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POLYMORPHISMS IN *PAAN-AG* PROMOTER INFLUENCES NF-κB BINDING AND TRANSCRIPTION ACTIVITY IN HEK293 CELLS

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The human leukocyte antigen-G (HLA-G), a protein highly expressed at the human maternal-fetal interface during pregnancy, is thought to be critical for the survival of the semi-allogenic fetus. Current evidence suggest that HLA-G programs immune cells at the maternal-fetal interface into immunosuppressive phenotypes, but definitive proof remai elusive since *in vivo* experiments in humans are not possible due to ethical concerns. In the search for an appropriate animal model, we have identified the olive baboon (*Papio anubis*) as a potential candidate. This primate expresses an HLA-G-like protein termed Paan-AG in the placenta. Preliminary data shows that *Paan-AG* gene shares many characteristics with *HLA-G*, including limited polymorphism, alternative splicing of the mRNA, and restricted tissue expression of the protein. Restricted tissue expression suggested that the two genes might share tissue-specific re_ atory elements. We previously identified a number of putative regulatory elements in the proximal promoters of two *Paan-AG* alleles, 5'UTAG-1 (AG1) and 5'UTAG-2 (AG2). The objective of the current study was to assess binding of the transcription factor NF-κB to *Paan-AG* κB elements and determine the effects of binding on *Paan-AG* promoter activity. Both alleles contained two κB elements, κB1 and κB2. Binding was assessed using electrophoretic mobility shift assays and functional activity using luciferase reporter assays. NF-κB bound both κB1 and κB2 element in the AG1 allele. In contrast, only κB1 of the AG-2 allele bound to NF-κB; κB2 did not bind. The AG2 κB1 element bound NFκB with a stronger affinity compared to AG-1 κB1. Mutagenesis studies showed that the difference in bi-ding was due to two nucleotide differences in the 3' end of κB1. The functional activity of the two alleles also di_ed; AG2 consistently showed higher luciferase activity compared to AG1. Mutating the last two nucleotides in th 3' end of κB1 resulted in an increase of luciferase activity to levels comparable to that of AG2. Overall, these results suggest that variations in the proximal promoter may influence transcription rates of *Paan-AG* as reported recently for *HLA-G*, and provide further evidence of the potential usefulness of the baboon as a model for *in vivo* HLA-G studies.
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