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Characterization of a novel splicing variant in the *RAPTOR* gene

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Abstract

The mammalian target of rapamycin (mTOR) plays an essential role in the regulation of cell growth, proliferation and apoptosis. Raptor, the regulatory associated protein of mTOR, is an important member in this signaling pathway. In the present report, we identified and characterized a novel splicing variant of this gene, *RAPTOR*_{v2}, in which exons 14–17, 474 bp in total, are omitted from the mRNA. This deletion does not change the open reading frame, but causes a nearly complete absence of HEAT repeats, which were shown to be involved in the binding of mTOR substrates. Real time PCR performed on 48 different human tissues demonstrated the ubiquitous presence of this splice variant. Quantification of mRNA levels in lymphoblastoid cell lines (LCL) from 56 unrelated Hap Map individuals revealed that the expression of this splicing form is quite variable. One synonymous SNP, rs2289759 in exon 14, was predicted by ESEfinder to cause a significant gain/loss of SRp55 and/or SF2/ASF binding sites, and thus potentially influence splicing. This prediction was confirmed by linear regression analysis between the ratio of *RAPTOR*_{v2} to total *RAPTOR* mRNA levels and the SNP genotype in the above 56 individuals ($r=0.281$ and $P=0.036$). Moreover, the functional evaluation indicated that this splicing isoform is expected to retain the ability to bind mTOR, but is unlikely to bind mTOR substrates, hence affecting signal transduction and further cell proliferation.

Keywords

RAPTOR; Alternative splicing; Expression; mTOR pathway

1. Introduction

The mammalian target of rapamycin (mTOR) pathway is important in cell growth, proliferation, and apoptosis [1] and alteration in this pathway can induce the onset of cancer [2,3]. Two multiple protein complexes, mTORC1 and mTORC2, constitute the core of this pathway [1]. The former one is the target of and sensitive to rapamycin, an immunosuppressant and anticancer agent, while the latter is not [1]. mTORC1 transfers the proliferation signal to the downstream proteins by phosphorylating two substrates, ribosomal protein S6 kinase 1 (S6K1) and the eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) [1]. mTORC1 is composed of three proteins, mTOR, G protein β -subunit-like protein (G β L) [4], and the regulatory associated protein of mTOR (raptor) [5], which works both as a scaffold and a regulator protein [4,6]. Indeed, 4E-BP1 and S6K1 bind to raptor through their TOR signaling (TOS) domain [7–9] and mTOR binds through its own HEAT repeat [5]. In the

Conflict of interest

The authors declare that there are no conflicts of interest.

absence of raptor, the activity of the mTOR pathway is predominantly reduced or inhibited [5]. It has been shown that the raptor protein includes three domains, *RAPTOR* N-terminal conserved (RNC), HEAT repeat, and WD40 repeat, all of which are conserved from yeast to human [5].

Alternative splicing plays an important role in increasing proteomic diversity [10–12]. It has been proposed that up to ~70% of the genes in the human genome show alternative splicing [10,12]. Some incorrect splicing events may cause severe diseases ([13], and references therein). An extreme example is the gene coding for the constitutive androstane receptor (CAR), which has as many as 22 different splicing forms [14]. Most of the splicing variants identified in human result in a functional alteration relative to the common and/or complete type. Some splice variants even show a reversal of function compared with the full-length counterparts. This is the case, for example, for the vascular endothelial growth factor receptor 1 (VEGFR-1, or Flt-1), which in its full-length form can bind VEGF and then transfer the signal to downstream proteins. In contrast, the soluble form of VEGFR-1 (soluble Flt-1), generated from alternative splicing of the same pre-mRNA used to produce the full-length Flt-1, contains the extracellular VEGF-binding domains, but does not include the transmembrane and intracellular catalytic domains [15]. As a consequence, soluble Flt-1 can bind to VEGF with a high affinity, but cannot transfer the signal [15]. Therefore, it inhibits the activity of VEGF, thus leading to a reverse function compared with the complete type [15].

In the present study, we identified and characterized a novel splicing isoform of the *RAPTOR* gene and its expression was described in multiple human tissues and within human populations. Moreover, one SNP in the first skipped exon was predicted to cause the gain/loss of a SRp55 (splicing factor, arginine/serine-rich 6) and/or SF2/ASF (splicing factor, arginine/serine-rich 1) binding site. This prediction was verified by a significant association between this SNP genotype and the expression ratio between splice form and the full-length in *RAPTOR*, suggesting that this SNP regulates *RAPTOR* splicing process.

2. Material and methods

2.1. Identification of splicing variant

Human lymphoblastoid cell line (LCL) mRNA was extracted by RNeasy Mini Kit (Qiagen, USA) and was reverse transcribed using the Super Transcript III kit (Invitrogen, USA). The segment that surrounds the deleted exons was amplified by primer pair Raptor_2U1 (5'-CCTCCGTCGATGAAAACTG-3') and Raptor_2L1 (5'-CTTGTAGGCGATGCTGTTGA-3'). After electrophoresis on 3.5% polyacrylamide gel, each band was eluted from the gel and directly sequenced using the BigDye terminator v3.1 chemistry (Applied Biosystems, USA).

2.2. Real time-PCR analysis of *RAPTOR* full-length and splicing variant

56 unrelated HapMap LCL (24 YRI, 22 CEU, and 10 ASN; repository number is listed in Table S1) were purchased from the Coriell Institute for Medical Research. Total mRNA was extracted and cDNA was synthesized by abovementioned method. Real time PCR to amplify the splicing variant was performed by using primers RAPT_F4 (5'-CAGTGGACAGCGAGCTGGTGGT-3'), which was designed to span the breakpoint of the two flanking constitutive exons, and RAPT_R4 (5'-GCTGTCTCGCACTGGGGTCAAA-3') whereas real time PCR to amplify the total *RAPTOR* transcript was done by using primers RAPT_F3 (5'-CGGGGAGGTCTGGGTCTTCAA-3') and RAPT_R3 (5'-CTCCTGCTCCCGCTGTAGTGC-3').

To investigate the distribution of the splicing variant across different human tissues, real time-PCR was also performed on a Tissuescan plate (OriGene Inc., USA), which includes cDNA from 48 different human tissues. All real time PCR was performed using SYBR green (Applied Biosystems, USA) and normalized based on β -actin levels by using the following primers: 5'-ACGTGGACATCCGCAAAGAC-3' and 5'-CAAGAAAGGGTGTACGCAACTA-3' [16].

2.3. Computational prediction of exon splicing enhancer (ESE) and statistical analysis

ESE is a *cis*-element that exists in most exons and is essential for correct splicing. Most ESEs are supposed to be binding sites for serine/arginine-rich (SR) proteins, a protein family that is involved in splicing process [17]. To identify potential regulatory elements for this splicing variant, all the known SNPs in the 500 bp region flanking the start site of the first skipped exon were included in ESE analysis. Both alleles at each SNP were evaluated by ESEfinder (<http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home>) with default parameters [18,19] and the outputs were compared in a pairwise manner. This software collected the binding matrices of multiple SR proteins and could be used to identify putative ESEs [18].

To examine the effect of SNP genotype on expression levels of the splice variants, three continuous integers were assigned to the three different genotypes (AA, AG, and GG) and linear regression analysis was performed between genotype and expression level. All statistical analysis was performed in SPSS 15.0 (SPSS Inc., USA) and the null hypothesis was rejected when $P < 0.05$.

Multiple databases were searched for significant associations between SNP rs2289759 and common human diseases. These databases included: SCAN (<http://genemem.bsd.uchicago.edu/newscan/>), dbGaP (<http://www.ncbi.nlm.nih.gov/gap>), GWAS catalog (<http://www.genome.gov/26525384>), and the Genetic Association Database (<http://geneticassociationdb.nih.gov/>).

3. Result and discussion

cDNA from the LCLs of 24 unrelated Hapmap individuals (8 YRI, 8 CEU, and 8 ASN) were used as templates for resequencing, with the goal of identifying novel coding SNPs. Amplification and sequencing of the *RAPTOR* transcript in LCL revealed the presence of double sequence peaks starting at position 2315 (relative to reference sequence NM_020761), which coincides with the start site of exon 14. Deconvolution of the double sequences showed that one sequence corresponds to the beginning of exon 14 while the other sequence to the beginning of exon 18 (Fig. 1), thus suggesting the existence of a splice variant in which exons 14–17 were omitted. To confirm this prediction, we ran the PCR product on a polyacrylamide gel and this revealed the presence of two bands (Fig. 2). The longer one was the complete type, 1812 bp, while the other one was approximately 500 bp shorter. Sequencing of the shorter band and alignment to the full-length cDNA confirmed that exons 14–17, 474 bp in total, were not included in this splicing variant, which will be referred as *RAPTOR_v2*. This deletion does not alter the open reading frame, but causes a deletion of 158 amino acids (position 504–661) in the raptor protein, which spans almost the entire HEAT repeat region (position 550–677) [5].

Recent studies have demonstrated that gene expression varies substantially within human populations and that human LCL is a good model to understand the genetic basis for this variation [20–22]. To evaluate the expression spectrum, we performed real time PCR in 56 unrelated LCLs of *RAPTOR_v2* and *RAPTOR* (Fig. 3; Table S1). It can be concluded that both transcripts showed as much as ~25-fold inter-individual variation. The real time PCR also revealed that the Ct values differed significantly between the full-length and the splice variant (Ct value, mean $\pm$ S.D., 30.77 ± 1.39 for *RAPTOR_v2* vs. 26.48 ± 1.34 for total *RAPTOR*, $n =$

56, $P < 0.0001$), which indicated that *RAPTOR_v2* does not account for a large portion of the total *RAPTOR* mRNA in LCLs.

It was also proposed that gene expression varies noticeably among different ethnic populations [22,23]. To investigate whether *RAPTOR_v2* is expressed differently in three major ethnic populations, we compared the expression level among CEU, YRI and ASN by ANOVA, but no significant difference was observed in *RAPTOR* ($P = 0.257$), *RAPTOR_v2* ($P = 0.149$), or the ratio of them ($P = 0.324$).

Gene expression is also not uniform across tissues in the human body and high expression levels suggest that the gene may play an important role in a given tissue. To describe the expression pattern of *RAPTOR_v2* within the human body, we performed real time PCR on multiple tissues (Fig. 4). It can be concluded that this splicing variant is widely expressed in the human body, which suggests that it is likely to be functional. However, its expression levels vary substantially across tissues, unlike what was reported for the full-length *RAPTOR* transcript (<http://symatlas.gnf.org/SymAtlas/>). The highest expression level for *RAPTOR_v2* was observed in nasal mucosa and pituitary, and the lowest in the spleen (Fig. 4; Table S2).

Recent studies have shown that some SNPs in the gene, especially those near exon skip sites, may regulate the expression of or even induce the appearance of splicing variants ([24] and references therein; [25,26]). To identify potential regulatory SNPs, we used our resequencing data for the *RAPTOR* cDNAs (data not shown) and used ESEfinder [18,19] to look for potential ESE in coding sequence in the skipped exon. In exon 14–17, we identified 4 SNPs at position 2323 (rs2289759), 2449 (rs34848699), 2575 and 2671. Three of them showed an extremely low frequency (1 out of total 48 sequenced chromosomes) and could not account for the wide and variable expression in human population. The remaining SNP, rs2289759, at the 8th nucleotide of exon 14, may influence splicing by inducing significant gain/loss of SRp55 and/or SF2/ASF binding sites. The A allele at this SNP was associated with a predicted SRp55 binding site (score = 3.06999, threshold = 2.676) while the G allele abrogated it (score = 2.14750). However, allele G created a new binding site for SF2/ASF (score = 2.86452, threshold = 1.867) [19] compared with allele A (score = 1.48077). The frequency of the minor allele (G) is 16%, 22%, 42%, and 19% in YRI, CEU, ASN and our 56 individuals, respectively.

As a member of SR family, SRp55 plays an important role in the splicing process [27]. It can recognize exons by binding to specific ESE element in them and then recruit other proteins to constitute the spliceosome. If a SRp55 binding site is destroyed by a mutation, the skipping event of the exon may be induced. This was observed, for example, in a recent study [28] in which a mutation in exon 5 of *PDHA1* was shown to disrupt the binding site of SRp55 and further induce the skipping of this exon. Thus, based on this prediction, the G allele at rs2289759 is expected to correlate with higher expression levels of *RAPTOR_v2*.

SF2/ASF is another important member of SR family. The mutations within its binding sites have also been shown to induce exon skipping and thus be associated with some diseases ([19] and references therein). Moreover, it was recently proposed that SF2/ASF can influence mTORC1 activity [29]. In this case, however, the allele G introduces a new binding site for SF2/ASF, which could potentially enhance the inclusion of this exon. In combination with the prediction for SRp55, it was difficult to foresee the actual effect of this SNP in alternative splicing.

To test the potential regulatory role of this SNP in alternative splicing, we retrieved genotype data for these 56 HapMap individuals (<http://www.hapmap.org>) and performed a linear regression analysis between genotype and expression level. The result indicated that this SNP did not correlate with *RAPTOR_v2* expression ($P = 0.486$), but significantly with the ratio of *RAPTOR_v2* to *RAPTOR* ($r = 0.281$, $P = 0.036$; Fig. 5). This result can be easily explained by

the fact that this SNP does not influence the transcription of *RAPTOR*, but can alter the splicing efficiency of exons 14. Moreover, the G allele was correlated with a relatively higher expression level (Fig. 5), which suggests that the prediction for SF2/ASF was redundant. However, the omission of exon 15–17 from *RAPTOR_v2* remains unclear. One possibility is that the deletion of exon 14 induces that of the following three exons by some unknown mechanism.

Another question concerns the function of this splicing variant. The omission of exons 14–17 does not change the open reading frame in *RAPTOR_v2*, but causes a nearly complete absence of HEAT repeat. HEAT repeat has been proposed to be involved in protein–protein interactions [30]. This is also the case in raptor, where the HEAT repeat domain is thought to be important in binding of S6K1 and 4EBP-1 [8]. The deletion of the HEAT repeat is highly likely to affect the binding of these substrates. However, since binding to mTOR requires multiple domains [5], the binding ability to mTOR may be retained or only attenuated to some extent in this splicing variant. Therefore, this isoform can bind to mTOR, but not to its substrates. Similar with the abovementioned soluble Flt-1, the *RAPTOR_v2* isoform can potentially down-regulate or even inhibit the signal transfer of this pathway by competitively binding to mTOR. Further studies are necessary to determine whether this is indeed the case.

Recent genome-wide association studies (GWAS) have provided valuable information on genetic variants that influence common disease phenotypes. To investigate whether SNP rs2289759 is associated with risk to the disease and non-disease phenotypes studied to date, multiple databases were searched for reports of a genotype-phenotype correlation. Since rs2289759 was not included in the most commonly used genotyping platforms, we searched for proxy SNPs, i.e., SNPs strongly correlated with rs2289759. No association was found at genome-wide significance levels. With regard to expression levels in lymphoblastoid cell lines, the most significant association was found for proxy SNP rs1468030 ($r^2 = 0.6446$ in Caucasian and $r^2 = 0.7369$ in Asian) and the *ERAS* gene on chromosome X. Additional studies are necessary to determine if this association can be replicated in other samples. It is well established that risk variants found by GWAS only explain a small fraction of the total genetic susceptibility to a given disease and that GWAS may have limited power to detect small genetic effects. Therefore, failure to find an association at genome-wide significance levels between rs2289759 and the phenotypes investigated so far does not exclude the possibility that this SNP has functional and phenotypic effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.mrfmmm.2009.01.001.

References

1. Wullschlegel S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006;124:471–484. [PubMed: 16469695]
2. Dann SG, Selvaraj A, Thomas G. mTOR Complex1-S6K1 signaling: at the crossroads of obesity, diabetes and cancer. *Trends Mol. Med* 2007;13:252–259. [PubMed: 17452018]
3. Chiang GG, Abraham RT. Targeting the mTOR signaling network in cancer. *Trends Mol. Med* 2007;13:433–442. [PubMed: 17905659]
4. Kim DH, Sarbassov DD, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H, Tempst P, Sabatini DM. GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Mol. Cell* 2003;11:895–904. [PubMed: 12718876]
5. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 2002;110:163–175. [PubMed: 12150925]
6. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* 2002;110:177–189. [PubMed: 12150926]
7. Choi KM, McMahon LP, Lawrence JC Jr. Two motifs in the translational repressor PHAS-I required for efficient phosphorylation by mammalian target of rapamycin and for recognition by raptor. *J. Biol. Chem* 2003;278:19667–19673. [PubMed: 12665511]
8. Nojima H, Tokunaga C, Eguchi S, Oshiro N, Hidayat S, Yoshino K, Hara K, Tanaka N, Avruch J, Yonezawa K. The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif. *J. Biol. Chem* 2003;278:15461–15464. [PubMed: 12604610]
9. Schalm SS, Fingar DC, Sabatini DM, Blenis J. TOS motif-mediated raptor binding regulates 4E-BP1 multisite phosphorylation and function. *Curr. Biol* 2003;13:797–806. [PubMed: 12747827]
10. Johnson JM, Castle J, Garrett-Engele P, Kan Z, Loerch PM, Armour CD, Santos R, Schadt EE, Stoughton R, Shoemaker DD. Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. *Science* 2003;302:2141–2144. [PubMed: 14684825]
11. Kwan T, Benovoy D, Dias C, Gurd S, Serre D, Zuzan H, Clark TA, Schweitzer A, Staples MK, Wang H, Blume JE, Hudson TJ, Sladek R, Majewski J. Heritability of alternative splicing in the human genome. *Genome Res* 2007;17:1210–1218. [PubMed: 17671095]
12. Modrek B, Resch A, Grasso C, Lee C. Genome-wide detection of alternative splicing in expressed sequences of human genes. *Nucleic Acids Res* 2001;29:2850–2859. [PubMed: 11433032]
13. Wang GS, Cooper TA. Splicing in disease: disruption of the splicing code and the decoding machinery. *Nat. Rev. Genet* 2007;8:749–761. [PubMed: 17726481]
14. Lamba JK, Lamba V, Yasuda K, Lin YS, Assem M, Thompson E, Strom S, Schuetz E. Expression of constitutive androstane receptor splice variants in human tissues and their functional consequences. *J. Pharmacol. Exp. Ther* 2004;311:811–821. [PubMed: 15194709]
15. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc. Natl. Acad. Sci. U.S.A* 1993;90:10705–10709. [PubMed: 8248162]
16. Liu W, Innocenti F, Wu MH, Desai AA, Dolan ME, Cook EH Jr, Ratain MJ. A functional common polymorphism in a Sp1 recognition site of the epidermal growth factor receptor gene promoter. *Cancer Res* 2005;65:46–53. [PubMed: 15665278]
17. Blencowe BJ. Exonic splicing enhancers: mechanism of action, diversity and role in human genetic diseases. *Trends Biochem. Sci* 2000;25:106–110. [PubMed: 10694877]
18. Cartegni L, Wang J, Zhu Z, Zhang MQ, Krainer AR. ESEfinder: a web resource to identify exonic splicing enhancers. *Nucleic Acids Res* 2003;31:3568–3571. [PubMed: 12824367]
19. Smith PJ, Zhang C, Wang J, Chew SL, Zhang MQ, Krainer AR. An increased specificity score matrix for the prediction of SF2/ASF-specific exonic splicing enhancers. *Hum. Mol. Genet* 2006;15:2490–2508. [PubMed: 16825284]

20. Cheung VG, Conlin LK, Weber TM, Arcaro M, Jen KY, Morley M, Spielman RS. Natural variation in human gene expression assessed in lymphoblastoid cells. *Nat. Genet* 2003;33:422–425. [PubMed: 12567189]
21. Stranger BE, Nica AC, Forrest MS, Dimas A, Bird CP, Beazley C, Ingle CE, Dunning M, Flicek P, Koller D, Montgomery S, Tavaré S, Deloukas P, Dermitzakis ET. Population genomics of human gene expression. *Nat. Genet* 2007;39:1217–1224. [PubMed: 17873874]
22. Zhang W, Duan S, Kistner EO, Bleibel WK, Huang RS, Clark TA, Chen TX, Schweitzer AC, Blume JE, Cox NJ, Dolan ME. Evaluation of genetic variation contributing to differences in gene expression between populations. *Am. J. Hum. Genet* 2008;82:631–640. [PubMed: 18313023]
23. Spielman RS, Bastone LA, Burdick JT, Morley M, Ewens WJ, Cheung VG. Common genetic variants account for differences in gene expression among ethnic groups. *Nat. Genet* 2007;39:226–231. [PubMed: 17206142]
24. Cartegni L, Chew SL, Krainer AR. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat. Rev. Genet* 2002;3:285–298. [PubMed: 11967553]
25. Hull J, Campino S, Rowlands K, Chan MS, Copley RR, Taylor MS, Rockett K, Elvidge G, Keating B, Knight J, Kwiatkowski D. Identification of common genetic variation that modulates alternative splicing. *PLoS Genet* 2007;3:e99. [PubMed: 17571926]
26. Lu ZX, Peng J, Su B. A human-specific mutation leads to the origin of a novel splice form of neuropsin (KLK8), a gene involved in learning and memory. *Hum. Mutat* 2007;28:978–984. [PubMed: 17487847]
27. Screaton GR, Caceres JF, Mayeda A, Bell MV, Plebanski M, Jackson DG, Bell JI, Krainer AR. Identification and characterization of three members of the human SR family of pre-mRNA splicing factors. *EMBO J* 1995;14:4336–4349. [PubMed: 7556075]
28. Boichard A, Venet L, Naas T, Boutron A, Chevret L, de Baulny HO, De Lonlay P, Legrand A, Nordman P, Brivet M. Two silent substitutions in the PDHA1 gene cause exon 5 skipping by disruption of a putative exonic splicing enhancer. *Mol. Genet. Metab* 2008;93:323–330. [PubMed: 18023225]
29. Karni R, Hippo Y, Lowe SW, Krainer AR. The splicing-factor oncoprotein SF2/ASF activates TORC1. *Proc. Natl. Acad. Sci. U.S.A* 2008;105:15323–15327. [PubMed: 18832178]
30. Andrade MA, Bork P. HEAT repeats in the Huntington's disease protein. *Nat. Genet* 1995;11:115–116. [PubMed: 7550332]

Fig. 1. Sequence of *RAPTOR* transcript. The upper sequence read is from *RAPTOR* while the below one from *RAPTOR_v2*.

Fig. 2.
The polyacrylamide gel electrophoresis of *RAPTOR* and *RAPTOR_v2*. M indicates the marker. The 1–4 lanes in the gel represent cDNA samples from different lymphoblastoid cell lines. The bands around 1800 bp are *RAPTOR* complete type while the ones around 1200 bp are *RAPTOR_v2*.

Fig. 3.

The expression boxplot of *RPTOR*, *RPTOR_v2*, and their ratio in 56 lymphoblastoid cell line from HapMap. All genes are normalized to β -actin and log-transformed. The values for the different transcripts are not comparable.

Fig. 4.

The *RAPTOR_v2* expression in 48 different human tissues. The data are multiplied by 5, normalized to β -actin, and log-transformed.

Fig. 5. The relationship between rs2289759 genotype and the ratio of *RAPTOR_v2* to *RAPTOR* (Pearson $r=0.281$ and $P=0.036$). The data are normalized to β -actin and log-transformed.