Characterization of a New Gene Causing Male Infertility in the M366 Mouse Model

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Abstract

Spermatogenesis is a complex process involving hundreds of genes, and a malfunction of any protein involved in gamete maturation could potentially result in nonfunctional sperm. Spermatogenesis is very similar between mice and humans, making mice good models for infertility studies. The M366 mouse model involves a mutation in a novel gene called *Nup210l*.This mutation causes infertility in homozygous male mice. Preliminary studies suggest the mutation may affect the function of Sertoli cells, which are vital to spermatogenesis. The purpose of this study is to further explore the nature of this novel gene and the infertility-causing mutation in the M366 mouse model. Characterization of the normal *Nup210l* gene will include identification of splice variants using mouse testicular RNA and confirmation of the existence of a *NUP210L* transcript in human testis. The nucleotide sequence of the mutated *Nup210l* gene carried by affected M366 mice will be compared to the nucleotide sequence of the wild-type *Nup210l* gene so that the exact nature of the mutation may be determined. These preliminary studies to characterize both wild-type and mutant versions of the *Nup210l* gene and their expression will form the basis of future work to define the precise role of the *Nup210l* protein in spermatogenesis.

Introduction

- The mouse strain M366 carries a mutational insertion in a novel gene called *Nup210l*. The exact nature of the mutation is unknown.
- Homozygous affected male M366 mice are infertile and have defects in Sertoli cells and spermatozoa.
- The *NUP210L* gene in humans has been predicted but not yet shown to produce transcripts.

Nup210l Locus and M366 Mutation

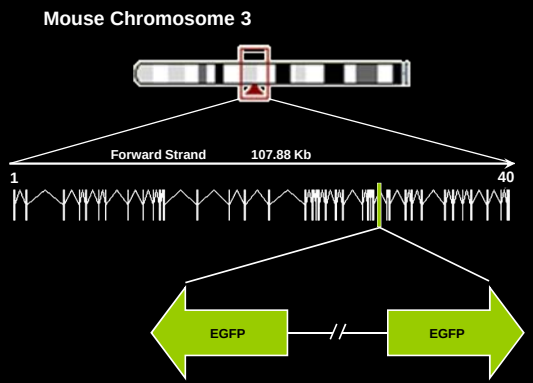

Figure1. *Nup210l* gene locus. In mice, the *Nup210l* gene is located on Chromosome 3 and contains 40 exons. This information was obtained from ENSEMBL, transcript ID ENSMUST00000029548. The mutation in the M366 strain is due to the insertion of an EGFP transgene flanked by inverted repeats within intron 27.

Nup210l Transcript Sequence Alignment

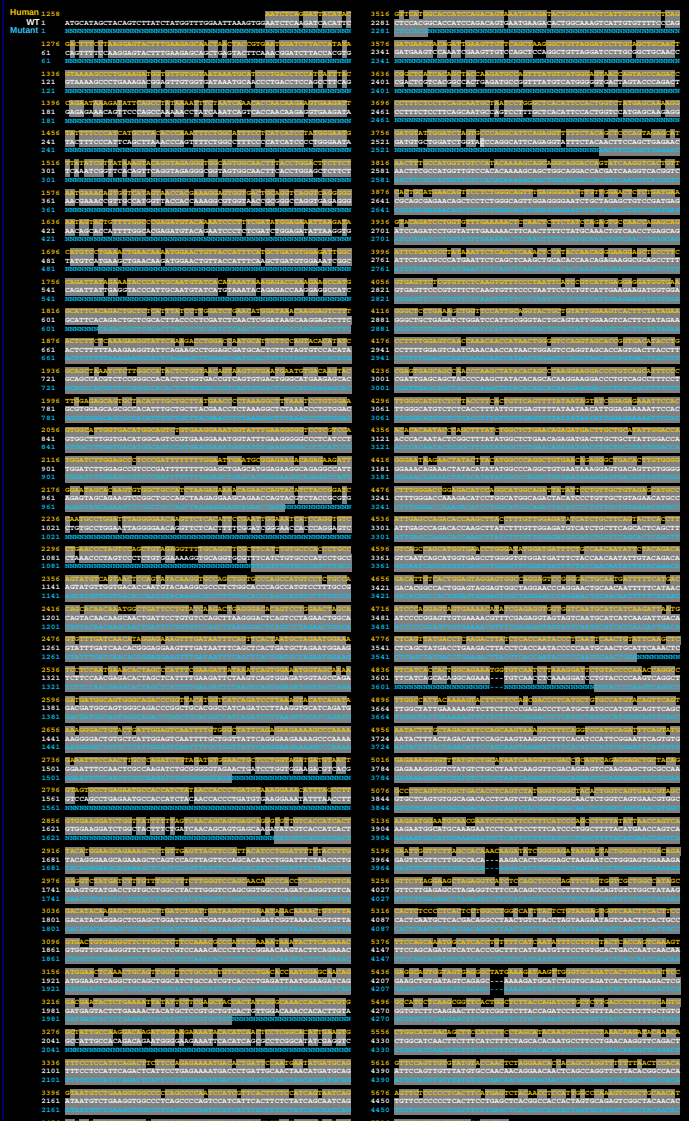

Figure 2. Alignment of human, wild-type mouse, and M366 transcript nucleotide sequences. The known wild-type mouse *Nup210l* transcript (white) is aligned with both the mutant (blue) and human (gold) transcripts. Areas of homology between sequences are signified by gray highlighting. Nucleotide bases that are unknown are signified with a "N". The human sequence is from ENSEMBL, transcript ID ENST00000368559. Only the portion of the human transcript with homology to the mouse transcript is shown due to space constrictions. The wild-type mouse transcript was determined experimentally by nucleotide sequence analysis of mouse testicular cDNA. mRNA from the testis of M366 mice was used in RT-PCR to generate cDNA in order to sequence the mutant transcript. Mutant *Nup210l* sequencing is not completed. The wild-type mouse and human transcripts share 82% nucleotide homology.

Evidence for Mouse *Nup210l* Splice Variants

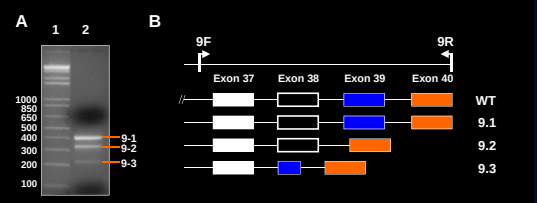

Figure 3. Gel electrophoresis of wild-type mouse RT-PCR products. A. Primers corresponding to nucleotides 4116-4139 (9F), and 4515-4485 (9R) of the mouse *Nup210l* gene (ENSMUST00000029548) gave multiple amplicons in RT-PCR reactions. Nucleotide sequence analysis of the amplicons was performed. Lane 1: 1Kb+ ladder (Invitrogen); Lane 2: primer set 9 amplicons labeled 9.1-9.3. **B.** In this schematic of the last 4 exons of mouse *Nup210l*, the position of the forward and reverse primer set 9 primers are indicated by the arrows. The exon content of the 3 primer set 9 amplicons are indicated. 9.1 has all 4 exons present, 9.2 is missing exon 39, and 9.3 is missing exon 38 and a portion of exon 39.

Confirmation of Human *NUP210L* Transcript

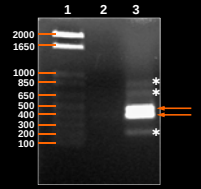

Figure 4. Gel electrophoresis of human RT-PCR products. Primers were designed to amplify the region corresponding to nucleotides 2239-26123 of the predicted human *NUP210L* gene (ENST00000368559). Lane 1: 1Kb+ ladder (Invitrogen); Lane 2: no template (negative) control; Lane 3: human RT-PCR products. A major amplicon of 375 bp in size was expected. Instead, two slightly larger amplicons were detected (arrows). In addition, three less intense amplicons of various size were also detected (✱).

Conclusions

- Nucleotide sequence analysis done to date does not show any major differences between the M366 allele of *Nup210l* and the wild-type allele.
- There is evidence that mouse *Nup210l* is alternatively spliced in testis.
- We have demonstrated experimentally the presence of a human *NUP210L* transcript.

Acknowledgements

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