NEURONAL CEROID LIPOFUSCINOSIS IN DEVON CATTLE – PEDIGREE ANALYSIS AND PRELIMINARY MOLECULAR GENETIC INVESTIGATION

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INTRODUCTION

The neuronal ceroid lipofuscinoses (NCLs) are a group of fatal inherited neurodegenerative diseases characterised by the accumulation of autofluorescent lipopigment in a variety of tissues.

At least eigth different forms of NCL occur in humans and the disease has been identified in various animal species, including sheep and cattle. Although seven of the disease-causing genes have been identified in sheep, mice and humans, the pathomechanisms of this group of diseases is still not fully understood.

In Australia, NCL was first described in 1988 in a Devon herd with approximately 150 cows joined per year (Harper *et al.,* 1988). The disease is characterised predominantly by blindness and behavioural changes and appears to be inherited in an autosomal recessive mode of inheritance. After exclusion of affected and known or predicted carrier animals from the breeding herd, the prevalence of affected animals was initially reduced from 1-4 calves per year (1977-1992) to no affected calves for six years. However, the occurrence of three affected calves in 2001 and the need for well-characterised large animal models for medical research instigated this project to identify the gene involved in bovine NCL. Animal models have been very useful in the past to enhance the understanding of the disease and will be needed in the future to develop and validate treatments including enzyme replacement or gene therapy.

MATERIAL AND METHODS

Identification of affected animals.

Clinical signs. Affected Devon cattle are born normal and develop progressive blindness at an age of 9-14 months. When disturbed, they tend to walk or trot in circles and may show a mild head tilt. They are reported to die of misadventure but can live up to at least 9 years of age in a safe environment.

Pathology. At necropsy, brains of affected Devon cattle show mild atrophy and yellow brown discoloration. Histological findings include severe retinal atrophy with almost complete loss of rods and cones, and intracytoplasmatic accumulation of storage material in neurons and many extraneural cell types. The storage material stains strongly with luxol fast blue, PAS and Sudan black B and shows autofluorescence in ultraviolet light (Harper *et al.,* 1988 ; Jolly *et al.,* 1992).

Biochemistry. The predominant component of the storage material is mitochondrial ATP synthase subunit c (Martinus *et al*., 1991). Enzyme levels for *TPP1*, the enzyme deficient in human *CLN2,* are normal in affected Devon cattle (M. Fietz, *pers. communication*).

Pedigree analysis. Initial pedigree analysis by Harper *et al.* (1988) and Jolly *et al.* (1992) implied inheritance as an autosomal recessive trait, but the available pedigree information did not allow all cases to be traced back to a common ancestor. In 2001, pedigree information available from the herd management program Herd Magic (Saltbush, Armidale) and information from herd books was put together to be analysed with the segregation analysis program 'Long Jumping Descent Graph Sampler' (LJDGS) (Henschell *et al*., 2001 ; Tier and Henshall, 2002 - computer paper at this conference). Information on a total of 1248 (including 24 affected and 28 known carrier) animals was analysed to identify a common ancestor and to predict genotype probabilities for the current herd. The founder allele frequency was set to 0.01. We could not assume that animals with unknown genotype were not affected, thus their genotypes were set to be unknown (either normal, affected or carrier).

Molecular Genetics.

Animal material. Samples for DNA analysis were available from 15 affected (including 9 tissue samples from initial cases), 6 known carriers, 42 likely carriers (genotype probability > 70 %) and 6 unrelated control animals.

Mapping approach. The currently available animal material is not sufficient for linkage analysis and the number of candidate genes is rather large. The thorough clinical, pathological and biochemical characterisation of the disease (see above) could exclude *CLN1*, *CLN2* and *cathepsin D* as causative genes. The designation *CLN7* is considered to be allelic to *CLN8* and *CLN4* represents a rare form of NCL with adult onset for which no genes have been identified. Thus, *CLN3*, *CLN5*, *CLN6* and *CLN8* remain as likely candidate genes for the bovine condition.

Sequencing all candidate genes for mutation detection was not considered a favoured option. Therefore, a combined homozygosity mapping and candidate gene approach (Tammen *et al.,* 1999) was used in this study : Comparative mapping information was used to predict the location of the four candidate genes in cattle and a set of 25 microsatellite markers was identified for corresponding chromosomal locations. An initial screen for homozygosity was performed using a set of four affected, four carrier and two control animals. *In-silico* techniques using EST data available for the candidate genes from GenBank (Benson *et al*., 2000) were used for further analysis. The bovine *CLN3* gene was assembled and alignment of several *CLN3*-ESTs identified several single nucleotide polymorphisms (SNPs) in the bovine *CLN3* gene (Tammen *et al*., in prep.). A C/G-SNP in exon 2 causing a change of an *Aci*I restriction site was analysed using the international bovine reference panel (IBRP, Barendse *et al.,* 1997) to map the gene. The SNP and microsatellite markers in the vicinity of bovine *CLN3* were screened for homozygosity in an enlarged set of 16 Devon cattle (6 affected, 5 carriers and 5 controls).

RESULTS AND DISCUSSION

Identification of the disease gene and of the causative mutation is potentially the most reliable way to eradicate the disease. A DNA test will allow unambiguous identification of all carriers and early diagnosis of affected animals. Furthermore, to provide a useful animal model for

medical research, the causative gene needs to be identified. This appears to be the only reliable method to identify which human form of NCL is homologous to bovine NCL.

Pedigree analysis. Traditional pedigree analysis and consequent exclusion of affected and known or predicted carrier animals from the breeding flock appeared initially to be very successful. For six years (1994–2000) not a single affected animal was diagnosed. Nevertheless, this approach didn't allow identification of all carriers and this lead to the diagnosis of three affected animals in 2001 (a total of 123 reared calves). Neglecting non-random mating and understatement of the number of affected animals due to late onset of the disease, the frequency of the NCL allele (q) was estimated to be 0.15 under Hardy- Weinberg equilibrium. Under complex pedigree analysis with (Henschell *et al*., 2001 ; Tier and Henshall, 2002) a common founder could not be identified, which could be due to incomplete pedigree information (records went back to animals imported from England) or due to false parentage records. Nevertheless, the genotype probabilities estimated by LJDGS, allow the breeder to make informed breeding decisions in the current generation and will help to reduce the frequency of the disease allele.

The five affected, six known carriers and further 32 likely carriers are being used in a breeding program to generate family material suitable for linkage analysis to map the disease gene and to maintain animals for medical research.

Molecular Genetics. Comparative mapping was used to predict the location of four known candidate genes *CLN3*, *CLN5*, *CLN6* and *CLN8* to bovine chromosomes 25, 12, 10 and 8, respectively. Initial homozygosity mapping using a set of 25 microsatellite markers and ten DNA samples did not reveal a specific area of interest. The limited number of only four affected, four carrier and two control animals available for this initial screen and the fact that the comparative mapping can lead to rather imprecise localisation of the candidate genes, did not allow us to exclude any of the candidate genes from further investigation.

To achieve an accurate location of the candidate genes, we commenced mapping these on the bovine linkage map, starting with *CLN3*. An *Aci*I restriction polymorphism was identified by analysis of EST information available at GenBank and was used to map bovine *CLN3* to chromosome 25 (table 1).

Table 1. Results of two-point linkage analysis between *CLN3* and markers on chromosome 25

CLN3	BP28	RM134	BM737	EPO	INRA222	PAI	AF5
LOD score	3.11	4.35	3.91	4.52	6.02	4.55	4.78
θ	0.21	0.17	0.19	0.00	0.16	0.05	0.18

In humans *CLN3* is mapped to chromosome 16p12.1 (Lerner *et al.,* 1994). Therefore, our prediction from comparative mapping information (Band *et al*., 2000) suggested that CLN3 could be located in the proximal half of cattle chromosome 25, which is homologous to human chromosome 16p11.2 –16p13.2. Our linkage mapping placed *CLN3* to the distal end of cattle chromosome 25 (not proximal as predicted), which is homologous to parts of human chromosome 7. The initial homozygosity screen, which was performed with markers from the

proximal half of cattle chromosome 25 (*IDVGA71, ILSTS102, BMS744, BM4005, BM737* and *ILSTS46*) was therefore not very informative.
Six new microsatellite markers from the distal part of cattle chromosome 25 (*BMS 1353, PAI, RM24, HAUT39, AF5 and BM1864*) and the *CLN3*-SNP were analysed on an enlarged set of 16 Devon cattle in a second homozygosity screen. *PAI, RM24* and the *CLN3*-SNP were not informative and the other four microsatellite markers did not indicate a trend towards homozygosity in affected animals in comparison to carrier and control animals. Further SNPs in the CLN3 gene identified in this study will be investigated for informativeness in Devon cattle.
Further research will include the mapping of *CLN5, CLN6* and *CLN8* in cattle. If none of these candidate genes can be associated with NCL in cattle, a whole genome scan will be initiated, which may lead to the identification of a new gene causing NCL.

CONCLUSION

The use of the LJDGS package to predict genotypes in this herd provided an advanced breeding tool that will help to reduce the NCL allele frequency in Devon cattle and allowed us to set up a breeding program for gene identification and further medical research.
The combined homozygosity mapping and candidate gene approach initiated here is a very cost efficient method to identify the causative gene in cattle for NCL against known homologues from other species. However, an as yet unidentified NCL gene could also be responsible for the bovine NCL described here. The establishment of a NCL research herd will provide the appropriate animal material to map and identify such a gene, which would be of significant interest for medical research.

ACKNOWLEDGEMENTS

This work is funded by the Batten Disease Support and Research Association. We are grateful to W. Barendse for the linkage analysis, to R. Jolly and J. Dennis for providing archive samples for DNA extraction and to M. Fietz for *TPP1* enzyme activity analysis.

REFERENCES

Barendse, W., Vaiman, D., Kemp, S.J. *et al.* (1997) *Mam. Genome* **8** : 21-28.
Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Rapp, B.A. and Wheeler, D.L. (2000) *Nucleic Acids Res*. **28** : 15-18.
Harper, P.A.W., Walker, K.H., Healy, *et al*.. (1988) *Acta Neuropathol.* **75** : 632-636.
Henshall, J.M., Tier, B. and Kerr, R.J. (2001). *Genet. Res*. **78** : 281-288.
Jolly, R.D., Gibson, A.J., Healy, P.J., Slack, P.M. and Birtles, M.J. (1992) *New Zealand Vet. J.* **40** : 107-111.
Lerner, T.J., Boustany, R.-M.N., MacCormack, K., Gleitsman, J., Schlumpf, K., Breakefield, X.O., Gusella, J.F. and Haines, J.L. (1994). *Am. J. Hum. Genet*. **54** : 88-94.
Martinus, R.D., Harper, P.A.W., Jolly, R.D., Bayliss, S.L., Midwinter, G.G., Shaw, G.J. and Palmer, D.N. (1991) *Vet. Res. Commun*. **15** : 85-94.
Tammen, I., Cavanagh, J.A.L., Harper, P.A.W., Cook, R.W., Raadsma, H.W. and Nicholas, F.W. (1999) *Archives of Animal Breeding* **42** (Suppl) : 163-166.
Tier, B. and Henshall, J.M. (2002) *Proc 7th WCGALP.*