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5' UTR SNP RS111686073 MODIFIES EXPRESSION OF TAPASIN IN BLACKS

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Abstract

Tapasin (TAPBP) is a transmembrane glycoprotein mediating the binding of TAP and MHC-I within the peptide loading complex in the endoplasmic reticulum, which is important for loading of high affinity peptides. Absence of TAPBP in mice is known to affect MHC-I expression and CD8+ T cell development, and viruses such as cytomegalovirus are known to inhibit TAPBP gene transcription to impair antigen presentation. TAPBP mRNA is known to be differentially expressed across humans, which we have confirmed. We hypothesized that expression level of TAPBP would affect HIV pathogenesis, and the genetic markers of expression could potentially serve as indicators for risk in the context of HIV outcome. Thus, we examined the TAPBP gene to identify any single nucleotide polymorphisms (SNPs) that affected expression level and ascertain the underlying mechanisms.

Methods

After scanning TAPBP for common SNPs, rs111686073 (G/C) was identified as a variant found only in blacks that significantly correlated with expression via qPCR in two South African cohorts and by microarray in a third. To verify the effect on expression, reporter constructs were generated from the 5' UTR of TAPBP and examined with luciferase assays in HeLa cells. As this particular SNP is in the 5' UTR, we used the transcription factor predictor Alibaba2 to probe for binding sites overlapping the SNP and identified putative binding sites for AP-2a and Sp1. Electrophoretic mobility shift assays were then performed to determine if either transcription factor mediated the change in expression.

Results

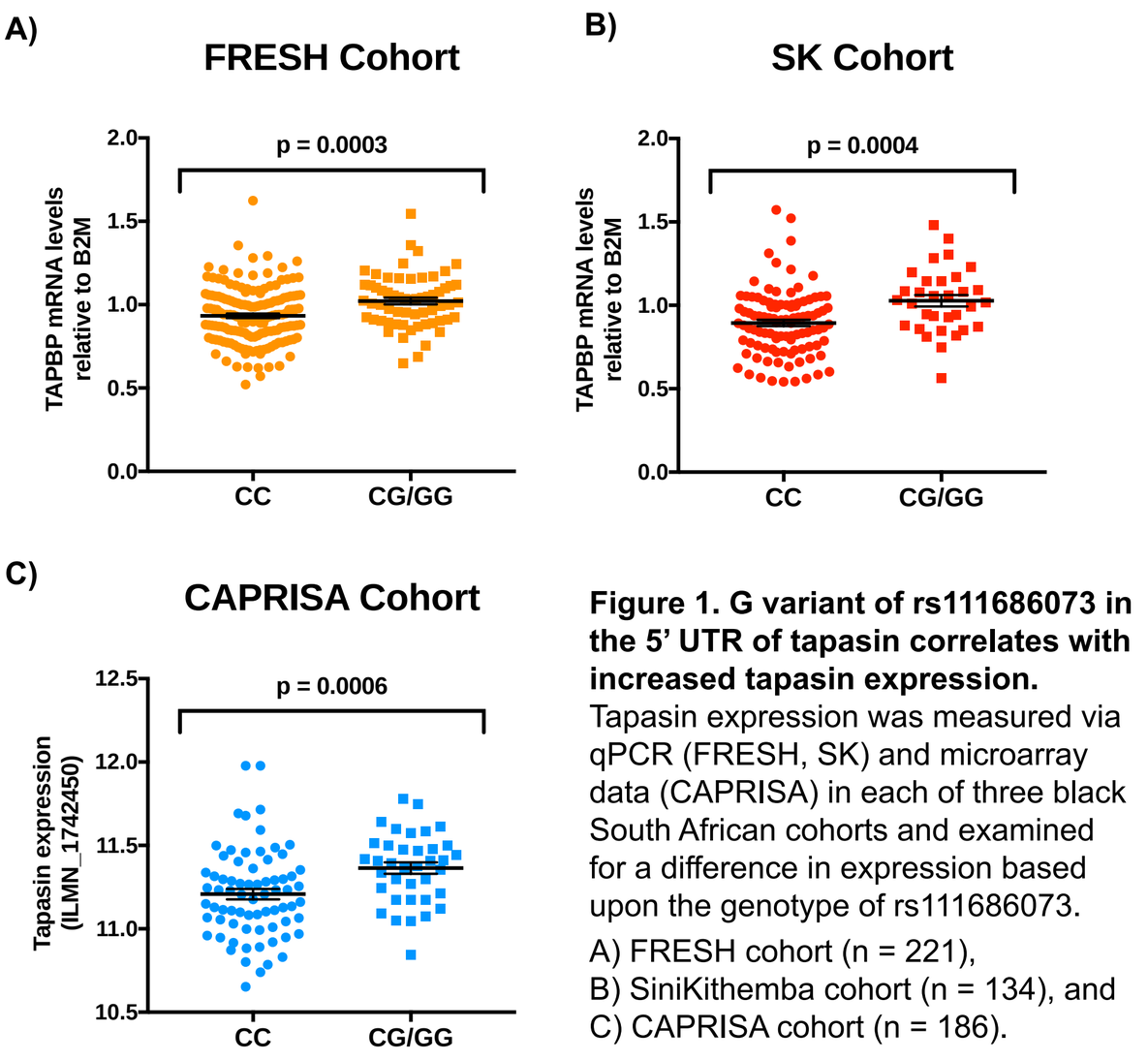


Figure 1. G variant of rs111686073 in the 5' UTR of tapasin correlates with increased tapasin expression. Tapasin expression was measured via qPCR (FRESH, SK) and microarray data (CAPRISA) in each of three black South African cohorts and examined for a difference in expression based upon the genotype of rs111686073.
A) FRESH cohort (n = 221),
B) SiniKithemba cohort (n = 134), and
C) CAPRISA cohort (n = 186).

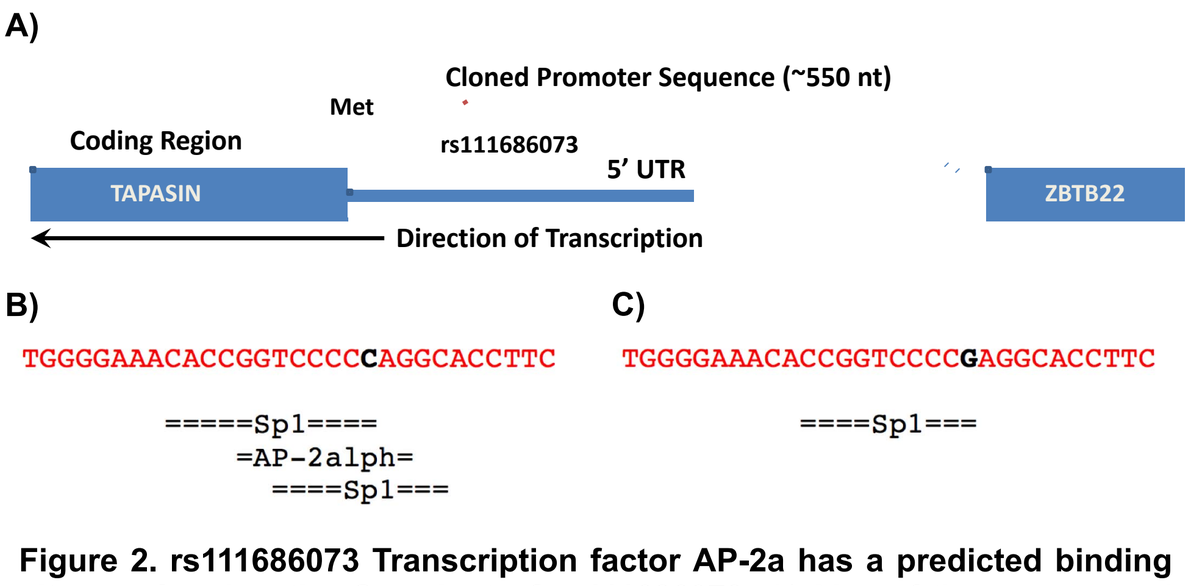


Figure 2. rs111686073 Transcription factor AP-2a has a predicted binding site overlapping the C variant of rs111686073. A) Map of the area around rs111686073 with the promoter sequence for the luciferase assay indicated (not to scale). B,C) Web-tool Alibaba2 predicted that AP-2a (TFAP2A) bound an area in the 5' UTR of tapasin that overlapped rs111686073 when the C variant is present (B) but not when the G variant is present (C). The location of the SNP is indicated in black and bold.

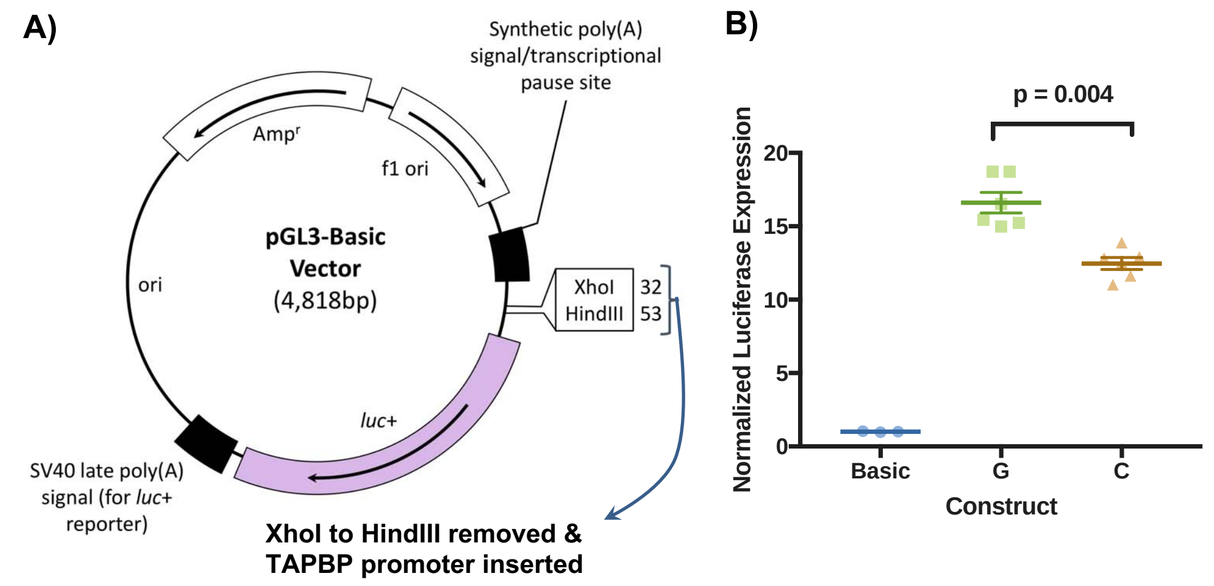


Figure 3. The G variant of rs111686073 causes higher luciferase expression than the C variant. A pGL3-based construct with a ~550 bp promoter sequence containing the transcription start site, core promoter, and SNP of interest was constructed via Gibson assembly and transfected into HeLa cells. After two days, cells were lysed and luciferase activity was read and normalized to the expression of Renilla and the empty pGL3-basic vector (Promega). A) pGL3-basic vector map. B) The construct with the G variant causes more normalized luciferase expression as compared to the one with the C.

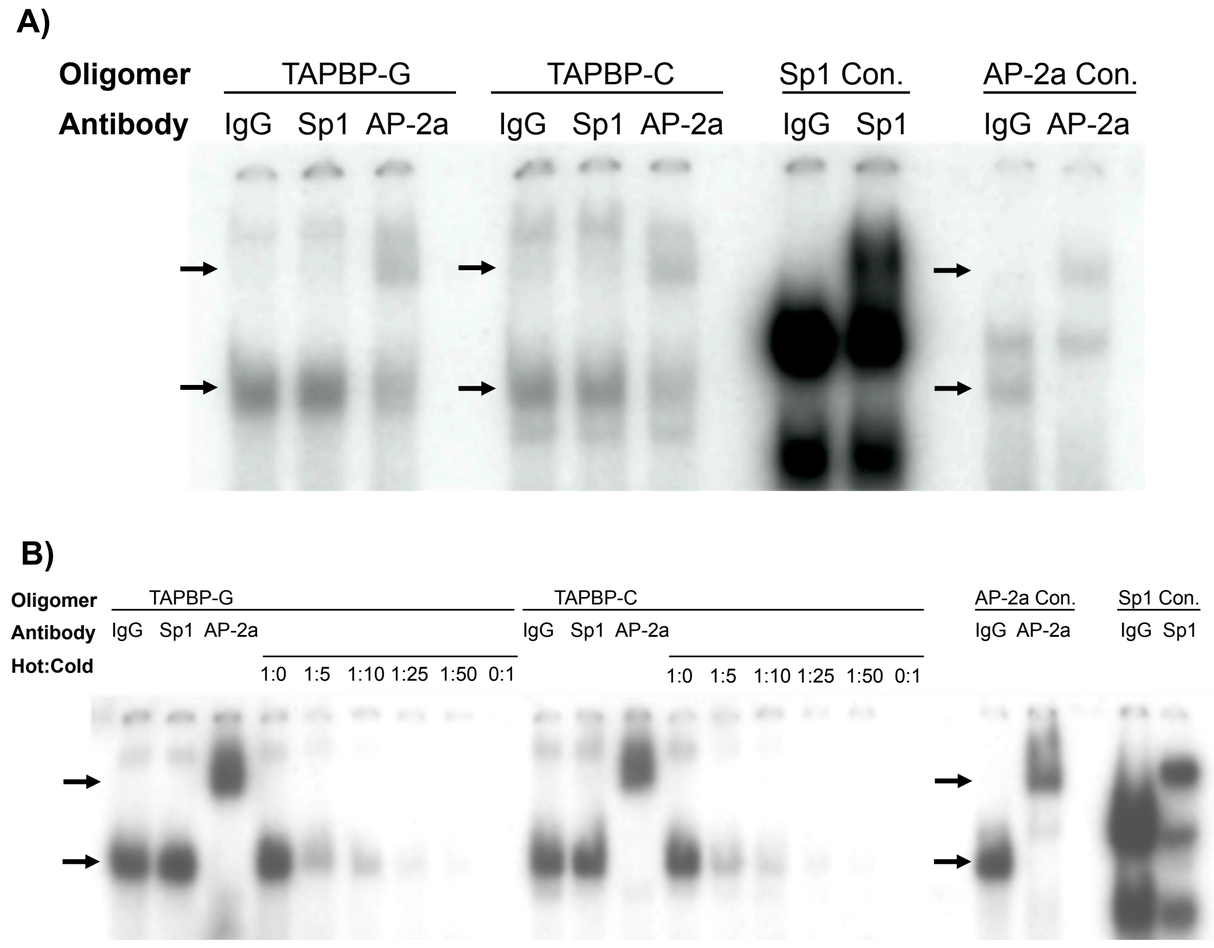


Figure 4. The G variant of rs111686073 binds AP-2a better than the C variant. Electrophoretic mobility shift assays (EMSA) were performed with oligonucleotides containing the two variants of the SNP and consensus sequences for Sp1 and AP-2a. A) Nuclear extract from 293Ts bound to both the C and G variant oligomers and shifts were observed in the presence of antibody to AP-2a (arrows), but not the control IgG or Sp1. Sp1 and AP-2a control oligomers show protein shifts with Sp1 and AP-2a, respectively. B) Cold competition assay with HeLa nuclear extract using the following ratios of hot oligomer to cold: 1:0, 1:5, 1:10, 1:25, 1:50, and 0:1. Both TAPBP oligomers bind AP-2a (arrows).

Conclusions

In summary, the SNP rs111686073 is significantly associated with TAPBP expression, and the G variant results in increased expression over the C variant in Luciferase assays. While AP-2a binds both variants based on EMSA analysis, our assay does not appear be sensitive enough to distinguish a clear difference in AP-2a binding on TAPBP expression. We hypothesize that lower expression of TAPBP may alter TAPBP-dependent HLA-B expression and antigen presentation, and thus affect CD8+ T cell responses. Future studies will focus on the effects of differential TAPBP expression in HIV pathogenesis.

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