

***SLC11A1* PROMOTER POLYMORPHISMS,  
GENE EXPRESSION AND ASSOCIATION  
WITH AUTOIMMUNE AND  
INFECTIOUS DISEASES**

**A Thesis Submitted for the Degree**

**of**

**Doctor of Philosophy**

**by**

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# **CERTIFICATE OF AUTHORSHIP/ORIGINALITY**

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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**Nicholas Steven Archer**

2012

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## ABSTRACT

Solute Carrier Family 11A Member 1 (SLC11A1) is a member of a highly conserved group of ion transporters and has restricted localisation to the phagosomal membrane of monocytes/macrophages. SLC11A1 plays an immunomodulatory role in influencing macrophage activation status and the T helper 1/T helper 2 bias. As such it modulates susceptibility to infectious/autoimmune diseases. A polymorphic (GT)<sub>n</sub> promoter microsatellite repeat is known to alter *SLC11A1* promoter activity. Of the nine (GT)<sub>n</sub> alleles identified, alleles 3 and 2, which account for a combined allele frequency of greater than 95%, drive high and low *SLC11A1* expression, respectively. The increased *SLC11A1* expression, driven by (GT)<sub>n</sub> allele 3 is hypothesised to result in a heightened activation status of classically activated macrophages, affording resistance to infectious disease, but conferring susceptibility to pro-inflammatory autoimmune diseases. Conversely, decreased *SLC11A1* expression in the presence of allele 2 would confer susceptibility to infectious disease, but resistance to autoimmune disease.

A large number of studies assessing the association between the presence of specific (GT)<sub>n</sub> promoter alleles with the incidence of infectious and autoimmune disease have produced inconsistent associations. Meta-analyses are powerful analytical tools which combine individual association studies to estimate the strength of an association, therefore, meta-analyses of case control association studies (from 1991-2006) analysing the association of *SLC11A1* promoter (GT)<sub>n</sub> alleles 2 and 3 with the incidence of autoimmune disease were performed. The meta-analyses found a weak predominance of disease in the absence of allele 2, with a fixed effects pooled OR of 0.80 (95% CI = 0.22), however, a random effects pooled odds ratio (OR) of 0.88 (95% CI = 0.66) for allele 3 suggested no association with the incidence of autoimmune disease.

The publication of additional case control studies between 2006 and the present allowed a more comprehensive meta-analysis to be completed. This analysis, which included additional *SLC11A1* polymorphisms, represents the largest study assessing the association of *SLC11A1* polymorphisms with disease occurrence to date. Allele 2 of the (GT)<sub>n</sub> microsatellite was associated with increased and reduced incidence of infectious [OR=1.32 (1.20-1.46)] and autoimmune diseases [OR=0.90 (0.81-1.00)], respectively. Allele 3 was significantly associated with reduced incidence of infectious disease



[OR=0.82 (0.76-0.88)], however, the association with susceptibility to autoimmune disease occurrence did not reach statistical significance [OR=1.11 (0.98-1.26)]. The findings of the meta-analysis challenges the hypothesis that allele 3 is the disease causing variant at the (GT)<sub>n</sub> microsatellite repeat.

The results of these meta-analyses highlight small sample sizes as a major limitation of case control association studies. Completion of large-scale studies has been impractical because conventional *SLC11A1* (GT)<sub>n</sub> genotyping methodologies are time consuming and cannot differentiate all (GT)<sub>n</sub> variants. A high resolution melt curve methodology has been designed and optimised to genotype two *SLC11A1* polymorphisms, the (GT)<sub>n</sub> and (CAAA)<sub>n</sub> microsatellite repeats. Assay validation yielded a 100% success rate for genotyping of the (GT)<sub>n</sub> and (CAAA)<sub>n</sub> microsatellites. The designed methodology is the first to enable accurate, sensitive and high-throughput genotyping of these microsatellites and will enable the completion of sufficiently large association studies required to determine the association between the *SLC11A1* (GT)<sub>n</sub> and (CAAA)<sub>n</sub> polymorphisms and disease occurrence.

In addition to the (GT)<sub>n</sub> microsatellite, the -237C/T polymorphism has also been shown to modulate *SLC11A1* expression, with the T variant driving low expression in the presence of (GT)<sub>n</sub> allele 3. Little is known about *SLC11A1* transcription or the mechanism by which the (GT)<sub>n</sub> and -237C/T promoter polymorphisms modulate *SLC11A1* expression. Bioinformatic studies were completed to identify putative regulatory elements involved in transcription and promoter constructs, containing different lengths of the *SLC11A1* promoter, were prepared and used to assess promoter function. A 581bp promoter region (-532 to +49) that controlled *SLC11A1* expression in monocytes was identified. Within this region was identified a 148bp minimal promoter region (-99 to +49) containing the core elements for the formation of the basal transcriptional complex. The greatest transcriptional enhancement was identified within a 170bp region (-532 to -362) containing a novel IRF-Ets composite sequence for the recruitment of transcription factors IRF-8 and PU.1. Additionally, the promoter constructs suggested that the *SLC11A1* promoter may mediate bidirectional transcription. It was further determined that, in monocytic cells, the ability of (GT)<sub>n</sub> alleles 2 and 3 to differentially modulate *SLC11A1* expression was not due to their differing abilities to form Z-DNA, but to monocyte-specific factor(s) binding to a 165bp

region (-362 to -197) of the *SLC11A1* promoter. Additional bioinformatic and functional assays suggested that the T variant of the -237C/T polymorphism reduced *SLC11A1* promoter activity independently of the (GT)<sub>n</sub> microsatellite repeat.

Infectious and autoimmune diseases are major contributors to morbidity and mortality. *SLC11A1* is instrumental in regulating macrophage function and hence susceptibility to infectious and autoimmune diseases. This study has provided insight into the association of *SLC11A1* with disease incidence, has developed a novel genotyping methodology to allow the completion of large association studies and has elucidated mechanisms of transcriptional regulation of *SLC11A1* and the influence of polymorphisms on *SLC11A1* expression.

# PUBLICATIONS ARISING FROM THE WORK DESCRIBED IN THIS THESIS

## (A) PUBLICATIONS IN PEER-REVIEWED JOURNALS

**Nicholas S. Archer**, Najah Nassif & Bronwyn A. O'Brien (2012) "The *SLC11A1* (GT)<sub>n</sub> promoter polymorphism modulates expression through monocyte specific factor(s) to alter susceptibility to infectious and autoimmune diseases", (Manuscript in preparation).

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**Nicholas S. Archer**, Melinda Sirmias, Stephanie Dowdell, Najah Nassif & Bronwyn A. O'Brien (2012) "Genotyping disease-associated *SLC11A1* microsatellite repeats by high resolution melt analysis", (Submitted).

**Nicholas S. Archer**, Najah Nassif & Bronwyn A. O'Brien (2010) "Discrimination of microsatellite repeat polymorphisms of the *SLC11A1* promoter by melting curve analysis using the Eppendorf Mastercycler ep *realplex*", Eppendorf Technical Application Note 206.

Bronwyn O'Brien, **Nicholas S. Archer**, Fraser Torpy & Najah Nassif (2008) "Association of *SLC11A1* Promoter Polymorphisms with the incidence of autoimmune and Inflammatory Diseases: A Meta-Analysis", *Journal of Autoimmunity*, 31(1): 42-51.

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## **(C) AWARDS**

Awarded the John Hambly Award for the best UTS presentation at the Combined RNSH/UTS/USyd/KIMR Scientific Research Meeting 2010.

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## LIST OF ABBREVIATIONS

<b>γ-IRE</b>	interferon-γ response element
<b>ALL</b>	acute lymphocytic leukaemia
<b>AML</b>	acute myeloid leukaemia
<b>AMML</b>	acute myelomonocytic leukaemia
<b>API</b>	Activator protein 1
<b>ARNT</b>	aryl hydrocarbon receptor nuclear translocator
<b>bp</b>	base pairs
<b>BRE</b>	TFIIB-recognition element
<b>BSA</b>	bovine serum albumin
<b>CCF2-AM</b>	coumarin cephalosporin fluorescein
<b>C/EBP</b>	CCAAT/enhancer binding protein
<b>CI</b>	confidence interval
<b>Ct</b>	cycle threshold
<b>DCE</b>	downstream core element
<b>DMEM</b>	Dulbecco's modified eagle medium
<b>DNA</b>	deoxyribonucleic acid
<b>DPE</b>	downstream promoter element
<b>EDTA</b>	ethylenediaminetetraacetic acid
<b>EMSA</b>	electrophoretic mobility shift assays
<b>FBS</b>	fetal bovine serum
<b>GM-CSF</b>	granulocyte macrophage colony-stimulating factor
<b>h</b>	hours
<b>HBSS</b>	Hanks buffered salt solution
<b>HIF-1</b>	Hypoxia inducible factor 1
<b>HIV</b>	Human immunodeficiency virus
<b>Idd</b>	insulin dependant diabetes (murine)
<b>IDDM</b>	insulin dependant diabetes mellitus (human)
<b>IECS</b>	IRF-Ets composite sequence
<b>IFN-γ</b>	interferon-gamma
<b>IL</b>	interleukin
<b>iNOS</b>	inducible nitric oxide synthase
<b>Inr</b>	Initiator element
<b>IRF</b>	interferon regulatory factors
<b>ISRE</b>	IFN-stimulated response element
<b>kb</b>	kilobase
<b>KLF</b>	kruppel-like factor
<b>l</b>	litre
<b>Lamp1</b>	lysosome-associated membrane protein 1
<b>LB</b>	Luria Bertani
<b>LD</b>	linkage disequilibrium
<b>LPS</b>	lipopolysaccharide
<b>MHC</b>	major histocompatibility complex
<b>min</b>	minutes
<b>MTE</b>	motif ten element
<b>NF-IL6</b>	nuclear factor IL-6
<b>NF-κB</b>	nuclear factor kappa-light-chain-enhancer of activated B cells
<b>NO</b>	nitric oxide

<b>Nramp</b>	natural resistance-associated macrophage protein
<b>NTC</b>	no template control
<b>Oct-1</b>	octamer binding protein 1
<b>OR</b>	odds ratio
<b>PAS</b>	periodic acid-schiff
<b>PBS</b>	phosphate buffered saline
<b>PCR</b>	polymerase chain reaction
<b>PMA</b>	phorbol myristate acetate
<b>PMN</b>	polymorphonuclear
<b>pol II</b>	RNA polymerase II
<b>PU.1</b>	protein encoded by <i>SPI-1</i> gene
<b>RES</b>	reticuloendothelial system
<b>RNA</b>	ribonucleic acid
<b>RNase</b>	ribonuclease
<b>RPMI</b>	Roswell Park Memorial Institute
<b>RT</b>	room temperature
<b>s</b>	seconds
<b>SBB</b>	Sudan black B
<b>SLC11A1</b>	Solute carrier family 11A member 1 (Human protein)
<b><i>SLC11A1</i></b>	Solute carrier family 11A member 1 (Human gene)
<b>Slc11a1</b>	Solute carrier family 11A member 1 (non-human protein)
<b><i>Slc11a1</i></b>	Solute carrier family 11A member 1 (non-human gene)
<b>SLC11A2</b>	Solute carrier family 11A member 2 (Human protein)
<b><i>SLC11A2</i></b>	Solute carrier family 11A member 2 (Human gene)
<b>SNP</b>	single nucleotide polymorphism
<b>Sp1</b>	Specificity protein 1
<b><i>SPI-1</i></b>	spleen focus by forming virus proviral integration 1
<b>TAF</b>	TBP associated factor
<b>TBP</b>	TATA binding protein
<b>TESS</b>	Transcription Element Search Software
<b>TFIID</b>	transcription factor II D
<b>TFBS</b>	transcription factor binding site
<b>Th1</b>	T helper 1
<b>Th2</b>	T helper 2
<b>T<sub>m</sub></b>	melting temperature
<b>TNF-<math>\alpha</math></b>	tumour necrosis factor-alpha
<b>TSS1</b>	transcription start site 1
<b>TSS2</b>	transcription start site 2
<b>UV</b>	ultraviolet
<b>XCPE1</b>	X core promoter element 1
<b>YY1</b>	Ying-Yang 1
<b>ZBP-1</b>	Z-DNA binding protein 1