Melanocortin-3-receptor promoter polymorphism associated with tuberculosis susceptibility does not influence protein expression

Marlene Eggert*, Martina Pfob and Ortrud K Steinlein

Abstract

Background: The melanocortin-3-receptor (MC3R) is a member of the G-protein coupled receptor family that mediate cellular response through the cyclic adenosine monophosphate signalling pathway. In the promoter region of MC3R the polymorphism rs6127698 has previously been shown to be strongly associated with tuberculosis susceptibility. It is predicted to generate an alternative transcription factor binding site.

Findings: We investigated the functional impact of rs6127698 by luciferase assay to assess if this polymorphism is capable of altering protein expression. Our results did not show any significant protein expression changes when comparing the two alleles of rs6127698.

Conclusions: Our experiments demonstrate that the rs6127698 polymorphism does not influence protein translation. A functional role of the predicted alternative transcription factor binding site could therefore not be confirmed. These results suggest rs6127698 has no direct role in tuberculosis susceptibility. The possibility remains that this polymorphism is linked to an adjacent functional genetic variant, acting as a surrogate marker for disease risk.

Keywords: Melanocortin-3-receptor, MC3R, Polymorphism, Tuberculosis susceptibility, Luciferase reporter assay

Findings

Introduction

Tuberculosis still poses a severe health problem worldwide and is classified as the second most frequently cause of death in respect of infectious diseases according to the World Health Organization [1]. Great effort has been made to find genomic regions and specific genes within these regions that play a role in tuberculosis susceptibility, respectively in protection against the disease [2-4]. Cook et al. found two genomic loci linked to tuberculosis susceptibility by sibling pair analysis and could further narrow it down to two genes, one of them being the melanocortin-3-receptor gene (MC3R) [2]. This G-protein coupled receptor has been implicated in a broad spectrum of physiological processes such as energy homeostasis, fat metabolism and immune response [5,6]. In a recently performed study by Adams et al. the major allele guanine (G) of the single nucleotide polymorphism (SNP) rs6127698 in the promoter of MC3R showed a highly significant association with tuberculosis susceptibility. In this work, an in silico predicted alternative transcription factor binding site generated by the G allele was discussed as a possible reason for the association with tuberculosis [7]. The aim of the present study was to investigate the hypothesis that the G allele of SNP rs6127698 is able to regulate protein expression due to the creation of a transcription factor binding site.

Material and methods

Firefly luciferase vector pGL4.10 (AY738222.1) and renilla luciferase vector pGL4.74 (AY738230.1) were purchased from Promega (Mannheim, Germany). The promoterless vector pGL4.10, encoding the firefly luciferase reporter gene luc2, contains a multiple cloning site upstream of luc2 to enable promoter studies. Vector pGL4.74, harbouring an HSV-TK promoter and the renilla luciferase reporter gene hRluc, is used as an expression control to normalize for transfection differences. The
The experiment was repeated independently three times with triplicate samples. A two-tailed t-test was used to compare the values of the test samples and the control samples. A p value of p < 0.05 was considered statistically significant. The data are expressed as fold change and as mean ± SEM.

**Results and conclusion**

The two constructs MC3R-G and MC3R-T were compared to each other by luciferase assay. The results showed no significant differences in protein expression between the two SNP allele variants (MC3R-G 0.35 ± 0.05; MC3R-T 0.38 ± 0.09; p = 0.816) (Figure 1). Thus, our findings do not support the previously reported hypothesis that the major allele G of SNP rs6127698 creates an alternative transcription binding site [7]. At least in our experimental setting, rs6127698 did not show any significant impact with regard to MC3R promoter function. However, we cannot rule out the possibility that the predicted transcription factor binding site created by the major allele G only becomes functional in certain cell types. Such a phenomenon has been reported in the literature before, e.g. for a transcription site in the human glutathione transferase kappa promoter [8]. Cell-specific transcription factors or other non-ubiquitously expressed proteins might be possible reasons for such a selective mechanism. Furthermore, we cannot exclude the possibility that the major allele G of SNP rs6127698 initiates a different, yet unknown, biological mechanism not detectable by the methods used here. Such an unknown mechanism could account for the reported association. A third possibility would be that the SNP allele itself might be non-functional but linked to a nearby
polymorphic site that itself affects protein expression or function. Further research is needed to elucidate the precise role of MC3R and its variants in terms of tuberculosis susceptibility.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
ME designed the study, carried out the experiments, analyzed the data and drafted the manuscript. MP helped to perform the experiments, to acquire the data and to draft the manuscript. OS designed the study, interpreted the data and critically revised the manuscript. All authors have given their final approval of the submitted version.

Acknowledgments
We thank Eva Hermann and Franz Jansen for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft [STE16511-2].

Received: 5 February 2013 Accepted: 12 March 2013
Published: 15 March 2013

References
1. Global Tuberculosis Report. 2012. http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf.
2. Cooke GS, Campbell SJ, Bennett S, Lienhardt C, McAdam KP, Snugio G, Sow O, Gustafson P, Mwangulu F, van Helden P, Fine P, Hoal EG, Hill AV: Mapping of a novel susceptibility locus suggests a role for MC3R and CTSZ in human tuberculosis. Am J Respir Crit Care Med 2008, 178(2):203–207.
3. Cervino AC, Lakiss S, Sow O, Bellamy R, Beyer N, Hoal-Ev Helden E, van Helden P, McAdam KP, Hill AV: Fine mapping of a putative tuberculosis-susceptibility locus on chromosome 15q11-13 in African families. Hum Mol Genet 2002, 11(14):1599–1603.
4. Miller EN, Jamieson SE, Joberty C, Fakbiola M, Hudson D, Peacock CS, Cordell HJ, Shaw MA, Lins-Lainson Z, Shaw JL, Ramos F, Silveira F, Blackwell JM: Genome-wide scans for leprosy and tuberculosis susceptibility genes in Brazilians. Genes Immun 2004, 5(1):63–67.
5. Hruby VJ, Cai M, Cain JP, Mayorov AV, Dedek MM, Trivedi D: Design, synthesis and biological evaluation of ligands selective for the melanocortin-3 receptor. Curr Top Med Chem 2007, 7(11):1107–1119.
6. Tao YX: Mutations in the melanocortin-3 receptor (MC3R) gene: impact on human obesity or adiposity. Curr Opin Invest Drugs 2010, 11(10):1092–1096.
7. Adams LA, Moller M, Nebel A, Scheiber S, van der Merwe L, van Helden PD, Hoal EG: Polymorphisms in MC3R promoter and CTSZ 3’UTR are associated with tuberculosis susceptibility. Eur J Hum Genet 2011, 19(8):676–681.
8. Shield AJ, Murray TP, Cappello JY, Coggan M, Board PG: Polymorphisms in the human glutathione transferase Kappa (GSTK1) promoter alter gene expression. Genomics 2010, 95(5):299–305.

doi:10.1186/1756-0500-6-99
Cite this article as: Eggert et al.: Melanocortin-3-receptor promoter polymorphism associated with tuberculosis susceptibility does not influence protein expression. BMC Research Notes 2013 6:99.