Molecular Characterisation of the Candidate Region for *bronx waltzer* : a Mouse Model of Hearing Impairment

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Declaration

I declare that this thesis is the result of my own work and has not, whether in the same or different form, been presented to this or any other university in support of an application for any degree other than that for which I am now a candidate

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Abbreviations

ABR	Auditory brainstem response
AHCs	Auditory hair cells
BAC	Bacterial artificial chromosome
bHLH	basic Helix Loop Helix
BLAST	Basic local alignment search tool
bp	Base pairs
CAPs	Compound action potentials
cDNA	Complementary DNA
cM	centiMorgan
CMs	Cochlear microphonics
CO_2	Carbon dioxide
dATP	2'-deoxyadenosine-5'-triphosphate
dbSPL	Decibels sound pressure level
dCTP	2'-deoxycytidine-5'-triphosphate
ddH_2O	Double distilled deionised water
dGTP	2'-deoxyguanosine-5'-triphosphate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
dpc	days *post coitum*
dpf	days post fertilisation
DPOEs	Distortion product otoacoustic emissions

ds	Double stranded
dTTP	2'-deoxythymidine-5'-triphosphate
E	Embryonic day
EDTA	Ethylenediaminetetraacetic acid
EMBL	European molecular biology laboratory
ENU	N-ethyl-N-nitrosourea
ESTs	Expressed sequence tags
EUCIB	European collaborative interspecific backcross mapping panels
g	Grams
GER	Greater epithelial ridge
HCl	Hydrochloric acid
HMM	Hidden Markov model
hpf	hours post fertilisation
HPLC	High performance liquid chromatography
HTGS	High throughput genome sequence
IHCs	Inner hair cells
IVF	*in vitro* fertilisation
K^+	Potassium ion
Kb	Kilobase
L	Litres
LB	Luria-Bertani medium
LER	Lesser epithelial ridge
LOD	Log of odds
Mb	Megabase
mg	milligrams

$MgCl_2$	Magnesium chloride
ml	millilitres
MO	Morpholino oligonucleotide
MRC	Medical Research Council
mRNA	Messenger RNA
Na^+	Sodium ion
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NCBI	National center for biotechnology information
OD	Optical density
OHCs	Outer hair cells
OMIM	Online Mendelian Inheritance in Man
OTOTO	Osmium tetroxide-thiocarbohydrazide procedure
P	Post-natal day
PBS	Phosphate buffer saline solution
PCR	Polymerase chain reaction
pg	picograms
pH	potential of Hydrogen
QTL	Quantitative trait locus
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNAi	RNA interference
RNase	Ribonuclease
rpm	Revolutions per minute
RT-PCR	Reverse transcription-PCR

s.e.	standard error
SCs	Supporting cells
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscopy/microscope
SNP	Single nucleotide polymorphism
SPs	Summating potentials
ss	Single stranded
SSCP	Single strand conformation polymorphism
ssDNA	Single stranded DNA
SSLP	Simple sequence length polymorphisms (microsatellites)
STS	Sequence tagged sites
T0.1E	10mM Tris/0.1mM EDTA buffer
TBE	Tris/borate/EDTA buffer
TE	10mM Tris/1mM EDTA buffer
TEM	Transmission electron microscopy/microscope
Tris	Tris(hydroxymethyl)aminomethane
U	Enzyme units
μg	micrograms
μl	microlitres
μm	micrometre
UTR	Untranslated region
UV	Ultraviolet
WGS	Whole genome shotgun
YAC	Yeast artificial chromosome

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Abstract

bronx waltzer is an autosomal recessive mouse mutation causing abnormalities in the inner ear which result in mutant mice having deficiencies in both the auditory and vestibular systems. Homozygous mice exhibit hyperactivity, circling behaviour, head tossing and failure to respond to sound. Hearing loss in these mice is due to degeneration of the inner hair cells in the organ of Corti, while the vestibular phenotype is a result of sensory hair cell degeneration in the maculae and cristae. This phenotype is visible from E17.5, shortly after the hair cells differentiate, making *bronx waltzer* an interesting model for the understanding of the molecular basis of development and function of the inner ear as well as for hereditary deafness.

The mutation was previously localised to a 2.8Mb region of chromosome 5 using a backcross of 1073 mice to the inbred strain 101/H. There remained 17 backcross mice with recombinations within this interval, and thus new polymorphic markers have been sought in order to reduce the size of the candidate region. With the identification of two new proximal flanking markers, the size of the critical region has been reduced to 2.45Mb, with the exclusion of seven candidate genes.

The remaining 52 genes currently annotated within the region have been systematically assessed and functional studies carried out on those thought most likely to be the causative agent for *bronx waltzer*. These have included expression screening using inner ear cDNA and gene knockdown using morpholinos designed to target zebrafish orthologs. In addition, large scale exon resequencing of all the coding regions within the interval has been carried out and 90.63% coverage achieved overall, with complete coding sequence obtained for 23 candidate genes. These new data have provided a means of critically evaluating the candidacy of genes within the *bronx waltzer* critical region.