

# An ovine CFTR variant as a putative cystic fibrosis causing mutation

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### Abstract

This report describes a DNA variant in the ovine cystic fibrosis transmembrane conductance regulator (CFTR) gene that has been previously reported as a putative cystic fibrosis causing mutation in humans. The variant is a guanine to adenine base change at position 1019 of the ovine CFTR cDNA, corresponding to an arginine (R) to glutamine (Q) amino acid substitution at position 297 in the predicted CFTR polypeptide. The equivalent R297Q mutation in exon 7 of the human CFTR gene has been reported in a CF patient. This is the first putative cystic fibrosis mutation to be detected in another animal species.

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Key words: cystic fibrosis; CFTR mutations; animal homologues.

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Cystic fibrosis (CF) is associated with mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene,<sup>1</sup> which encodes a small conductance chloride ion channel,<sup>2</sup> and may have other additional functions as a regulator of other ion channels.<sup>3</sup> Extensive analysis of the CFTR gene in patients and carriers has shown over 600 DNA changes, including point, frameshift, and splice site mutations (CF Genetic Analysis Consortium, unpublished data). However, only a few mutations

have been shown to influence CFTR Cl<sup>-</sup> channel activity, and much work is still required to understand how specific mutations cause disease.

No natural animal models of CF have been identified, although several lines of transgenic CF mice have been generated that reflect the pathology of the disease to a greater or lesser extent.<sup>4</sup> Recently, more advanced techniques of homologous recombination and mouse embryonic stem cell manipulation have resulted in the generation of transgenic CF mice carrying the ΔF508 CFTR mutation, the most frequent CF associated mutation.<sup>5</sup> Nevertheless, CF mice models have limitations and there is still a need for a natural animal model to help study the pathology of CF lung disease and for evaluating pharmacological approaches to CF treatment. We are investigating sheep as a potential model for CF.<sup>6</sup> Genetic screening for polymorphisms of ovine CFTR in natural sheep populations has identified several DNA sequence changes. Here, we describe the identification of one such DNA variant that has a putative CF causing equivalent in humans.

Single stranded conformation polymorphism (SSCP) analysis was carried out on ovine genomic DNA, using primers specific for ovine CFTR intron sequences either side of the exon (ov7i5: GGAAAGTATATAAGCACC and ov7i3: AGAGAGTTTGCTCATGAC) which corresponds to the human exon 7 sequence. Methods were based on those of Shackleton *et al.*<sup>7</sup> SSCP mobility shifts were detected in several sheep DNA samples (fig 1), and one SSCP was found to be the result of a guanine to adenine substitution at base pair position 1019 in the ovine CFTR cDNA sequence.<sup>6</sup> This substitution destroys a *MspI* site, resulting in a 387 bp fragment instead of the two component fragments (303 and 84 bp) observed when the site is present. *MspI* restriction analysis on amplified exon 7 DNA from sheep families carrying the variant confirmed Mendelian inheritance (fig 1). Ewe "G" is heterozygous for the R297Q variant.

To confirm the expression of this mutation, reverse transcriptase (RT) PCR was performed on blood lymphocyte mRNA from ewe "G". A specific fragment of the ovine CFTR cDNA was amplified using the method of Chalkley and Harris.<sup>8</sup> The two primer sets were as follows: first set 5' AST2R: GCAGATGAGAATAGCTATG 3' (and RT primer) BST1L: CTCCATCACTACATCTG; second (nested) set 5' AST3R: GATGAAGGACTT-GCACTGG 3' A2LA: CAATGCAGAATGAGATGG. *MspI* restriction analysis on the purified PCR product was consistent with the

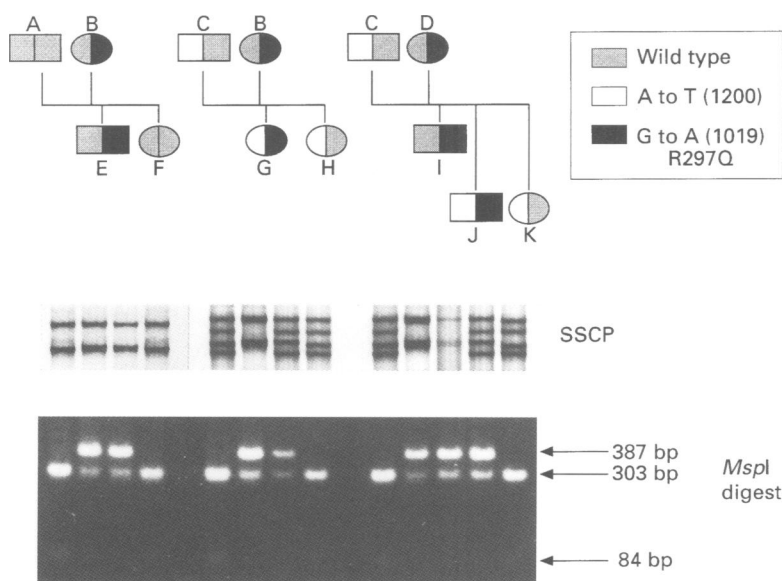


Figure 1 Pedigree structure of sheep carrying the G1019A (R297Q) CFTR exon 7 gene variant in the heterozygote state (also a silent polymorphism, A1200T, unpublished data). SSCP analysis of exon 7 is shown, as well as the *MspI* restriction analysis.

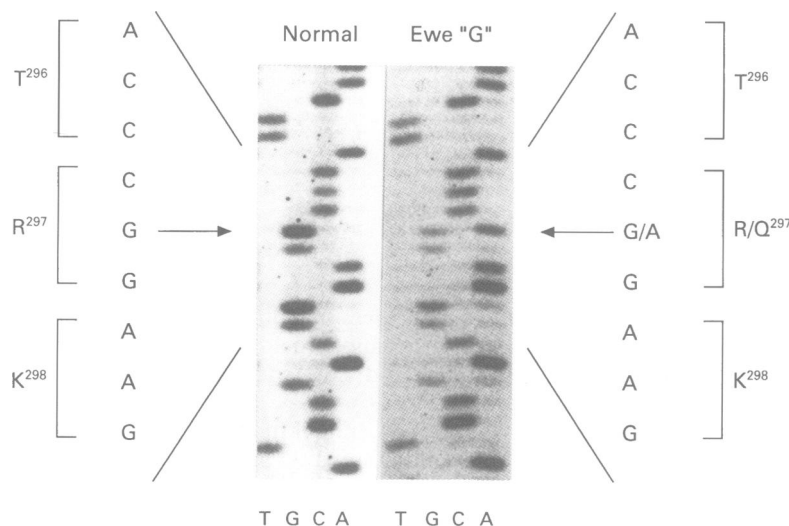


Figure 2 Sequence analysis of exon 7 of the CFTR gene transcripts from "normal" and a G1019A carrying sheep (ewe "G"). RT-PCR products were directly sequenced. Ewe "G" is heterozygous for a G to A base change at ovine CFTR cDNA position 1019, resulting in a predicted arginine to glutamine (R to Q) amino acid substitution.

exon 7 variant being an expressed allele (data not shown). DNA sequence analysis was used to confirm that the R297Q allele is efficiently transcribed (fig 2).

The ovine DNA variant reported here (G to A at position 1019) causes an arginine (R) to glutamine (Q) change at codon 297 in the predicted CFTR polypeptide. This is homologous to a putative cystic fibrosis causing mutation (R297Q) reported in a Northern Ireland family.<sup>9</sup> Interestingly, the arginine (R297) residue lies in the first membrane spanning domain of the predicted CFTR protein, specifically part of the cytoplasmic loop between the putative transmembrane helices 4 and 5.<sup>1</sup> The threonine-arginine-lysine (TRK) peptide in this region is entirely conserved in the predicted polypeptides from all animal species in which the CFTR gene has been characterised,<sup>10</sup> including human, sheep, cattle, mouse, *Xenopus*, and dogfish. This seems consistent with a non-conservative amino acid change at R297 eliciting a functional change in the CFTR protein, perhaps leading to a cystic fibrosis phenotype.

The two human CF patients reported to carry the R297Q mutation were aged 6 and 8 years (in 1991). Clinically, both patients were similarly affected with mild to moderate chest symptoms and both were receiving pancreatic

enzyme supplements.<sup>9</sup> A recent study of a French CF family<sup>11</sup> has suggested that R297Q represents a rare polymorphism, rather than a disease causing mutation, or that the length of a T tract in intron 8 may play a role in influencing the severity of the R297Q allele.<sup>12</sup> We are currently investigating any equivalent T tract length variation in intron 8 of the ovine CFTR gene. At present, it is uncertain if R297Q is a disease causing mutation. Nevertheless, if R297Q in the human CFTR protein does indeed contribute to the cystic fibrosis phenotype, it is tempting to consider that another animal species, carrying R297Q in the homozygous state, might also show some of the symptoms of CF. The ewe "G", who is heterozygous for R297Q, seems perfectly healthy. We are establishing a breeding programme to obtain lambs that are homozygous for the R297Q variant.

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