGF2 rs7924316 genotype (Table 15, p. 49, Figure 12).

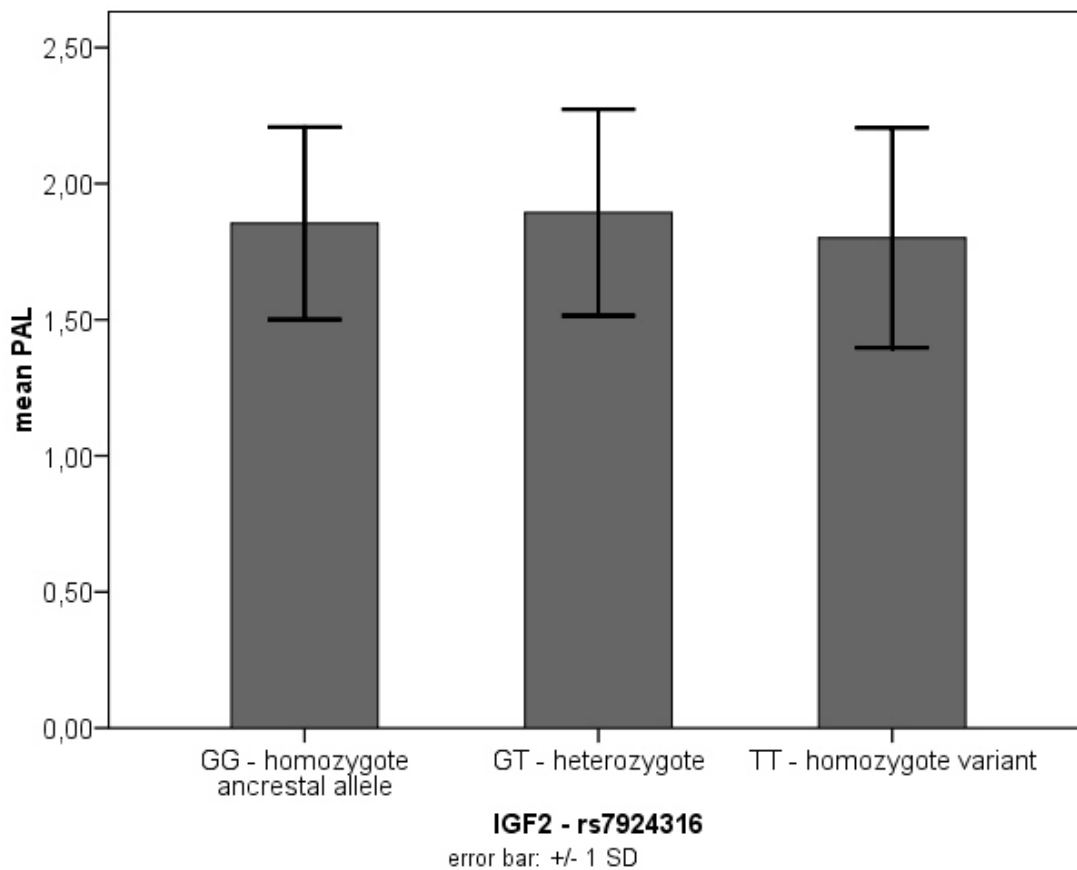


Figure 12: Mean PAL values in the IGF2 (rs7924316) genotype groups

The IGF2 rs7924316 genotype distribution was the only one of the five analysed polymorphism that was in Hardy-Weinberg Equilibrium. As the assumption was made, that the remaining polymorphisms might have an influence on fitness phenotypes (see chapter 4.1) due to the absence of Hardy-Weinberg Equilibrium concerning the genotype distribution (Ryckman & Williams, 2008), this was not true for the IGF2 rs7924316 polymorphism. It can be speculated that the IGF2 rs7924316 polymorphism might not have any impact on the fitness phenotype.

Devaney et al. (2007) found a link between differences in response to strength training and the IGF2 rs7924316 genotype, but only in men: Strength losses immediately after maximal isotonic eccentric strength exercise in the elbow flexor muscle were greater in homozygotic men of both variants (GG and TT) than in male heterozygotes. Effects lasted for 10 days. Muscle soreness 3 and 4 days postexercise was significantly higher in TT homozygotic men than in men carrying the other two genotypes. Myoglobin and creatine kinase activity was significantly higher in male TT homozygotes than in heterozygotic men indicating higher muscle damage in individuals carrying the TT

genotype. Mean value of the GG genotype was between but not significantly different to the other two genotype groups. The population studied consisted three-quarter of Caucasians and was in Hardy-Weinberg equilibrium for the IGF2 rs7924316 polymorphism (Devaney et al., 2007).

Findings of Devaney et al. (2007) indicate a higher sensitivity of individuals homozygotic for the IGF2 rs7924316 polymorphism to strength exercise in comparison to heterozygotes. Contradictory, in this analysis the overall activity level and not the effect of a special training input was examined and the athlete group consisted of endurance athletes. Therefore, there is no analogism within the results.

4.2.5 The myostatin K153R (rs18050586) polymorphism

The K153R polymorphism is an allele change from AAG to AGG, which results in an amino acid exchange of lysine to arginine (NCBI, 2009c). Although both amino acids belong to the group of amino acids with charged and polar side chains (Loeffler, 2001), the exchange results in a missense function (NCBI, 2009c).

No significant differences ($p > 0.05$) between cohorts (neither between quartiles nor between athletes and controls) of different activity level could be found concerning the distribution of the MSTN K153R genotype (Table 15, p. 49, Figure 13).

Interestingly, there were no athletes carrying the variant allele (Table 15, p. 49), whereas some of the controls did. In fact, the variant allele R of the MSTN K153R polymorphism is rather rare in Caucasians (minor allele frequency 0.03 (Applied Biosystems, 2009a)). Studies on the influence of the MSTN K153R polymorphism on physical performance in Caucasians failed, because there were no participants carrying the rare RR genotype (Ivey et al., 2000; Seibert et al., 2001; Thomis et al., 2004).

As the athlete cohort consisted of endurance specialists, the absence of a variant allele is not surprising. Various analyses found the KK genotype of the MSTN K153R polymorphism more advantageous for endurance performance (Gonzalez-Freire et al., 2008; Muniesa et al., 2008; Ruiz, Gomez-Gallego, Santiago, Gonzalez-Freire, Verde, Foster & Lucia, 2009; Santiago, Ruiz, Muniesa, Gonzalez-Freire, Gomez-Gallego & Lucia, 2009). However, hypotheses, that the KK genotype favours endurance performance must be interpreted with caution: Although in world-class runners there were hardly athletes carrying the RR genotype (Gonzalez-Freire et al., 2008; Muniesa et al., 2008), this might be a consequence of the extreme low RR genotype frequency,

especially in the analysed Spain (Caucasian) sample. As the world's best middle and long distance runners are mostly of African origin (Yang et al., 2007), the analyses of that ethnic group would be of interest. Ferrell et al. (1999) found a significant higher frequency of the rare RR genotype in African-Americans than in Caucasians. There might be similar differences between ethnic groups as they were found for the ACTN3 R577X genotype. Whereas some analyses in Caucasian endurance athletes indicated a favourable role of the XX genotype for endurance performance (Yang et al., 2003; Niemi & Majamaa, 2005; Ahmetov et al., 2008), the hypothesis could not be confirmed in a East and West African endurance runners. The XX genotype was hardly present, neither in the athlete nor in the control group (Yang et al., 2007).

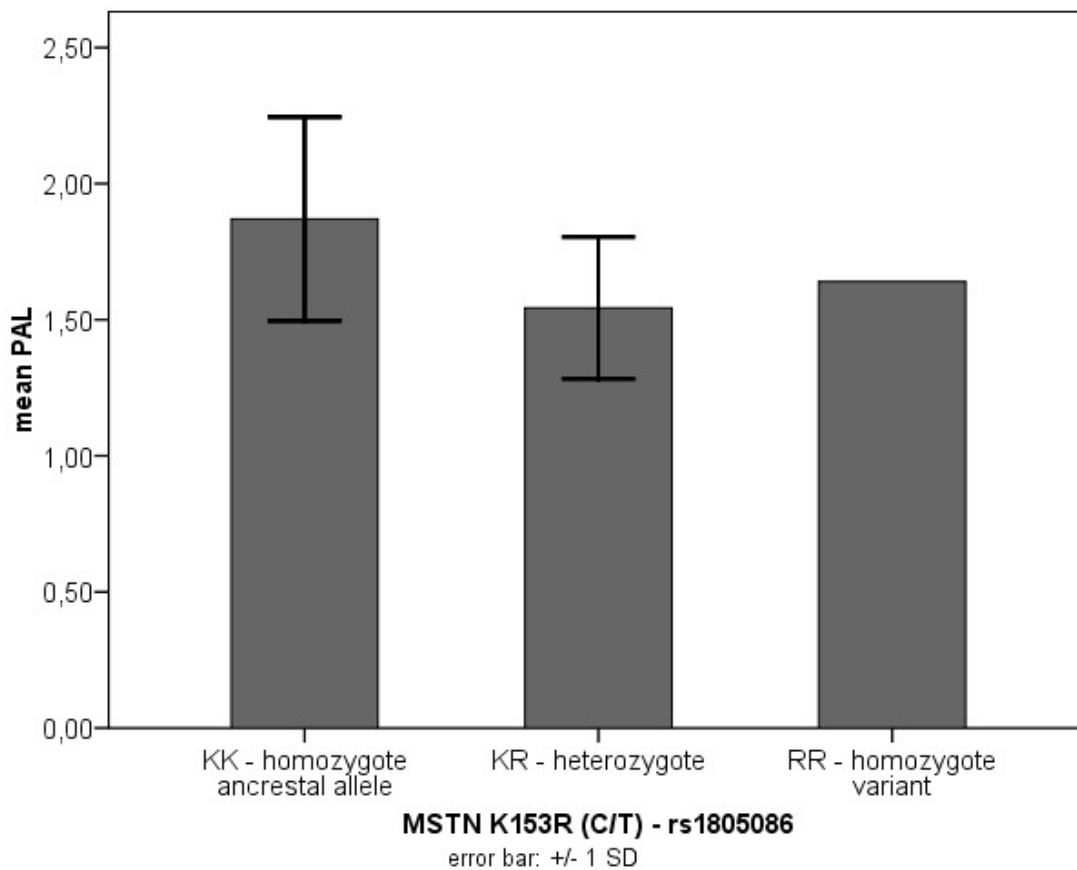


Figure 13: Mean PAL values in the MSTN K153R (rs1805086) genotype groups

We had hypothesised that the MSTN K153R polymorphism might influence skiing performance as strength and endurance as well as partly body composition are important components for success. In elite soccer players (Juffer, Furrer, Gonzalez-Freire, Santiago, Verde, Serratosa, Morate, Rubio, Martin, Ruiz, Arenas, Gomez-Gallego & Lucia, 2009), there was no impact of the K153R polymorphism found on

success, nor was there a difference between the genotype frequency of male sedentaries, endurance athletes and soccer players (Juffer et al., 2009).

4.3 Gender differences

Neither in the whole study population nor within the three study cohorts (sedentary, active, athlete) gender differences could be detected ($p > 0.05$). Although results were not significant, only female athletes carried the GG (variant) genotype in the IGF1 (rs35767) gene (Table 15, p. 49). This is so much more demonstrative as only 3% (12 individuals) of the athletes are female (Table 6, p. 28) whereas gender distribution was balanced in the sedentary and active control groups. Insignificance might be the consequence of the low number of subjects in the categories (36 endurance athletes are divided by sex and genotype).

The impact of the IGF1 rs35767 and other polymorphisms (mainly in the IGF2 gene) on muscle generation after bouts of strength exercise was determined in Caucasian males and females (Devaney et al., 2007). There were no associations between muscle generation and the IGF1 rs35767 polymorphism and no gender differences could be observed. Considering all analysed polymorphisms (in the IGF1 and IGF2 gene) there were more significant associations in men than in women but no apparent explanation could be found (Devaney et al., 2007). A not significant trend for lower body height and relative mortality risk was observed only in female inhabitants of the Netherlands carrying the non-dominant (A) allele of the IGF1 CA dinucleotide microsatellite polymorphism (van Heemst et al., 2005). Other researches on genetic variants in the IGF1 gene included either only men or only women in their analyses (Jernstrom et al., 2001; Allen et al., 2002; Lopez-Alarcon et al., 2007) or did not find any gender differences (Rosen et al., 1998; Sun et al., 1999; Vaessen et al., 2001; Kostek et al., 2005; Morimoto et al., 2005; Canzian et al., 2006). Lower IGF2 serum levels and higher body weight and height was found in GG (ancestral allele) homozygotic men of the IGF2 Apal (rs680) polymorphism whereas the genotype effect was not observed in women (Sayer et al., 2002). Results of O'Dell et al. (1997) confirmed these genotype effects in men, whereas women were not included in the analysis. Muscle performance (strength parameters) was found to be higher in GG homozygotes of the IGF2 Apal (rs680) polymorphism only in women but not in men (Schrager et al., 2004).

However, gender differences were partly observed concerning different gene variants but study results lack a conclusive explanation. An impact of the ACTN3 R577X

polymorphism (rs1815739) on increase in muscle strength in response to resistance training was found in females but not in males (Clarkson et al., 2005). In a similar strength training study, gender differences of the ACTN3 R577X polymorphism (rs1815739) could not be confirmed (Roth et al., 2005). Baseline strength parameters were found to be different between female genotype (rs1815739) groups whereas no differences were observed in men (Walsh et al., 2008).

Highly significant differences concerning the between ACTN3 R577X (rs1815739) allele frequency was assessed between female sprint and endurance athletes (Yang et al., 2003). As the effect could not be confirmed in men, gender effects of the ACTN3 R577X (rs1815739) genotype on performance were suggested. Contrary, in a sample of East and West-African elite athletes and controls, no gender differences of variants in the ACTN3 gene (rs1815739) were detected (Yang et al., 2007).

The effect of the MSTN K153R (rs1805086) on muscle response to resistance training was assessed in men and women (Ivey et al., 2000). As the rare R-allele was only found in female individuals, results indicate gender differences concerning the MSTN (rs1805086) genotype frequency.

4.4 The Total Genotype Score (TGS)

The study aimed to assess combined genetic effects on physical performance because it can be considered that not one polymorphism alone but the combination of several favorable gene variants causes a real genetic advantage concerning sports performance.

To evaluate polygenetic profiles, Williams and Folland (2008) generated the model of the Total Genotype Score (TGS). The model assigned a genotype score for every included polymorphism, whereas the preferable genotype for a certain phenotype was equated with “2”, the less optimal variant was equated with “0”. Heterozygotes got the value “1”. The maximum possible genotype score was calculated by multiplying the number of polymorphisms included with 2. The sum of each individual genotype score was expressed as part of 100 (Williams & Folland, 2008).

Although the five polymorphisms analysed in the present study are obviously not strongly associated with endurance, the comparison of the TGS between endurance athletes and controls would have been of interest. Unfortunately, the selected polymorphisms in the IGF1 (rs35767) and IGF2 (rs3213221, rs7924316) genes analysed have not adequately been documented and described in published data. As

our own data did not highlight any differences in the genotype frequency between endurance athletes and controls, it is not obvious whether the polymorphisms favoured endurance performance or not, and in the case they did, which variant were the optimal one for endurance performance. Therefore, it was impossible to calculate the TGS because genotypes cannot reasonably be assigned. Even if the calculation of the TGS would have been possible, it cannot be supposed that different TGS between controls and athletes were noted because differences in the genotype distribution of any analysed polymorphism were not detected either.

The study population was grouped according to the alleles of the ACTN3 R577X as well as of the MSTN K153R polymorphisms. 4 groups were designed: One with carriers of at least one copy of the ancestral allele of both polymorphisms, one with carriers of at least one copy of the variant allele of both polymorphisms, and two groups with carriers of at least one copy of the variant allele of one polymorphism and at least one copy of the ancestral allele of the second polymorphism (Table 16). Mean PAL values were calculated within the groups. Heterozygotes of both polymorphisms are part of each of the four groups because they carry every possible allele combination.

Table 16: ACTN3 R577X and the MSTN K153R polymorphisms and PAL

Allele of the ACTN3 R577X polymorphism (at least one copy; X = variant allele)	Allele of the MSTN K153R polymorphism (at least one copy; R = variant allele)	PAL (mean $\pm$ SD)
ACTN3-R + MSTN-K		1.86 $\pm$ 0.366
ACTN3-R + MSTN-R		1.54 $\pm$ 0.141
ACTN3-X + MSTN-K		1.85 $\pm$ 0.374
ACTN3-X + MSTN-R		1.57 $\pm$ 0.218

It is remarkable that in the groups with carriers of the MSTN K153R variant allele (R) the PAL is distinctly lower than in the other two groups. The R-allele of the MSTN K153R polymorphism is associated with power athlete status, the effect on endurance performance are discussed controversially (chapter 2.1.3.1, 4.2.5). In the present study population, only four individuals carried at least one copy of the very rare R-allele (Table 15, p. 49). None of them was classified as athlete, whereas all genotyped athletes were homozygotic for the ancestral K-allele of the MSTN K153R

polymorphism. Therefore, the lower PAL values in the groups with carriers of the R-allele (MSTN K153R polymorphism) are the logical result. The PAL values in the other two groups (with carriers of the K-allele of the MSTN K153R polymorphism) are comparable with the mean PAL values of the whole study population, which is 1.87 ± 0.380 .

No endurance athletes carried at least one copy of the variant allele (R) of the MSTN K153R polymorphism, which is in accordance with outcomes of other investigations (Gonzalez-Freire et al., 2008; Muniesa et al., 2008; Ruiz et al., 2009) but it might also be only the result of the very low allele frequency (Table 14, p. 47; chapter 4.2.5).

Williams & Folland (2008) calculated the hypothetical preferable TGS for endurance performance including 23 polymorphisms. The probability for an individual of the whole world's population to possess the optimal genetic profile concerning the 23 from Williams and Folland selected polymorphisms was 0.0005%. The calculation documents that genetic predisposition is the basis for excellent physical performance but many other factors must be co-determining for success. The same assumption is confirmed by the outcomes of Santiago et al. (2009). Seven candidate polymorphisms for better endurance performance using the TGS were analysed in 39 excellent and 15 national-class Caucasian lightweight rowers. The polygenetic profiles did not differ between rowers of the different levels. However, there was a trend observable that rowers had a better polygenetic profile indicated by a higher TGS than control subjects (Santiago et al., 2009). The comparison of 46 excellent endurance athletes and 123 Caucasian controls highlighted significantly higher TGS values in the athletes than in the controls (Ruiz et al., 2009). Polymorphisms analysed were the same in both researches, namely polymorphisms in the ACE, the ACTN3, the peroxisome proliferators-activated receptor γ coactivator 1 α , the creatine kinase, the adenosine monophosphate deaminase and the hereditary haemochromatosis gene (Ruiz et al., 2009; Santiago et al., 2009). Significance of results is limited because the seven selected candidate gene polymorphisms are only a part of genes and polymorphisms involved determining endurance performance (Williams & Folland, 2008; Ruiz et al., 2009; Santiago et al., 2009).

5 Conclusion

The aim of the study was to compare genotype frequencies of five candidate polymorphisms in cohorts of different activity level.

In recent years, the number of polymorphism discussed to influence fitness phenotypes and physical performance has emerged (Rankinen et al., 2006a). Despite many investigations according to the impact of various polymorphisms on physical performance were done, uncertainty remains concerning the effective influence of a certain gene variant on a certain phenotype (Savulescu & Foddy, 2005; Rankinen et al., 2006a).

The ACTN3 R577X polymorphism (rs1815739) causes a loss of function in the ACTN3 protein, which occurs in skeletal muscle (North et al., 1999; MacArthur & North, 2004). The RR genotype is the ACTN3 positive one and evidently influences performance in sprint competitions (Yang et al., 2003; Druzhevskaya et al., 2008; Papadimitriou et al., 2008; Massidda et al., 2009). The impact of the polymorphism on endurance performance is indistinct (Niemi & Majamaa, 2005; Lucia et al., 2006; Yang et al., 2007; Eynon et al., 2009).

The K153R polymorphism (rs 1805086) in the MSTN gene affects the expression of the negative growth factor myostatin (McPherron et al., 1997; McNally, 2004). Effects on strength performance were discussed but results are uncertain, at least due to the very low frequency of the RR variant in certain populations (Ferrell et al., 1999; Seibert et al., 2001; Thomis et al., 2004). The impact of the polymorphism on endurance performance was assessed, too. There are references that indicate a favourable role of the KK genotype but outcomes are inconsistent (Gonzalez-Freire et al., 2008; Muniesa et al., 2008; Ruiz et al., 2009; Santiago et al., 2009).

Variants in the IGF1 and IGF2 genes in the context of physical performance have hardly been analysed although polymorphisms might influence the function of IGF1 and IGF2 as growth hormones and therefore physical performance. Devaney et al. (2007) analysed the effects on strength outputs and strength training response of a number of polymorphisms in the IGF1 and IGF2 genes. Some associations were found but results were difficult to be interpreted evidently.

244 volunteers donated their DNA for genotyping of the ACTN3 R577X (rs1815739) polymorphism, of the MSTN K153R polymorphism (rs1805086), and of variants in the IGF1 (rs35767) and the IGF2 (rs 3213221, rs7924316) gene, respectively. All

participants gave written informed consent to the analyses and the study protocol was approved by the local ethic committee. DNA was isolated from saliva, which was collected using mouthwash. DNA was isolated using a prepared isolation kit from Qiagen. Allelic discrimination was assessed using real-time PCR and test kits from Applied Biosystems. Statistical analyses were calculated in SPSS for Windows using the significance level $\alpha = 0.05$.

48% of the probands were classified as active, meaning they averagely exercised more than 3 hours per week. 36% were sedentary (<3 hours exercise/week). 16% were endurance athletes competing on a national or international level.

Differences in the genotype distributions between cohorts could not be detected in any of the five analysed polymorphisms. Additionally, genotype frequencies were compared between the quartiles of physical activity indicated by the PAL value. Differences could not be observed, either. No gender differences were detected.

Whereas the effects of the ACTN3 R577X (rs1815739) and the MSTN K153R (rs1805086) on endurance performance are contrary discussed in present data, no results dealing with variants in the IGF1 (rs35767) and in the IGF2 (rs3213221, rs7924316) gene have been published yet.

Although the control group had an acceptable sample size, the athlete group was rather small for genetic analysis. In fact, it is difficult to get an elite athlete sample from very homogenous sport disciplines, which is also large enough to detect genetic effects, especially in Austria. The national ski team might have been the best possible Austrian athlete cohort. Moreover, alpine skiracers have not been the object of genetic analyses and findings would have been helpful in the discussion whether strength or endurance is the dominating skill in alpine skiing disciplines.

Negative results concerning the effects of the analysed polymorphisms might indicate that the impact of these variants on sport performance was overestimated. Success in competition is multifactorial and genetic predisposition is only one part. As we cannot exactly assess the extent of genetics, neither be sure about the direction of genetic effects, every result is helpful in this large field of research.

On the other hand, the sample might have been too inhomogenous to highlight genetic effects. In fact, large enough sample cohorts and a very rigorous classification of sample cohorts were the necessary fundament for the prediction of significant genetic effects. Requirements that were not completely met by this analysis, neither by many

other published works. Often they simple cannot be achieved. It is important to evaluate results of genetic analyses in the context of the used sample population as, for example, the occurrence of the minor allele highly differs between different ethnic groups. Ethnic effects might have an impact on the interpretation of outcomes.

To sum up, present data indicates that the ACTN3 R577X (rs1815739) polymorphism, the MSTN K153R polymorphism (rs1805086), and variants in the IGF1 (rs35767) and the IGF2 (rs 3213221, rs7924316) gene do not influence endurance performance in Austrians because the genotype distribution was not different to the control cohort.

Polygenetic profiles are more useful to be analysed because it is evident that not one polymorphism alone decides about success or failure in competition but combined gene effects are critical or at least additive for success in competition. However, present data did not allow the calculation of the Total Genotype Score because advantageous genotypes could not be identified. Complementary effects of the ACTN3 R577X (rs1815739) and the MSTN K153R (rs1805086) polymorphism on the PAL value were difficult to interpret due to the very low frequency of the rare R-allele of the MSTN K153R (rs1805086) polymorphism.

Although the knowledge about genetic predisposition for and the impact of gene variants on sport performance is advanced by current works with analyses of larger cohorts and highly excellent athletes, future research is needed to get evident data. Conclusive data on genetic variants and their effects on performance-related phenotypes would complement possibilities in talent scouting and allow developing individual training schedules – for elite athletes as well as for sedentaries and recreational sportspeople.

6 Summary

The study aimed to assess differences of the genotype frequency of five candidate polymorphisms in cohorts of different activity level in the context of a larger study on genetics and physical performance. Analysed polymorphisms were the ACTN3 R577X (rs1815739) polymorphism, the MSTN K153R (rs1805086) polymorphism as well as variants in the IGF1 (rs35767) and the IGF2 (rs3213221, rs7924316) gene. It was hypothesised that genotype frequencies differ between study cohorts; especially it was supposed to find different genetic profiles in athletes.

DNA was isolated of saliva samples, which were collected using mouthwash. Samples were genotyped using TaqMan® SNP Genotyping assays and real-time PCR. All participants gave written informed consent and the study was approved by the local ethics committee.

244 probands were classified as sedentary (<3 hours exercise/week; 36%), active (>3 hours exercise/week, 48%) and athletes (competing on national or international level of different sport disciplines, 16%). Additionally, the whole study group was divided (quartiles) according to the activity level indicated by the PAL, which was assessed using a Physical Activity Questionnaire. Group differences were calculated on the significance level $\alpha = 0.05$ using SPSS for Windows.

Differences in the genotype distributions of any of the five analysed polymorphisms were not detected, either between the cohorts “sedentary”, “active” and “athletes” or between quartiles of different physical activity. Gender differences could not be observed.

Results indicate that the analysed polymorphisms do not harbour any advantage for endurance performance in Austrians. The calculation of polygenetic effects was intended but not possible.

Assessed genetic variants are rather associated with strength and power parameters, whereas their impact on endurance performance is discussed controversially, and even unexplored, respectively. Present results contradict a possible impact of the five analysed polymorphism on endurance performance but future research is reasonable.

7 Zusammenfassung

Die Verteilung der genetischen Varianten ACTN3 R577X (rs1815739), MSTN K153R (rs1805086), sowie Varianten im IGF1 (rs35767) und IGF2 (rs3213221, rs7924316) Gen wurde in Gruppen unterschiedlicher körperlicher Aktivität im Rahmen eines größeren Projekts zum Einfluss genetischer Varianten auf die Leistungsfähigkeit bestimmt. Es wurde vermutet, dass sich die Gruppen in der Häufigkeit der verschiedenen Genotypen unterscheiden. Insbesondere wurde angenommen, in der Athletengruppe Genotypenunterschiede zu finden.

DNA wurde aus mittels Mundspülung gesammelten Speichelproben isoliert. Die Allelverteilung wurde mittels TaqMan® SNP Genotyping Tests und Real-Time PCR bestimmt. Alle Probanden erklärten sich schriftlich mit der Untersuchung einverstanden und die Studie wurde von der lokalen Ethikkommission genehmigt. Die Stichprobe wurde in die Gruppen „Untrainierte“ (<3 Stunden Bewegung/Woche; 36%), „Aktive“ (>3 Stunden Bewegung/Woche, 48%) und „Athleten“ (Mittel- und LangstreckenläuferInnen und StraßenradfahrerInnen auf nationalen und internationalen Niveau) geteilt. Zusätzlich wurden die Quartilen, aufgeteilt nach dem PAL als Indikator für körperliche Aktivität (erhoben mittels Fragebogen zur körperlichen Aktivität), miteinander verglichen. Gruppenvergleich wurden mit SPSS für Windows auf einem Signifikanzniveau $\alpha = 0.05$ durchgeführt.

Unterschiede hinsichtlich der Genotypen-Häufigkeit zwischen den Probandengruppen bzw. der Aktivitätsquartilen konnten bei keinem der untersuchten Polymorphismen festgestellt werden. Geschlechtsunterschiede wurden nicht beobachtet.

Die vorliegenden Ergebnisse weisen darauf hin, dass keiner der untersuchten Polymorphismen die Ausdauerleistungsfähigkeit in ÖsterreicherInnen begünstigt. Die Berechnung polygenetischer Effekte war angedacht, jedoch leider nicht möglich.

Der derzeitigen Datenlage zufolge, scheinen die ausgewählten genetischen Varianten eher Einfluss auf die Schnelligkeits- und Kraftleistung zu haben, die Begünstigung der Ausdauerleistungsfähigkeit durch eine Ausprägung der Polymorphismen wird aber kontrovers diskutiert bzw. ist noch nicht erhoben worden. Die vorliegenden Daten sprechen gegen einen Einfluss der fünf analysierten Polymorphismen auf die Ausdauerleistung.

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Appendix

Notice on the Austrian Institute for Sports Medicine, Vienna

Zentrum für Sportwissenschaft und Universitätssport
Abteilung Sport- und Leistungsphysiologie



universität
wien

Probanden und Probandinnen für wissenschaftliche Studie gesucht !

- mindestens 18 Jahre alt
- männlich oder weiblich
- bei guter Gesundheit
- Spitzensportler/in, Hobbysportler/in oder untrainiert

Wenn die oben genannten Punkte auf Sie zutreffen, dann laden
wir Sie ein an der Studie

**„Einfluss genetischer Varianten auf Leistungsfähigkeit
und Fitness - ein Vergleich zwischen Spitzenathleten,
Hobbysportlern und Untrainierten“**
teilzunehmen.

Der Zeitaufwand bei Teilnahme beträgt ca. 30 min.

Nähere Informationen
unter der Tel-Nr. 01/4277 – 48875 oder
per e-mail an barbara.wessner@univie.ac.at

Written informed consent

Probandeninformation und Einwilligungserklärung zur Teilnahme an dem Forschungsprojekt

Einfluss genetischer Varianten auf Leistungsfähigkeit und Fitness –

Ein Vergleich zwischen SpitzenathletInnen, HobbysportlerInnen und untrainierten Personen

Sehr geehrte/r ProbandIn!

Wir laden Sie ein, am oben genannten Forschungsprojekt teilzunehmen. Die Aufklärung darüber erfolgt in einem ausführlichen ärztlichen Gespräch.

Die Teilnahme am Forschungsprojekt ist freiwillig und kann jederzeit ohne Angabe von Gründen durch Sie beendet werden, ohne dass Ihnen hierdurch Nachteile entstehen.

Forschungsprojekte sind notwendig, um verlässliche neue Forschungsergebnisse zu gewinnen. Unverzichtbare Voraussetzung für die Durchführung eines Forschungsprojektes ist jedoch, dass Sie Ihr Einverständnis zur Teilnahme an diesem Forschungsprojekt schriftlich erklären. Bitte lesen Sie den folgenden Text als Ergänzung zum Informationsgespräch mit Ihrem Arzt sorgfältig durch und zögern Sie nicht, Fragen zu stellen.

Bitte unterschreiben Sie die Einwilligungserklärung nur

- wenn Sie Art und Ablauf des Forschungsprojektes vollständig verstanden haben,
- wenn Sie bereit sind, der Teilnahme zuzustimmen und
- wenn Sie sich über Ihre Rechte als Teilnehmer an diesem Forschungsprojekt im Klaren sind.

Zu diesem Forschungsprojekt, sowie zur Probandeninformation und Einwilligungserklärung wurde von der zuständigen Ethikkommission eine befürwortende Stellungnahme abgegeben.

1. Was ist der Zweck des Forschungsprojektes?

Im Rahmen des oben genannten Projekts sollen ausgewählte, erbliche Faktoren mit Einfluss auf die Leistungsfähigkeit in Gruppen unterschiedlicher sportlicher Aktivität erhoben werden. Die menschliche Leistungsfähigkeit wird durch viele Faktoren wie zum Beispiel Training, soziale Aspekte, Ernährung und mehr bestimmt. Ein Teil wird auch erblich, durch Gene, festgelegt. Durch den Vergleich der Häufigkeiten der untersuchten genetischen Variationen können Rückschlüsse auf deren Wirkungen gezogen werden. Kennt man die Auswirkung genetischer Variationen, können Training und Lifestyle-Interventionen gezielt auf die jeweilige Person

abgestimmt werden. Dies ermöglicht eine effektive Planung von sowohl gesundheitsorientiertem als auch leistungsorientiertem Training.

2. Wie läuft das Forschungsprojekt ab?

Dieses Forschungsprojekt wird am Zentrum für Sportwissenschaft und Universitätssport durchgeführt. Untersucht werden sowohl jeweils 250 österreichische SpitzensportlerInnen auf internationalem Niveau als auch 250 untrainierte Personen bzw. GesundheitssportlerInnen.

Voraussetzungen für die Teilnahme an der Studie:

- gute Gesundheit
- kein manifester Diabetes mellitus Typ I (insulinpflichtig) oder Typ II
- kein Vorliegen von koronaren Herzkrankheiten
- kein Vorliegen von chronischen Erkrankungen
- Vollendetes 18. Lebensjahr

In die Gruppe der GesundheitssportlerInnen/untrainierte Personen fallen all jene, die

- maximal 3 Stunden/Woche sportliche Aktivität oder anstrengende Freizeitbeschäftigung ausüben und früher nicht professionell sportlich aktiv waren.

Für die LeistungssportlerInnen aus den verschiedenen Disziplinen gelten folgende Einschlusskriterien:

- Ski alpin: Zugehörigkeit zum Österreichischen Skiverband National-, A-, B- oder C-Kader
- Snowboard: Zugehörigkeit zum Österreichischen Skiverband National-, A-, B- oder C-Kader
- Skilanglauf: Zugehörigkeit zum Österreichischen Skiverband National-, A-, B- oder C-Kader
- Biathlon: Zugehörigkeit zum Österreichischen Skiverband National-, A-, B- oder C-Kader
- Nordische Kombination: Zugehörigkeit zum Österreichischen Skiverband National-, A-, B- oder C-Kader
- Sprunglauf: Zugehörigkeit zum Österreichischen Skiverband National-, A-, B- oder C-Kader
- Leichtathletik: Mittel- und LangstreckenläuferInnen (800 Meter bis Marathon, inklusive Hindernis) sowie TeilnehmerInnen an den Wurfdisziplinen (Speer, Kugel, Diskus, Hammer) mit einer aktuellen oder früheren Leistung, die 800 oder mehr Punkten laut IAAF Punktetabelle entspricht.

- Straßenradfahren: Teilnahme an Weltcup-Rennen der Internationalen Radsportunion in der aktuellen oder einer früheren Saison und Zugehörigkeit zu einem professionellem Radteam
- Gewichtheben: aktuelle oder frühere Leistung von mind. 2kg/kg Körpergewicht (Frauen) bzw. mind. 2,5kg/kg Körpergewicht (Männer)
- Kraftdreikampf: Aktuelle oder ehemalige Punkteleistung für die Angehörigkeit am internationalen A- oder B-Kraftdreikampfkader (Männer: >409 Punkte; Frauen: >369 Punkte)

Ihre Teilnahme an dieser Studie erfolgt einmalig durch die Abnahme einer Speichelprobe und Ausfüllen eines Fragebogens zur Erhebung des Trainingsumfanges bzw. der körperlichen Aktivität.

Speichelproben werden mittels speziellen Bürstchen zur Speichelsammlung von der Mundschleimhaut entnommen.

Bei dieser Form der Probenziehung bestehen für die ProbandInnen keine Risiken und nur ein Minimum an Aufwand. Die Probenabnahme erfolgt zweimal direkt nacheinander (einmal an der rechten Wange, einmal an der linken Wange). Die Dauer der Probenabnahme beträgt nur wenige Minuten, davor soll für eine Dauer von 30 Minuten nicht gegessen oder getrunken werden. Die Probenabnahme erfolgt an einem von Ihnen frei wählbaren, angenehmen Zeitpunkt.

Die in den Zellen der Speichelprobe enthaltene Erbinformation (DNA) wird anschließend gereinigt und isoliert.

Mittels „real-time polymerase chain reaction“, einer molekularbiologischen Methode, wird die DNA vervielfältigt und die Abfolge der darin enthaltenen Basen bestimmt. Dabei können individuelle Unterschiede (so genannte genetische Varianten oder Polymorphismen) sichtbar gemacht werden. Die Häufigkeit der gefundenen Unterschiede wird zwischen den verschiedenen Untersuchungsgruppen (SpitzensportlerInnen und untrainierte Personen) mittels statistischer Verfahren verglichen.

Die Studie verfolgt rein wissenschaftliche Zwecke und wird anonymisiert durchgeführt. Das bedeutet, dass Proben im Nachhinein nicht mehr mit dem/der SpenderIn in Verbindung gebracht werden können. Deshalb können individuelle Studienergebnisse nicht mitgeteilt werden. Informationen über allgemeine Studienergebnisse können Sie jedoch bei Univ.-Prof. Dr. med. Norbert Bachl oder DI Dr. Barbara Wessner erhalten.

3. Was sind Genvarianten (Polymorphismen)? Welche Gene werden untersucht?

Die Studie dient zur Erforschung möglicher erblicher Faktoren, die Einfluss auf die Leistungsfähigkeit haben.

Obwohl der Großteil der genetischen Information bei gleichgeschlechtlichen Personen identisch ist, treten kleine Unterschiede, so genannte Genvarianten oder Polymorphismen, auf. Diese sind angeboren und können im Lebensverlauf nicht mehr verändert werden. Interessanter Weise können sie aber die Wirkungsweise von Eiweißstoffen im Körper und somit unter anderem die Leistungsfähigkeit beeinflussen.

Diese wird in jedem Fall von vielen Faktoren, wie Training, Ernährung und Umwelt, beeinflusst. Die Vererbbarkeit ist ein weiterer Einflussfaktor und wird nach derzeitigen Schätzungen für 25-40% der Leistungsfähigkeit verantwortlich gemacht.

Es ist anzunehmen, dass bestimmte Genvarianten, die sich günstig auf die Leistungsfähigkeit auswirken, vermehrt bei Hochleistungssportlerinnen auftreten. Dies soll durch den Vergleich mit einer untrainierten bzw. Gesundheitssportgruppe nachgeprüft werden.

Die sportliche Leistung als auch die Wirkung von Training und die Bereitschaft zu sportlicher Aktivität werden von vielen Faktoren bestimmt. Es gibt schon zahlreiche Studien, die den Zusammenhang zwischen einzelnen genetischen Varianten und der Leistungsfähigkeit untersuchten. Da letztere jedoch ein sehr komplexes System darstellt, sollen im Rahmen dieses Projekts insgesamt 22 verschiedene Genvarianten, die als Einflussfaktoren in Frage kommen, untersucht und verglichen werden. Diese können in die Gruppen Ausdauer, Kraft, Ausdauer&Kraft, Sportliche Betätigung und Kohlenhydratstoffwechsel unterteilt werden:

Getestete Genvarianten	Funktion
Ausdauer	
Adrenozeptor α -2A	Rezeptor für Epinephrin (Stresshormon)
Adrenozeptor β -1	Steuerung der Herzkraft und -frequenz
Adrenozeptor β -2	Entspannung glatter Muskulatur
Nuclear respiratory factor 2	Transkriptionsfaktor (spezifische Aktivierung des Proteinaufbaus)
PPARG coactivator 1 α	Bildung von Mitochondrien (Kraftwerke der Zelle)
Kraft	
Myostatin	Negativ regulierender Wachstumsfaktor
Insulin-ähnlicher Wachstumsfaktor 1	Stimulation der Zellteilung
Insulin-ähnlicher Wachstumsfaktor 2	Zellteilung und -entwicklung
Hepatische Triglycerid Lipase	Fettabbauendes Enzym
Vitamin D Rezeptor	Vermittlung der Vitamin D Wirkung
Ausdauer & Kraft	
Angiotensin I-konvertierendes Enzym	Blutdruck-, Wasser- und Elektrolythaushaltregulierung
α -Actinin 3	Ermöglicht kräftige Kontraktionen bei hohen Geschwindigkeiten in Muskelfasern
Adenosine-Monophosphat-Deaminase	Energiebereitstellung bei intensiven Belastungen
Kreatinkinase	Schnelle, kurzzeitige Erholung von Energielieferanten

Sportliche Betätigung	
Leptin receptor	Wirkungsvermittlung des Sättigungshormons Leptin
Melanocortin 4 receptor	Signalvermittlung an das Sättigungszentrum im Gehirn
Kohlenhydratstoffwechsel	
Adrenergic β -2 receptor	Regulierung des Energiestoffwechsels
Adrenergic β -3 receptor	Fettstoffwechsel und Wärmebildung
AMP-activated protein kinase γ 3	Energiebereitstellung
Leptin	Steuerung der Nahrungsaufnahme
Peroxisome proliferative activated receptor γ	Regulierung des Fett- und Zuckerstoffwechsels
Uncoupling protein 2	Wärmebildung und erhöhter Energieverbrauch

Falls sie genauere Informationen zu den einzelnen Genvarianten wünschen, klären wir etwaige Fragen gerne in einem persönlichen Gespräch.

4. Worin liegt der Nutzen einer Teilnahme an dem Forschungsprojekt?

Es ist nicht zu erwarten, dass Sie aus Ihrer Teilnahme an dieser Studie einen unmittelbaren gesundheitlichen Nutzen ziehen werden.

Die Ergebnisse dieser Studie können dazu beitragen, in Zukunft individuelle Trainingspläne für jeden einzelnen zu gestalten. Der Vorteil darin liegt in einer besseren Ausschöpfung der maximal möglichen Leistungsfähigkeit im Spitzensport als auch dem gezielten Entgegenwirken von Risikofaktoren und der Planung effizienterer Gesundheitsmaßnahmen für untrainierte Personen bzw. HobbysportlerInnen (z.B. bessere Wirkung von Kraft- oder Ausdauertraining für die entsprechende Person).

5. Gibt es Risiken, Beschwerden und Begleiterscheinungen?

Es gibt keine gesundheitliche Risiken, Beschwerden und Begleiterscheinungen, die durch die Teilnahme an der Studie entstehen.

6. In welcher Weise werden die im Rahmen dieses Forschungsprojekts gesammelten Daten verwendet?

Das Untersuchungsmaterial wird ausschließlich für die hier angeführten Untersuchungen herangezogen. Es werden keine anderen Tests damit durchgeführt. Nach Abschluss der Untersuchungen und Veröffentlichung der Ergebnisse wird das Probenmaterial verworfen (voraussichtlich Ende 2011). Während der Untersuchung wird das Probenmaterial am Institut für Sportwissenschaften, Universität Wien, unter der Verantwortung von Univ.-Prof. Dr. med. Norbert Bachl aufbewahrt. Der Schutz vor dem Zugriff Unbefugter ist durch einen beschränkten Zugang zum Labor gewährleistet.

Die Datenaufarbeitung erfolgt in anonymisierter Form, das heißt, nachträglich ist für niemanden eine Verknüpfung des genetischen Materials mit der Identität der entsprechenden Person möglich. Auch der Prüfarzt und Studienleiter kann die Probe nicht der Identität einer Person zuordnen. Spätere Änderungen einer einmal getroffenen Entscheidung sind daher nicht möglich, die Anonymisierung bietet das höchste Maß an Sicherheit. Auch individuelle Studienergebnisse können daher nicht mitgeteilt werden.

Die Weitergabe der Daten im In- und Ausland erfolgt ausschließlich zu statistischen Zwecken und Sie werden ausnahmslos darin nicht namentlich genannt. Auch in etwaigen Veröffentlichungen der Daten dieses Forschungsprojektes werden Sie nicht namentlich genannt. Weiters ist es nicht möglich, Sie auf Grund anderer Informationen als Person zu identifizieren.

7. Entstehen für die Teilnehmer Kosten? Gibt es einen Kostenersatz oder eine Vergütung?

Durch Ihre Teilnahme an diesem Forschungsprojekt entstehen für Sie keine zusätzlichen Kosten. Von unserer Seite gibt es keinen Kostenersatz und keine Vergütung.

8. Möglichkeit zur Diskussion weiterer Fragen

Für weitere Fragen im Zusammenhang mit diesem Forschungsprojekt stehen Ihnen Ihr Prüfarzt und seine MitarbeiterInnen gern zur Verfügung. Auch Fragen, die Ihre Rechte als ProbandIn und TeilnehmerIn an diesem Forschungsprojekt betreffen, werden Ihnen gerne beantwortet.

Studienleiter: Univ.-Prof. Dr. med. Norbert Bachl

Erreichbar unter: 0664/602 77-488 70

Name der Kontaktperson: DI Dr. Barbara Wessner

Ständig erreichbar unter: 0664/3154930

Einwilligungserklärung

Name des/der ProbandIn in Druckbuchstaben:

.....

Ich erkläre mich bereit, an dem Forschungsprojekt „**Einfluss genetischer Varianten auf Leistungsfähigkeit und Fitness - ein Vergleich zwischen Spitzenathleten, Hobbysportlern und Untrainierten**“ teilzunehmen. Ich bin von Dr. med. Norbert Bachl ausführlich und verständlich über genetische Analysen, mögliche Risiken, sowie über Wesen, Bedeutung und Tragweite des Forschungsprojektes aufgeklärt worden. Ich habe darüber hinaus den Text dieser Probandenaufklärung und Einwilligungserklärung, die insgesamt 7 Seiten umfasst, gelesen. Aufgetretene Fragen wurden mir vom Prüfarzt verständlich und genügend beantwortet. Ich hatte ausreichend Zeit, mich zu entscheiden. Ich habe zurzeit keine weiteren Fragen mehr.

Ich werde den ärztlichen Anordnungen, die für die Durchführung des Forschungsprojektes erforderlich sind, Folge leisten, behalte mir jedoch das Recht vor, meine freiwillige Mitwirkung jederzeit zu beenden. Nach Probenabnahme kann ich meine Entscheidung nicht mehr ändern, da meine Probe anonymisiert wurde. Die Anonymisierung bietet für mich als ProbandIn jedoch die höchste Sicherheit des Datenschutzes und ich kann danach nicht mehr als Person identifiziert werden.

Ich bin zugleich damit einverstanden, dass meine im Rahmen dieses Forschungsprojektes ermittelten Daten aufgezeichnet werden.

Beim Umgang mit den Daten werden die Bestimmungen des Datenschutzgesetzes beachtet.

Eine Kopie dieser Probandeninformation und Einwilligungserklärung habe ich erhalten. Das Original verbleibt beim Prüfarzt.

.....
(Datum und Unterschrift des/der ProbandIn)

.....
(Datum, Name und Unterschrift des verantwortlichen Arztes)

(Der/Die ProbandIn erhält eine unterschriebene Kopie der Probandeninformation und Einwilligungserklärung, das Original verbleibt im Forschungsprojektordner des Prüfarztes.)

Case report form

Genetische Varianten & Leistungsfähigkeit

CRF_Version 1

Code	_ _ _ _		
Geschlecht	☐ Männlich		
	☐ Weiblich		
Geburtsdatum	_ _ / _ _ / _ _ _ _		
Gruppe	☐ Untrainierte Personen/GesundheitssportlerInnen ☐ LeistungssportlerInnen		
Wenn Leistungs- sportlerInnen, dann	☐ Ski alpin		
Sportart	☐ Snowboard		
	☐ Langlauf		
	☐ Biathlon		
	☐ Nordische Kombination		
	☐ Sprunglauf		
	☐ LA: Mittel-/Langstreckenlauf		
	☐ LA: Wurf		
	☐ Straßenrad		
	☐ Gewichtheben		
	☐ Kraftdreikampf		
Wenn Leistungs- sportlerInnen, dann			
Hauptdisziplin	_____		
Diabetes	☐ Nein	☒ Ja	_____
Herzerkrankung	☐ Nein	☒ Ja	_____
Sonstige	☐ Nein	☒ Ja	_____
Erkrankung	_____		
Speichel -	Am _ _ / _ _ / _ _	um _ _ _ _ _ Uhr	
Abnahme	Ort: _____		
	Durch: _____		

Physical activity questionnaire for controls

Physical Activity Frequency Questionnaire

Mit diesem Fragebogen wollen wir das Ausmaß Ihrer körperlichen Aktivität im alltäglichen Leben (Beruf und Freizeit) sowie die von Ihnen ausgeübten Sportarten erfassen.

Denken Sie an eine **typische Woche innerhalb der letzten drei Monate (Ausnahme Wintersportarten; hier bitte das Winterhalbjahr heranziehen)** und versuchen Sie, die Fragen so genau wie möglich zu beantworten. Kreuzen Sie dazu die Anzahl der Tage pro Woche an und geben Sie die jeweilige Dauer der Aktivität in Stunden und Minuten pro Tag an.

Zur Erklärung:

- Unter **leichten Aktivitäten** versteht man Tätigkeiten, bei denen Sie nicht heftiger atmen als normal.
- **Moderate Aktivitäten** sind Tätigkeiten, bei denen Sie ein wenig stärker atmen als normal.
- Unter **anstrengenden Aktivitäten** versteht man Tätigkeiten, bei denen Sie deutlich stärker atmen als normal.

Alle Ihre Angaben werden anonym und streng vertraulich behandelt!

Teil 1: Persönliche Daten

Code _____	(vom Interviewer auszufüllen)
Geschlecht	☐ Männlich
	☐ Weiblich
Geburtsdatum (TT/MM/JJ)	
Gewicht (kg)	
Größe (m)	

Teil 2: Körperliche Aktivität am Arbeits-/Studienplatz

Aktivität	Tage pro Woche									Durchschnittliche Dauer pro Tag	
	0	1	2	3	4	5	6	7	Stunden	Minuten	
Vorwiegend sitzende Tätigkeit (z.B. Bürotätigkeit)											
Vorwiegend stehende oder gehende Tätigkeit (z.B. VerkäuferIn, LaborantIn)											
moderate körperliche Tätigkeit (z.B. RaumpflegerIn)											
Anstrengende körperliche Tätigkeit (z.B. BauarbeiterIn)											

Teil 3: Körperliche Aktivität zur Fortbewegung

Fortbewegungsart	Tage pro Woche									Durchschnittliche Dauer pro Tag	
	0	1	2	3	4	5	6	7	Stunden	Minuten	
Auto, Moped, Motorrad											
Öffentliche Verkehrsmittel											
Zu Fuß gehen											
Fahrrad fahren											
Sonstiges: _____											

Teil 4: Hausarbeit und Familienfürsorge

Aktivität	Tage pro Woche								Durchschnittliche Dauer pro Tag	
	0	1	2	3	4	5	6	7	Stunden	Minuten
Hausarbeit										
Kinderbetreuung										
Gartenarbeit										
Sonstiges: _____										

Teil 5: Freizeitaktivitäten

Aktivität	Tage pro Woche								Durchschnittliche Dauer pro Tag	
	0	1	2	3	4	5	6	7	Stunden	Minuten
Leichte körperliche Aktivität (z.B. Spazieren gehen)										
Moderate körperliche Aktivität (z.B. langsames Laufen / Fahrrad fahren / Schwimmen)										
Anstrengende körperliche Aktivität (z.B. schnelles Laufen / Fahrrad fahren / Schwimmen / Aerobic)										

Teil 6: Sport – Nähere Angaben (bitte ankreuzen, Mehrfachnennungen möglich)

Welche der folgenden Sportarten üben Sie regelmäßig (im Durchschnitt mind. 1x/Woche) aus?

Sportarten	Regelmäßigkeit		Seit wann?
	ja	nein	Jahre
☐ Laufen			
☐ Radfahren			
☐ Schwimmen			
☐ Rudern			
☐ Wandern			
☐ Skifahren (Wintermonate)			

Sportarten	Regelmäßigkeit		Seit wann?
	ja	nein	Jahre
☐ Snowboarden (Wintermonate)			
☐ Skiwandern/-langlaufen (Wintermonate)			
☐ Krafttraining/Gymnastik (Kleingeräte, eigenes Körpergewicht)			
☐ Krafttraining (Hanteltraining)			
☐ Aerobic			
☐ Yoga			
☐ Fußball			
☐ Tennis			
☐ Sonstiges: _____ _____ _____ _____			

Teil 7: Inaktiv verbrachte Freizeit

	Durchschnittliche Dauer pro Wochentag	
	Stunden	Minuten
Im Sitzen verbrachte Zeit		
Schlafdauer		

Vielen Dank für Ihre Teilnahme!

Physical activity questionnaire for athletes

Seite 1/3

Fragebogen für LeistungssportlerInnen

Mit diesem Fragebogen möchten wir neben persönlichen Daten Ihren Trainingsumfang erheben.

Dies ist nötig, um die Sportartengruppen untereinander und zur Gesundheitssport-Gruppe beschreibend unterscheiden zu können.

Weiters möchten wir gerne wissen, ob Sie Ihre Sportart eher der Ausdauer oder der Kraft zuordnen.

Teil 1: Persönliche Daten

Code _____	(vom Interviewer auszufüllen)
Geschlecht	☐ Männlich
	☐ Weiblich
Gewicht (kg; in aktiver Zeit)	
Größe (m)	

Teil 2: Sportart

Kategorie	☐ Ski alpin
	☐ Snowboard
	☐ Langlauf
	☐ Biathlon
	☐ Nordische Kombination
	☐ Sprunglauf
	☐ Mittel-/Langstreckenlauf
	☐ Leichtathletischer Wurf
	☐ Straßenrad
	☐ Gewichtheben
	☐ Kraftdreikampf
Disziplin(en)	_____

Seite 2/3

Teil 3: Erfolg

- | | |
|--|--|
| ☐ Sieg bei ... | ☐ Olympische Spiele |
| ☐ Medaillengewinn bei ... | ☐ Wettmeisterschaft |
| | ☐ Europameisterschaft |
| | ☐ Weltcup |
| | ☐ Europacup |

Bestes Ergebnis: Platzierung in der Weltrangliste unter den ...

- ☐ Top 10
- ☐ Top 50
- ☐ Top 100

Teil 4: Umfang

Seit wann bzw. in welchen Jahren waren sie im Wettkampfsport tätig? (Jahresangabe)	Beginn: _____
	Ende: ☐ noch aktiv ☐ _____

Wochentrainingszeit in Stunden Vorbereitungsperiode Sommer*	
Wochentrainingszeit in Stunden Vorbereitungsperiode Winter*	
Wochentrainingszeit in Stunden Wettkampfperiode Sommer*	
Wochentrainingszeit in Stunden Wettkampfperiode Winter*	

* LäuferInnen: bitte Umfang in Kilometer/Woche zusätzlich angeben

Seite 3/3

Teil 5: Persönliche Einschätzung

Wo würden Sie das Gesamttraining ihrer Sportart ansiedeln (bitte mit senkrechtem Strich markieren)?



Vielen Dank für Ihre Teilnahme!

Lebenslauf

Persönliche Daten

Zuname: Brandstetter
Vorname: Sabine
Geboren am: 25. Oktober 1983
Geburtsort: Wiener Neustadt
Staatsbürgerschaft: Österreich
Eltern: Ursula Brandstetter (Buchhalterin; geb. 5. Oktober 1957)
Hermann Brandstetter (Elektriker; geb. 5. Jänner 1955)
Kinder: Levi Marten, geb. 15. Juni 2009

Schulbildung

September 1990 - Juni 1994	Volkschule Pernitz
September 1994 – Juni 1998	Bundesrealgymnasium Berndorf
September 1998 – Juni 2002	Oberstufenrealgymnasium Wiener Neustadt unter besonderer Berücksichtigung der sportlichen Ausbildung
Oktober 2002 – Juni 2006	Bakkalaureatstudium Leistungssport Universität Wien
Oktober 2002 – April 2008	Diplomstudium Ernährungswissenschaften Universität Wien
Seit Oktober 2006	Magisterstudium Sportwissenschaft Universität Wien

Praktika während des Studiums

Büro und Organisation Laufteam Burgenland Eisenstadt (2001 – 2003)
Nachwuchstrainerin Laufteam Burgenland Eisenstadt (2002 – 2005)
Obfrau des Sportvereins Jugend – Sport – Treff Wiener Neustadt (2003 – 2008)
Organisation und Durchführung von Schulsportveranstaltungen (2003 – 2005)
Angestellte Lauffachgeschäft „Lauftreff“/New Balance Österreich
ÖISM (Fit für 50+, etc.)
Pressereferentin Laufteam Burgenland Eisenstadt (seit 2006)
Praktikum in der Gemeinschaftspraxis TSM GmbH (Anthropometrie, Trainingsplanung,
Personal Coaching, 2006 – 2007)
Nachwuchs- und Konditionstrainerin Ruderverein STAW (2007 – 2008)