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ACTN3 R577X Genotypes Associate with Class II and Deep Bite Malocclusions

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Abstract

Introduction— α -actinins are myofibril anchor proteins which influence contractile properties of skeletal muscle. *ACTN2* is expressed in slow type I and fast type II fibers whereas *ACTN3* is expressed only in fast fibers. *ACTN3* homozygosity for the 577X stop codon (i.e. changing 577RR to 577XX - the R577X polymorphism) results in the absence of α -actinin-3 in about 18% of Europeans, diminished fast contractile ability, enhanced endurance performance and reduced bone mass or bone mineral density. We have examined *ACTN3* expression and genetic variation in masseter muscle of orthognathic surgery patients to determine genotype associations with malocclusion.

Methods—Clinical information, masseter muscle biopsies and saliva samples were obtained from 60 subjects. Genotyping for *ACTN3* SNPs, RT-PCR quantitation of muscle gene message and muscle morphometric fiber type properties were compared to determine statistical differences between genotype and phenotype.

Results—Muscle mRNA expression level was significantly different for *ACTN3* SNP genotypes ($p < 0.01$). The frequency of *ACTN3* genotypes was significantly different for sagittal and vertical classifications of malocclusion with the clearest association being elevated 577XX genotype in skeletal class II malocclusion ($p = 0.003$). This genotype also resulted in significantly smaller diameter of fast type II fibers in masseter muscle ($p = 0.002$).

Conclusion—*ACTN3* 577XX is overrepresented in skeletal class II malocclusion, suggesting a biologic influence during bone growth. *ACTN3* 577XX is underrepresented in deep bite malocclusion, suggesting muscle differences contribute to variations in vertical facial dimensions.

Malocclusion often develops as a complex trait condition which is influenced by combinations of transcription and growth factors acting on bone, teeth and skeletal muscle.¹ Complex traits are quantitative or continuous conditions with a broad spectrum of presentations. For humans, variations in height, IQ, blood pressure and birth weight are complex quantitative traits which result from the interplay of genetic and environmental

influences. One approach for identifying genes that contribute to the development of malocclusion is to consider those already known to influence musculoskeletal growth and function. Malocclusion is a complex musculoskeletal trait because masticatory muscle contributes to variations in the vertical dimension of facial growth.² Specifically, vertical facial dimensions are influenced by the size and proportion of muscle fiber types in masticatory muscles, with the majority of these studies being conducted by direct biopsy or indirect imaging studies of masseter muscle.²⁻⁴ Genome-wide association analysis of skeletal muscle fiber types is underway,⁵ and should add important information to the current Human Gene Map for Performance and Health-Related Fitness Phenotypes compendium, which summarizes gene variations that influence muscle size and strength.⁶ Overall these gene association studies demonstrate that fiber type properties are influenced by genetic variation, which most commonly are single nucleotide polymorphisms (SNPs) in gene sequences which have functional consequences.⁷ Unlike limb muscle, which is highly responsive to training and displays wide phenotypic variability with exercise and other environmental factors,⁸ cranial muscles show less activity-related changes and are not typically subject to maximum force recruitments.⁹⁻¹⁰

Alpha-actinin-3 (*ACTN3*) is a particular gene of interest that influences muscle performance and fiber type proportions.¹¹ Alpha-actinins are cytoskeletal proteins which bind actin filaments in a variety of cell types. In skeletal muscle, α -actinin-2 and -3 crosslink actin filaments to dense bodies located in the Z-disk of the sarcomere, to help order the myofibril array during sarcomere contraction. Alpha-actinin-2 is found in all skeletal muscle fiber types, while Alpha-actinin-3 is restricted to most type II fast contracting fibers.¹² The genes that encode these two closely related isoforms are found on different chromosomes with *ACTN2* located on the long arm of chromosome 1, and *ACTN3* on chromosome 11.¹³ A common nonsense mutation R577X identified in the *ACTN3* gene results in a lack of protein expression due to the production of a stop codon at residue 577.¹⁴ About 18% of the European population is homozygous for the R577X change. Absence of α -actinin-3 is not associated with any obvious pathology, and since α -actinin-2 is still expressed in the fast fiber types, the functional role for the α -actinin-3 was first thought to be redundant.¹⁵ Shortly thereafter, it became apparent that *ACTN3* genotype variations are important in human elite athletic performance. In a study comparing Australian Olympic athletes to controls, both male and female elite sprint athletes had higher frequencies of the 577R allele. Among females, elite sprint athletes also had higher 577RX heterozygote frequency and elite endurance athletes had lower 577RX frequency.¹⁶ There was no comparable heterozygote genotype effect in male athletes. Subsequently, *ACTN3* allele and genotype frequencies have been investigated in at least ten other athletic and control populations.¹⁷ These investigations support the conclusion that the 577RR genotype is more common in sprint/power athletes, but not that the X allele enhances endurance capability.¹⁷ Overall, the literature indicates that the presence of α -actinin-3 enhances production of forceful, fast contraction in type II muscle fibers and that these genotypic effects may be influenced by gender.

Alpha-actinin-3 may also contribute to variations in muscle function by interaction with the signaling protein calcineurin to influence fiber type proportions during growth.¹¹ Alpha-

actinin-3 binds to calsarcin family signaling proteins located at the Z disc,¹⁸ that in turn, bind to calcineurin to activate fiber type specific gene expression pathways which determine fiber types and size.¹⁹ In a study of vastus lateralis muscle in young adult males, the RR genotype resulted in type IIX, fast contracting-fatigueable fibers, of larger size and greater number compared to the XX genotype.¹¹ Consequently, the men with RR genotype had significantly elevated leg muscle power. Type I, slow contracting fibers, also show variation with *ACTN3* genotype in vastus lateralis, with the percentage of type I fibers increasing in 577XX compared to RR genotype.²⁰ Therefore α -actinin-3 may act directly through structural functioning or cell-signaling pathways to alter composition and function of skeletal muscle.

Previous masseter muscle biopsy studies have demonstrated that increased size or proportion of type II fibers associates with skeletal deep bite malocclusion, and decreased type II fibers with skeletal open bite.¹⁻² To further explore how genetic variation might influence masticatory muscle function and skeletal shape, this study sought to associate *ACTN3* genotypes with malocclusion classification and masseter muscle fiber type properties. To do so, we looked at two SNPs located at rs1815739 and rs678397. The first SNP, rs1815739 (R577X), is a Cytosine to Thymine transition at nucleotide 1,586 in exon 16 which converts an arginine to a stop codon at residue 577 and produces three genotypes CC (normal), TC (heterozygote) and TT (no α -actinin-3). The second SNP, rs678397, is a Cytosine to Thymine transition at nucleotide 15,193 in an *ACTN3* gene intron, which has no reported functional changes, and produces three genotypes CC (normal), TC and TT.

MATERIAL AND METHODS

Participants

Sixty subjects undergoing orthodontic and maxillofacial surgery treatment for correction of malocclusion were recruited from the University of Lille Department of Oral and Maxillofacial Surgery. Subjects were recruited after they had signed an informed consent, and the research protocol was validated by the French Independent Ethical Committee (named CPP), the Temple University and the University of Pittsburgh IRB Committees. Malocclusion classification was based upon the sagittal and vertical jaw repositioning required to execute the surgical treatment plan. Subjects were classified into one of six craniofacial morphologic groups that included one variation of sagittal skeletal jaw malocclusion, either Class II or III, and one variation of vertical skeletal jaw malocclusion, open, deep or normal - bite relationship. Thirty one non-treated Class I control subjects without malocclusion came from the Dental Registry and DNA Repository at the University of Pittsburgh which included only subjects of European descent, to be comparable to the French subjects with malocclusion. Saliva samples from all subjects were stored in Oragene® kits and used for DNA extraction and posterior genotyping. For the malocclusion subjects, muscle samples were also collected from the deep anterior area of masseter muscle as a bilateral sagittal split osteotomy of the mandible was performed. After snap freezing, muscle samples were stored at -80°C prior to histologic and gene expression analysis.¹⁻²

Genotyping

Two SNPs in *ACTN3* were selected for genotyping on all subjects, rs1815739 (the R577X SNP) and rs678397 (a SNP not known to have functional consequences) and tested to determine if specific allelic variants are over-represented in subjects with malocclusion sub-classifications using TaqMan chemistry and end-point analysis in an automatic sequence-detection instrument (ABI Prism 7900HT, Applied Biosystems, Foster City, CA), as described previously.²¹ Thirty-three additional anonymous SNPs were genotyped to assess the presence of population substructure among the controls selected.

Fiber type histomorphometric analysis of masseter muscle

Frozen muscle was cryosectioned at 10 μ m thickness to obtain serial cross sectional slices, and sections were mounted on glass microscope slides for immunostaining with five antibodies specific for myosin heavy chain (MyHC) isoforms; anti-Type I, anti-Type II, anti-Type IIA, anti-Type neonatal and anti- α -cardiac (atrial) as described previously.²²⁻²³ We classified masseter fibers into 4 fiber type groups as type I, type hybrid (containing both type I and II MyHCs), type II containing only type IIA and/or IIX MyHCs and type neonatal - atrial that contained the neonatal and/or α -cardiac MyHCs in combination with other type I and II isoforms. Type I fibers are slow contracting and fatigue resistant, used most commonly to maintain postural freeway space. Type II fibers are fast contracting and are either fatigue resistant (type IIA) or fatigueable (type IIX). The hybrid fibers are a very unusual and distinctive fiber type found in masseter muscle, which combines slow and fast contractile properties.²³ Hybrid fibers are more commonly found in certain states of skeletal muscle pathology, although they are sometimes present in normal healthy limb muscle. In human masticatory muscles however, hybrid fibers are always present as a common fiber type. In addition, α -actinin-3 was identified across fiber types by staining with a rabbit IgG monoclonal antibody (EP2531Y), commercially available from Origene Technologies, Inc. For fiber type classification, only tissue section series with consistent antibody reactions for all stains and acceptable morphology of muscle fibers, which were clearly in transverse section, were used. All fibers within the selected areas were type - classified and their cross-sectional areas measured with Image J image - analysis software available from the National Institutes of Health. Tests for measurement error included intra-rater reliability in determination of fiber area (by repeating morphometric tracing of all fiber areas in one biopsy by one examiner), which resulted in an R^2 value of 0.94. We compared differences in fiber type properties between all of the malocclusion subjects that were genotyped as either 577XX or RR.

Quantification of Actinin mRNA

RNA was isolated from the remaining muscle after cryosectioning with TRIzol™ reagent and quantified by absorbance at A_{260} as described previously.²⁴ *ACTN2* and *ACTN3* were quantified by TaqMan® quantitative real time PCR (qRT-PCR). Reactions, in triplicate, contained RNA from masseter muscle or an adult skeletal muscle reference standard, TaqMan RNA-to-CT 1-Step reagent and an Applied Biosystems expression assay set for either *ACTN2* (#Hs01100111_g1), *ACTN3* (#Hs00153809_m1) or internal control HPRT1 (#Hs02800695_m1) for normalization. RNA was expressed as relative quantities determined

by the comparative C_T ($\Delta\Delta C_T$) method that measures fold - difference between normalized quantities of target in the sample and in the reference standard. A limited number of subjects were included in the mRNA quantification to determine if there were differences in message expression between *ACTN3* genotypes rs1815739 and rs678397 for 13 of the study subjects.

Statistical Analysis

Chi-square or Fisher's exact tests were used to assess Hardy-Weinberg equilibrium and compare allele and genotype distributions between individuals with malocclusion and non-treated Class I control subjects. Student's *t* tests were used to compare differences for fiber type mean fiber area, fiber number and percent occupancy measurements between *ACTN3* genotypes. An ANOVA was used to compare differences in malocclusion classifications for *ACTN3* genotypes between malocclusion and control subjects. A separate ANOVA was conducted to determine if *ACTN2* and 3 mRNA expression levels were different by R577X genotype.

RESULTS

ACTN3 Genotype

We recruited sixty orthognathic surgery patients who were systemically healthy and without genetic craniofacial syndromes, other growth disturbances or reported trauma. They were 42 females and 18 males with the average age of 23.5 ± 9.9 years, including 18 Class II open bite, 7 Class III open bite; 13 Class II deep bite, 6 Class III deep bite; 12 Class II normal bite and 4 Class III normal bite malocclusion. R577X genotype frequencies for the entire malocclusion group was 14% CC, 63% TC and 23% TT, with the reported European population frequency of 18% for TT.¹⁶ Lateral cephalograms of one Class III open bite subject with R577X CC genotype (A) and one Class II open bite subject with TT genotype (B) are shown in **Figure 1**. Because we hypothesized that the *ACTN3* SNPs would differ between sagittal and vertical malocclusion classification we compared SNP frequency for Class II vs Class III to Class I controls (**Table I**) and secondly between open, normal and deep bite (**Table II**). Overall, only a small number of genotypes were undetermined, ranging from 0 to 3 for each comparison.

No significant allele frequency differences were found between cases and controls in the 31 anonymous SNPs genotyped (data not shown), and we proceeded to test differences in *ACTN3* genotypes between cases and controls. There were no deviations from the Hardy-Weinberg equilibrium in the samples and no statistically significant differences between cases and controls in regards to age and sex. Chi-square tests revealed very significant differences between Class II malocclusion vs. controls for genotypes and alleles at rs1815739 and rs678397 (**Table I**). There were no significant differences for Class III malocclusion. In vertical dimension comparisons, there were very significant differences for genotypes and alleles at rs678397 (**Table II**). For rs1815739, the TT and CC genotypes were underrepresented in subjects with deep bite malocclusion.

RNA Expression Levels

Although the 577XX should result in no α -actinin-3, we assayed mRNA levels for *ACTN2* and *ACTN3* to determine if gene expression levels differed for this SNP. In doing so, we wanted to test whether *ACTN2* expression is increased when α -actinin-3 was absent. Relative RNA was quantified in masseter muscle from 13 malocclusion subjects with differing R577X genotypes (**Table III**). A three way ANOVA between genotypes found no difference in gene expression for *ACTN2* ($p = 0.84$) and significant differences in gene expression for *ACTN3* ($p = 0.003$). Based on this result it is expected that the R577X CC genotype should have a full complement of no α -actinin-3, the TC genotype an intermediate amount and TT no detectable no α -actinin-3, which supports previous findings.¹⁴ *ACTN2* expression remained relatively consistent regardless of changes in *ACTN3* genotype.

Fiber Type Differences Associated with ACTN3 R577X Genotype

We compared differences in fiber type properties from masseter muscle between 12 subjects genotyped at rs1815739 as TT and 8 subjects genotyped as CC to determine how normal or absent alpha-actinin-3 protein expression affected muscle phenotype. Since we know from previous studies that the size and occupancy of type II fibers influence the length of vertical anterior facial dimension, it was important to know what malocclusion types are being compared along with *ACTN3* genotypes. The TT subjects included 10 Class II, 3 Class III, 6 open bite, 5 normal bite and 2 deep bite malocclusions and the CC subjects included 4 Class II, 4 Class III, 5 open bite, 1 normal bite and 2 deep bite malocclusions. So for vertical dimension classification in both groups, open bite malocclusion was the most common feature, and was larger in proportion for the CC group. The mean type II fiber area and muscle percent occupancy composition was larger in subjects with CC genotype (**Table IV, Figure 2**). The mean type II fiber area was significantly smaller for the TT genotype, being on average less than half the size of type II fibers in the CC genotype ($p = 0.002$). (**Table IV, Figure 3**). There were however larger numbers of type II fibers in the TT genotype, which did not produce a significant difference in type II fiber numbers between groups. Although type II fibers had larger occupancy in the CC group, the differences were not significant. There was an almost significant difference between type I fiber occupancy, with the TT group having a higher percent occupancy for type I fibers. For CC genotype, fiber staining profiles demonstrate that no α -actinin-3 is expressed in all fibers that contain IIA or IIX MyHCs. This is demonstrated by the similar staining pattern and intensity for fast MyHC antibody (**Figure 2A**) and alpha-actinin-3 antibody (**Figure 2D**). For TT genotype, there was no detectable alpha-actinin-3 in masseter muscle (**Figure 3D**). There was also a lack of neonatal and/or alpha-cardiac MyHC proteins in masseter muscle from subjects with TT genotype. Of the 8 subjects studied with CC genotype, three had limited expression of alpha-cardiac MyHC and four had limited expression of neonatal MyHC.

DISCUSSION

The *ACTN3* 577XX genotype is a common nonsense mutation which has been studied throughout world populations, usually comparing elite athletes to the general population.^{17,20} There is consensus that the presence of alpha-actinin-3 protein is advantageous in elite athletic performance for sprinting and power lifting, and there is

limited evidence that lack of no α -actinin-3 protein enhances human endurance activities.¹⁷ In mouse models however, there is clear evidence that α -actinin-3 co-localizes with glycogen phosphorylase and enhances oxidation of carbohydrates during exercise. In knockout mice, type II muscle fibers have reduced free glucose and shift from glycolytic metabolism towards a more oxidative metabolism similar to type I fibers.²⁵ Since the human studies conducted so far have been on appendicular muscle function, one cannot rule out that there are significant shifts in cranial muscle energy metabolism between *ACTN3* genotypes.

We were able to compare α -actinin-3 and mRNA expression levels in masseter muscle to *ACTN3* R577X genotypes in 60 subjects to determine if differences in protein function might contribute to development of malocclusion. Although we hypothesized that the main effect of α -actinin-3 loss (TT) would be smaller type II fibers and open bite malocclusion, the most distinctive finding was that TT was overrepresented in Class II skeletal malocclusion ($p = 0.003$). There were too few Class III subjects, however, to determine genotypic effects for this malocclusion (**Table I**). It has recently been discovered that alpha-actinin- is expressed in bone osteoblasts. Both knockout mice and postmenopausal women with TT genotype have significantly decreased bone mineral density.²⁶ Unlike muscle, α -actinin-2 is not expressed in osteoblasts and therefore cannot become a functional substitute when α -actinin-3 is absent. Although the molecular role that α -actinin-3 might play in bone mineralization is at present unknown, osteoblasts cultured from *ACTN3* knockouts have abnormal gene expression with *Enpp1* (ectonucleotide pyrophosphatase/phosphodiesterase) increased by 1.45 fold. *Enpp1* is an important negative regulator of bone mineralization, and cultured osteoblasts with elevated *Enpp1* expression have reduced mineral formation.²⁷ It is possible that differences in bone growth that occur with TT genotype contribute to Class II skeletal malocclusions when *Enpp1* expression is elevated. A second possibility is that SNPs in *Enpp1* play an additional role, since *Enpp1* polymorphisms are associated with differences in height, hip geometric indices and facial morphology.²⁸⁻³⁰

As expected, loss of α -actinin-3 with R577X TT genotype did result in significantly smaller type II fiber diameter in masseter muscle ($p = 0.002$). Type II fiber number was increased for TT compared to CC, but these differences were insignificant (**Table IV**). Mouse knockout experiments have also demonstrated reduced diameter for type II fibers, but without changes in fiber numbers.²⁵ The TT genotype was underrepresented in deep bite subjects vs. controls ($p = 0.02$) (**Table II**). This most likely resulted in increased type II fiber percent occupancy in the deep bite group compared to the open and normal bite groups and provides a mechanical explanation for how the genotypes affect vertical facial dimension.

The *ACTN3* SNP at rs678397 is also associated with significant differences in sagittal and vertical malocclusion classifications (**Table I and II**). Genotypes and alleles at rs678397 are significantly different for Class II malocclusion (**Table II**) and for all vertical dimension bite malocclusions. The SNP is located in an intron area not known to change protein levels or functioning. The possibility remains that this intronic SNP alters protein levels. The initial hypothesis is that rs678397 is in linkage disequilibrium with another undermined *ACTN3* variant that impacts the function of α -actinin-3.

Experimental subjects were from Lille, France and control subjects were European Americans without malocclusion from Pittsburgh, Pennsylvania. *ACTN3* allele frequency varies with global latitudinal gradient, with higher frequency of 577XX occurring at increased latitude.³¹ Despite no evidence that there is undetected population substructure in the study, our comparison may be somewhat biased in that Lille is geographically 10⁰ latitude higher than Pittsburgh. Further studies which include Class I control subjects from Lille will be necessary to confirm that *ACTN3* genotypes contribute to development of malocclusion. Of course SNPs in many other genes are influencing the diversity of muscle and jaw properties and ultimately must be considered together to understand musculoskeletal heritability. *Enpp1* may be a key genetic influence and needs further careful consideration. In addition to the bone effects described above, it also binds directly to skeletal muscle insulin receptors, and a polymorphism is known to produce insulin resistance.³²⁻³³ Over time, insulin resistance alters the proportion of type I versus type II skeletal muscle fibers,³⁴ which could also influence the vertical dimension of malocclusion.²⁻⁴ How *ACTN3* and *ENPP1* gene expression levels interact in maintenance of muscle and bone is yet to be investigated, but could have insightful findings for conditions such as osteoporosis, obesity, diabetes and malocclusion.³⁵ In the future, orthodontists may use genetic findings derived from saliva tests of individual patients for a personalized medicine approach to treatment planning. If SNPs like the *ACTN3* R577X polymorphism are detected as part of routine diagnosis and treatment planning, a more personalized and specific treatment plan might be developed to produce a more favorable treatment result.

CONCLUSIONS

1. Alpha-actinin-3 enhances forceful, fast skeletal muscle contraction and strength. A common *ACTN3* polymorphism, R577X, results in alpha-actinin-3 protein absence, changes in fiber type proportions, muscle metabolism and bone mineralization. This study demonstrated that R577X associates with Class II and deep bite skeletal malocclusions.
2. Loss of alpha-actinin-3 was accompanied by significantly smaller type II fiber diameter in masseter muscle. Although we expected this decrease in diameter to result in an association between 577XX and open bite malocclusion, significant genotype associations were demonstrated with deep bite malocclusion versus controls. This is most likely resulted from increased type II fiber percent occupancy in masseter muscle, even though average type II fiber diameter was smaller.
3. RT-PCR experiments demonstrated that as *ACTN3* mRNA expression decreased to almost undetectable with 577XX genotype, *ACTN2* expression levels remained unchanged, suggesting that in masseter muscle alpha-actinin-2 may not compensate for loss of alpha-actinin-3.
4. Two SNPs in *ACTN3* at rs1815739 and rs678397 had statistically significant differences between Class II malocclusion and controls. These SNPs had no significant association with Class III malocclusion, which may be the result of limited numbers of Class III subjects in the study. These findings suggest that bone

growth may be altered with *ACTN3* genotype for Class II subjects in our population.

5. Another study has demonstrated that *ACTN3* knock-out mice have significant decreases in bone mineral density. Further, absence of alpha-actinin-3 in bone resulted in increased expression of *Enpp1*, a negative regulator of mineralization. These relationships may be important to the development of Class II malocclusions in humans and merit further investigation.

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Figure 1.

Lateral cephalograms of two *ACTN3* - rs1815739 genotyped subjects. Left subject (A) is a Class III open bite malocclusion with CC genotype and right subject (B) is a Class II open bite with TT genotype.

Figure 2.

Serial sections of masseter muscle from an *ACTN3* – rs1815739 CC genotyped subject stained for Myosin Heavy Chain (MyHC) and alpha-actinin-3 protein antibodies. A. anti-fast MyHC antibody stain, B. anti-IIA, C. anti-type I and D. anti – alpha-actinin-3. Arrows mark fibers by type classification. Alpha-actinin-3 protein is present in all type II and hybrid fibers, but not in type I fibers. Bar = 1000µm.

Figure 3.

Serial sections of masseter muscle from an *ACTN3* – rs1815739 TT genotyped subject stained for MyHC and alpha-actinin-3 antibodies. A. anti-fast MyHC antibody stain, B anti-IIA, C. anti-type I and D. anti – alpha-actinin-3. Arrows mark fibers by type classification. There is no alpha-actinin-3 antibody staining of hybrid or type II fibers. The type IIA fibers have relatively small fiber diameter, compared to other fiber types, and to type IIA fibers stained for the CC genotype subject (Fig. 2B). Bar = 1000µm.

Table I*ACTN3* Genotype % by Malocclusion Class Compared to Normal Controls

<i>SNP Marker</i>	<i>Phenotype</i>	<i>Genotypes</i>				<i>Total n</i>		<i>p value</i>	
		<i>CC</i>	<i>TC</i>	<i>TT</i>	<i>undetermined</i>	<i>n</i>		<i>Genotypes</i>	<i>Alleles</i>
<i>ACTN3 rs1815739</i>	Class III	4 (25%)	9 (56%)	3 (19%)	1	17		0.39	0.28
	Class II	4 (10%)	27 (66%)	10 (24%)	2	43	60	0.003 **	0.009 **
Control	Class I	14 (45%)	12 (39%)	5 (16%)		31			
<i>ACTN3 rs678397</i>		CC	TC	TT					
	Class III	4	9	3	1	17		0.11	0.04
	Class II	4 (10%)	24 (60%)	12 (30%)	3	43	60	0.003 **	0.000005 **
Control	Class I	17 (55%)	12 (39%)	2 (6%)		31			

**
 $p < 0.01$

Table II*ACTN3* Genotype % by Vertical Dimension Classification

<i>Marker (Gene)</i>	<i>Phenotype</i>	<i>Genotypes</i>				<i>Total n</i>		<i>p-value</i>	
		CC	TC	TT	undetermined	N		genotypes	alleles
<i>ACTN3</i> rs1815739	Normal bite	1 (7%)	10 (62%)	5 (31%)	0	16		0.6	0.4
	Open bite	5 (22%)	12 (52%)	6 (26%)	2	25		0.2	0.41
	Deep bite	2 (11%)	14 (78%)	2 (11%)	1	19	60	0.02 *	0.16
Control	Normal occlusion	14 (45%)	12 (39%)	5 (16%)		31			
		CC	TC	TT	undetermined				
<i>ACTN3</i> rs678397	Normal bite	1 (6%)	9 (56%)	6 (38%)	0	16		0.001 **	0.0002 **
	Open bite	5 (21%)	13 (54%)	6 (25%)	1	25		0.02 *	0.005 **
	Deep bite	2 (12%)	11 (69%)	3 (19%)	3	19	60	0.02 *	0.009 **
Control	Normal occlusion	17 (55%)	12 (39%)	2 (6%)		31			

*
 $p < 0.05$ **
 $p < 0.01$

Table IIIDifferences in gene expression for *ACTN2* and 3 by R577X Genotype

<i>Gene</i>	<i>Mean mRNA expression (average RQ)</i>			<i>p-value</i>
	(rs1815739) CC	TC	TT	
<i>ACTN2</i>	0.514 (n=4)	0.635 (n=5)	0.55 (n=4)	0.84
<i>ACTN3</i>	0.957 (n=4)	0.387 (n=5)	0.02 (n=4)	0.003**

**
 $p < 0.01$

Table IVFiber Type Differences between *ACTN3* R577X Genotype (TT vs. CC)

<i>Fiber Type</i>	<i>Mean Fiber Area (μm^2) $\pm$ CI</i>			<i>Fiber Number</i>			<i>Fiber Occupancy (%)</i>		
	TT	CC	<i>p</i> value	TT	CC	<i>p</i> value	TT	CC	<i>p</i> value
Type I	1575.8 $\pm$ 646	2360.9 $\pm$ 60.7	0.11	41.4 $\pm$ 26.9	70.5 $\pm$ 42.5	0.25	49.7 $\pm$ 10.8	48.09 $\pm$ 8.6	0.08
Hybrid	1313.8 $\pm$ 63.1	1277.3 $\pm$ 77.2	0.89	22.4 $\pm$ 10.3	31.9 $\pm$ 24.2	0.41	31.4 $\pm$ 10.3	19.42 $\pm$ 6.9	0.452
Type II	634.9 $\pm$ 244.8	1421.6 $\pm$ 06.3	0.002 **	58.2 $\pm$ 41.4	31.7 $\pm$ 13.6	0.344	17.1 $\pm$ 9.9	22.4 $\pm$ 7.75	0.439

**
p < 0.01