AKC Canine Health Foundation

REVIEW OF SCIENTIFIC PROGRESS

Grant No.: 247 **Date Report Received:**

Title of Grant: The Study of the Genetics of Cranial Cruciate Ligament Disease in the Dog

Amount Granted: $96,392.00 **Amount Paid: $96,392.00** **Amount Owed: $0.00**

Principal Investigator: Max Rothschild, PhD

Research Institution: Iowa State University

Start Date: 4/1/2004

Length of Grant: 2 **Progress Report:** Addendum to Final

Date of Review: 11/1/06 **Recommended for Approval: Approve**

Performance Summary: A genome scan and candidate gene approach were performed; very thorough work; analysis of heritability completed; work continuing
Objectives completed: all completed
Publications: 3 plus a dissertation
Significant Accomplishments: CFA 3, 10, 23 have number of microsatellites associated with CCLR clinical status; number of candidate genes analyzed

The Study of the Genetics of

Cranial Cruciate Ligament Disease in the Dog

Principal Investigators: *Dr. Max Rothschild and Dr. Mike Conzemius*

Iowa State University

Grant No. 247

Funds received: $53,996 year 1; $42,396 year 2; Total $96,392

Length of Project: 2 years
Initiated: April 1, 2004
Final Report provided March 31, 2006
Extended final report, October 14, 2006

I. NARRATIVE SECTION

A. Grant Objective One: **100% completed**
Establish level of genetic control of cranial cruciate ligament disease in Newfoundland dogs.

This objective has been completed and the manuscript published in the Journal of the American Veterinary Medical Association on January 1, 2006, volume 228, pp 61-64. In summary, we predicted 1) average inbreeding coefficient, defined as the probability that a mating pair's genes are identical because they were inherited from a common ancestor, as 1.19×10^{-4} in the pedigree overall, 2) heritability, the degree of resemblance for RCCL classification between parents and offspring in this Newfoundland population, was estimated to be 0.27, and 3) segregation analysis predicted a major gene effect with a recessive pattern of inheritance. The frequency of the recessive allele was 0.60 with 51% penetrance. The low penetrance makes finding the gene difficult.

B. Grant Objective Two: **overall objective approximately 95% completed**
Determine if cranial cruciate ligament disease in Newfoundlands is associated with individual gene defect(s).

a. Candidate Gene Analysis: 100% completed

Previously, we reported the sequence identification of single nucleotide polymorphisms (SNPs) identified in the biological candidate genes *COMP,* and *COL9A1, COL9A2,* and *FBN.* These genes are associated with primary arthritis formation in humans. Briefly, based on the individual gene SNP, a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) was designed. This PCR-RFLP allowed us to determine the genotype of an individual animal for a particular area of a gene. Then a χ^2 analysis was performed testing both allele frequency by status and genotype frequency by status to determine if a particular genotype and/or allele are associated with ruptured cranial cruciate ligament (RCCL) status. All of the results of the SNP association analyses were completed and the results accepted for publication by Animal Genetics in 2005, volume 36, pages 519 to 521. Since a trend towards significance was observed between allele frequency and RCCL status for *COL9A1*, further sequencing of this gene was performed. An additional SNP was identified, but no statistical association between RCCL status and SNP was determined.

b. Genome Scan: 90% completed

Selection of dogs: Pedigrees, determination of CCL status (normal or affected with CCLR), and DNA were collected from Newfoundland dogs in a study performed at Iowa State University (Wilke *et al.* 2006). Newfoundland dogs were classified as affected with CCLR based on signs of pain on hyperextension of the knee, knee effusion, decreased range of motion, positive cranial drawer sign or cranial tibial thrust, radiographic evidence of stifle effusion and osteoarthritis, and/or surgical confirmation of a ruptured CCL. From this pedigree, affected and normal dogs were chosen for genotyping based on their predicted

statistical likelihood of being genetically either homozygous normal (all unaffected, n=45) or homozygous recessive (all affected, n=45) (see Table 1). Details for the method are found in Macrossan *et al.* (2005).

Selection of markers: Two different and complimentary genome scans were conducted. An initial broad genome scan was performed utilizing 130 microsatellite markers (MSATs). Most of the MSATs were selected from Minimal Screening Set 2 (Guyon *et al.* 2003). Initially, primer optimization for the 130 microsatellite markers was performed on 8 dogs. Final selection of MSATs for the broad genome scan was based on ease of scoring and informativeness of marker, as determined by polymorphic content of alleles. The second phase allowed for the density of the genome scan to be increased by the inclusion of an additional 344 MSATs. This list was generated from MSATs currently utilized in the Veterinary Genetics Laboratory (VGL), University of California at Davis. Similarly, markers were selected for this second stage of the genome scan based on ease of scoring and ability to amplify. All MSATs were multiplexed based on established protocols of the laboratory performing the genotyping.

Statistical analyses. Initial analysis of the MSATs included number and frequency of alleles and genotypes, followed by a chi square analysis performed on each marker. Such analyses compared the allele and genotypic frequencies for each marker for each group of dogs; those assumed to be homozygous normal and those assumed to be homozygous susceptible. Some of the contingency tables (CCLR status by allele and CCLR status by genotype) had cell counts (observations) of less than five, a violation of an assumption of classical chi square tests. Instead, randomization chi square tests were used for all the markers. The null hypothesis of the randomization test is that CCLR status is independent of allele or genotype, so that the chi square statistic from the data should be consistent with chi square statistics generated after randomly shuffling the CCLR status relative to the markers. If the original chi square statistic is an outlier (infrequent) relative to the distribution of randomly generated chi square statistics (less than 5% are greater than the original statistic, i.e.; $P<0.05$), then the statistical test is considered nominally statistically significant. For each marker, 5000 random chi square statistics gave a stable distribution to compare with the original chi square statistic. Statistical tests were performed (one for each marker) so that the conservative Bonferroni and less conservative false discovery rate (FDR) adjustments were used to account for type I error inflation and fix the final cutoff for statistical significance.

Since we could not completely determine those that were expected to be genetically normal and those to be genetically susceptible we also selected a subset of dogs. This stratification of the dogs was based on age and a more specific cause of CCLR. This generated a list of 8 young, CCLR affected dogs (expected to have genetic susceptibility) and 9 old, CCLR normal dogs which are more likely to be genetically normal. Recalculation of the chi square statistic as described above was repeated for genotypes of these 17 dogs. This subpopulation of dogs was chosen to minimize errors associated with status ascertainment, or the variability created in analysis that could be due to a dog having an incorrect identified cause of CCLR. Based on our hypothesis that CCLR in young, large breed dogs is due to a genetic predisposition, we attempted to minimize the potential that our results from the genome scan could be skewed if a dog with a diagnosis of CCLR from a cause other than genetic predisposition was used in the analysis. Other potential causes for CCLR include

true trauma (strain to the CCL that exceeds four times the body weight of the dog) or from age related degeneration.

The original optimization results revealed that 107 of the 130 chosen MSATs could be reliably scored and of these 107 MSATs, 97 were polymorphic. These 97 MSATs provided genome coverage of the 38 autosomes in the canine genome with an average spacing of 28 cM. Initial analyses of the genotyping results for the selected 90 Newfoundland dogs revealed no association for a chromosomal region(s) and CCLR status. For the additional 344 MSATs, 320 were selected for genotyping based on ease of scoring and ability to amplify. This provided additional genome coverage with spacing of markers approximately every 6.7 cM (see Figure 1). Due to problems that were encountered in amplifying the original DNA at the VGL, new samples had to be collected. Unfortunately, new samples were only collected from 66/90 dogs (the remaining dogs were either deceased (n = 11) or the owners failed to respond (n=13). DNA was successfully amplified for the first 97 MSATs in the 90 dogs, but genotyping for the remaining 320 MSATs was completed for 66/90 dogs. Cumulatively, genotyping was performed for 417 MSATs for which 10 were duplicated to allow for error checking. Fifteen markers failed to amplify in the majority of dogs, and 18 markers were monomorphic, leaving 374 informative markers. Of these 374 markers, there was an average of 5 alleles per marker with a range of 2 to 20 alleles per marker.

Based on the nominal $P < 0.05$, 91 markers were considered nominally statistically significant (see Table 2). The 91 markers were located on 31 autosomes. Seven chromosomes (CFA 3, 5, 10, 14, 18, 23, and 27) had 4 or more markers with statistical significance. Based on FDR and Bonferroni correction, 14 markers (located on CFA 1, 3, 5, 9, 10, 11, 13, 16, 24, 30, and 31) were considered to be significantly associated with CCLR. All of the chromosomes with markers considered statistically significant for CCLR status based on FDR and Bonferroni correction had one marker considered significant except for three markers that were located on chromosome 10 and two markers located on chromosome 13.

Based on the stratification of young, CCLR affected dogs and old, unaffected with CCLR dogs, 72 markers were determined to be nominally statistically significant with $P < 0.05$ (see Table 2). These 72 markers were located on 33 autosomes. Five chromosomes had 4 or more markers with statistical significance; chromosomes 3, 8, 10, 23, and 24. Based on FDR correction, 6 markers were considered significant; located on chromosomes 5, 9, 13, 24, and 30. All of the chromosomes with markers considered statistically significant for CCLR status based on FDR correction had one marker considered significant except for the same two markers located on chromosome 13 that were also considered significant in the full data set.

We previously predicted a simple recessive mode of inheritance. However, in this study several chromosomal regions are statistically associated with CCLR status. This may come about by false identification of certain regions or, given our heritability estimate of 0.27 for this trait, it is possible that there are modifying genes that are being expressed that may explain trait variability.

A list of possible candidate genes based on adjacent location to statistically significant markers located on chromosomes 3, 10, and 23 is noted in Table 3. These chromosomes were chosen because they contained the largest number of significant markers

in common based on analyses of both the total population and the stratified population. The Map Viewer program of NCBI (National Center for Biotechnology Information, Bethesda, Maryland) was used to identify genes located in canine specific chromosomal regions identified as statistically significant from the genome scan analysis. Genes were selected as potentially involved in etiopathogenesis of CCLR based on known comparative function available in the Online Mendelian Inheritance of Man (OMIM, NCBI).

Potential candidate genes include several related growth factor receptor genes: fibroblast growth factor binding protein, fibroblast growth factor receptor substrate 2, insulin like growth factor-1 receptor, transforming growth factor alpha, transforming growth factor beta type II receptor, and the related bone morphogenetic protein 10 precursor. In addition, several proteoglycan related genes (versican, proteoglycan link protein 2 precursor, aggrecan core protein precursor, and chondroitin synthase 1) and interleukin receptors (interleukin-1 receptor type I and II and interleukin 2 receptor beta) were identified on these chromosomes. Fibroblast growth factor (FGF) has a prominent role in skeletal development and is known to bind to cell-surface heparan-sulfated proteoglycans (Ornitz *et al.* 2001). Insulin like growth factor I (IGFI) responds to growth hormone and exerts its effect on cellular function, specifically bone growth (Mohan *et al.* 1991). The IGFs cause hypertrophy of all cells involved in osteogenesis. Transforming growth factors (TGFs) are involved in wound healing and activation of signal transduction (Lawrence 1996). Bone morphogenic proteins (BMPs) are known as bone growth-regulatory factors and are osteoinductive (Mohan *et al.* 1991). Proteoglycans are major components of extracellular matrix of cartilage (Schwartz et al. 2002), whereas the interleukins are involved in immune modulation. Cartilage link protein stabilizes aggregates of aggrecan and hyaluronan, giving cartilage its tensile strength and elasticity (Watanabe *et al.* 1999).

In conclusion, we report that medical and surgical treatment for CCLR in the dog in the U.S. costs an estimated $1.32 billion. The CCLR disorder has a potential recessive mode of inheritance with 51% penetrance in a population of Newfoundlands and has an incidence rate of CCLR of 22%. The SNPs from a list of biological candidate genes that we examined were not associated with CCLR status but chromosomes 3, 10, and 23 have a large number of statistically significant microsatellite markers that are associated with CCLR status.

ADDENDUM and RECOMMENDATIONS

Completion of genome scan

Final genotyping data from the second genome scan were received from UC Davis in September, 2006. Data were reported on 33,557 genotypes (435 MSATs for 89/90 dogs) and are still being analyzed but final analysis and writing of the paper will be completed this year. However, preliminary qualitative data (data are still being checked for errors) were recorded and are listed in Table 4. The analyses performed will be the same as those reported on the genome scan results for 417 MSATs for 66/90 dogs (chi square compared to the randomization test).

Fine mapping

Based on analysis of the initial 417 MSATs for 66/90 dogs, 91 markers were considered statistically significant based on a nominal P value < 0.05. Six chromosomes had

four or markers that were statistically significant. Because this was a wide range of regions that need to be studied further, we attempted to narrow the range of regions even more. Analysis of the stratified population and comparison to the initial analysis revealed chromosomes 3, 10, and 23 to have a large number of microsatellite markers in common that were statistically associated with CCLR status. We subsequently performed fine mapping in these regions by genotyping additional gene markers in close proximity to and between the already genotyped microsatellite markers.

The list of positional candidate genes selected for analysis is listed in Table 3. We performed sequence analysis and SNP discovery in those genes. All of the genes listed in Table 3 were studied for SNPs but only the genes listed in Table 5 had SNPs identified. Association analyses of these SNPs and CCLR status (Table 5) has revealed some association to CCLR status. The associated genes will be studied further.

C. Presentations and Publications

a. Presentations

Wilke, V.L., Ruhe, A., Conzemius, M.G., Rothschild, M.F. Predisposition to rupture of the cranial cruciate ligament in the dog is genetically associated with chromosome 3. 33rd Annual Veterinary Orthopedic Society Conference, Keystone, Colorado, March 6, 2006.

Wilke, V.L., Ruhe, A., Kinghorn, B.P., Weiguo, C., Conzemius, M.C., Rothschild, M.F. Understanding the Genetics of Ruptured Cranial Cruciate Ligament in the Newfoundland Dog. 3rd International Symposium on Genetics of Animal Health, Ames, IA, July 13, 2005.

Wilke, V.L., Robinson, D., Evans, R.B., Kinghorn, B.P., Conzemius, M.C., Rothschild, M.F. Understanding the Genetics of Cranial Cruciate Ligament Disease in the Newfoundland Dog. 2nd International Conference on Advances in Canine and Feline Genomics, Utrecht, Netherlands, October 15, 2004.

Wilke, V.L., Kinghorn, B.P., Conzemius, M.G., Rothschild, M.F. Prediction of Inheritance for Cranial Cruciate Ligament Disease in the Newfoundland Dog. 2004 American College of Veterinary Surgeons Veterinary Symposium, Denver, CO, October 7, 2004.

Wilke, V.L., Kinghorn, B.P., Conzemius, M.G., Rothschild, M.F. Prediction for Inheritance for Cranial Cruciate Ligament Disease in the Newfoundland Dog. ASAS/ADSA Midwestern Meeting, Des Moines, IA, March 15, 2004.

Wilke, V.L., Kinghorn, B.P., Conzemius, M.G., Rothschild, M.F. Prediction for Inheritance for Cranial Cruciate Ligament Disease in the Newfoundland Dog. 31st Annual Veterinary Orthopedic Society Conference, Big Sky, Montana, February 24, 2004, p. 42.

b. Publications

Wilke, V.L., Conzemius, M.G., Kinghorn, B.P., Macrossan, P.E., Weiguo, C., Max F. Rothschild. (2006) Predicting the inheritance of rupture of the cranial cruciate ligament in the Newfoundland dog. J Am Vet Med Assoc. 228: 61-64.

Wilke, V.L., Evans, R.B., Robinson, D.A., Conzemius, M.G. (2005) Estimate of the annual economic impact of rupture of the cranial cruciate ligament in the dog in the United States. J Am Vet Med Assoc 227:1604-1607

Wilke, V.L., Conzemius, M.G., Rothschild, M.F. (2005) SNP association analyses in candidate genes for rupture of the cranial cruciate ligament in the dog. Anim Genet 36:519-520

Wilke, V.L. Analysis of genomic region(s) and gene(s) associated with cranial cruciate ligament rupture in the dog. PhD Dissertation, Iowa State University, Ames, IA, 2006.

D. Future Plans

a. Based on the results of this grant, the authors have submitted an AKC-CHF grant for March, 2006. The grant's focus is on additional extensive fine mapping of the chromosomal region(s) found to be significant from the results of this AKC-CHF grant's genome scan.

b. Since several chromosomal regions have been found to be associated with CCLR status based on preliminary analysis of 417 MSATs for 66/90 dogs, the authors felt that an additional genome scan was necessary to further narrow the chromosomal regions. Therefore, the authors have purchased 90 GeneChip Canine WGSA SNP arrays from Affymetrix. The canine SNP array contains approximately 26,000 SNPs to provide genome coverage with a marker approximately every 100,000 base pairs. The information gleamed from this study and analysis of the SNP arrays should sufficiently narrow the associated chromosomal region(s) associated with CCLR status and allow the identification of the causal mutation.

Table 1. Summary statistics for dogs used in genome scan.

	No. Males	No. Females	Avg Age (yrs)	Avg Age at CCLR (yrs)
CCLR unaffected	8	37	6.34	0
CCLR affected	16	29	7.15	4.7

Figure 1: Name and chromosomal position of microsatellites used in this study. The 417 MSATs provide markers at approximately 6.7 cM intervals.

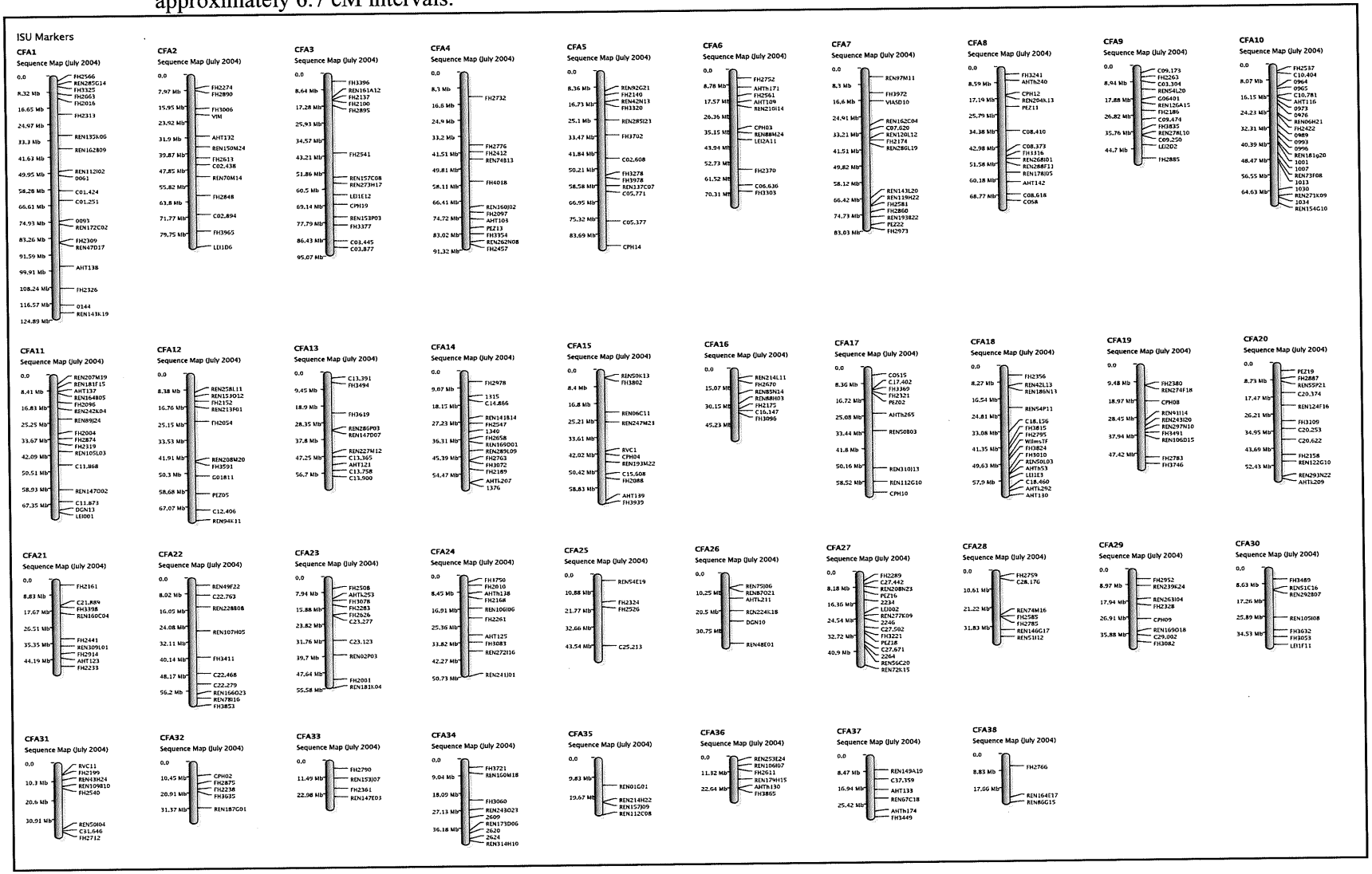

Table 2. List of MSATs significantly associated with CCLR status.

Total Population			
CFA	Location (Mb)	Marker	p-value
1	5.4	FH3325	0.0278
1	9.0	FH2663	0.0049
2	51.2	REN70M14	0.0051
2	60.8	FH2848	0.0322
3	4.5	FH3396	0.0171
3	41.7	FH2541	0.0062
3	64.0	LEI1E12	0.0129
3	68.5	***CPH19***	0.0001
3	75.0	REN153P03	0.0322
3	#	PEZ12	0.0442
4	#	GO7704	0.0133
4	81.0	PEZ13	0.0404
5	12.6	FH2140	0.0428
5	25.2	REN285I23	0.0323
5	32.9	***FH3702***	0.0003
5	42.0	C02.608	0.0163
6	10.0	AHTh171	0.0308
6	15.3	FH2561	0.0355
6	33.9	CPH03	0.0474
7	31.0	REN162C04	0.0220
7	31.5	C07.620	0.0398
8	45.0	C08.373	0.0292
8	48.3	FH3316	0.0144
8	70.0	COS8	0.0121
9	6.4	***FH2263***	0.0001
9	12.8	REN54L20	0.0024
9	34.4	FH3835	0.0179
10	4.0	C10.404	0.0011
10	12.0	964	0.0012
10	24.9	RENO6H21	0.0421
10	62.7	DTR10.5	0.0330
10	70.8	**REN154G10**	0.0012
11	23.3	***REN89J24***	0.0005
11	39.0	REN105L03	0.0054
11	71.2	DGN13	0.0040
12	44.5	FH3591	0.0130
13	22.9	***FH3619***	0.0003
13	32.8	***REN147D07***	0.0001
13	55.0	C13.758	0.0302
14	25.1	REN141B14	0.0036
14	36.0	REN169D01	0.0024
14	36.3	FH2658	0.0109

Stratified Population			
CFA	Location (Mb)	Marker	p-value
1	5.4	FH3325	0.0267
1	9.0	FH2663	0.0058
2	41.0	C02.466	0.0459
2	51.2	REN70M14	0.0060
3	4.5	FH3396	0.0197
3	41.7	FH2541	0.0067
3	64.0	LEI1E12	0.0218
3	87.4	C03.445	0.0030
3	#	FH2137	0.0113
4	#	GO7704	0.0135
5	32.9	***FH3702***	0.0004
6	15.3	FH2561	0.0342
8	21.3	PEZ11	0.0031
8	45.0	C08.373	0.0044
8	48.3	FH3316	0.0134
9	6.4	***FH2263***	0.0001
9	12.8	REN54L20	0.0030
9	34.4	FH3835	0.0165
10	24.9	RENO6H21	0.0372
10	39.4	REN181g20	0.0432
10	48.0	REN73F08	0.0178
10	62.7	DTR10.5	0.0347
10	#	1013	0.0103
10	#	1007	0.0189
11	71.2	DGN13	0.0035
12	44.5	FH3591	0.0105
12	74.1	REN94K11	0.0012
13	22.9	**FH3619**	0.0005
13	32.8	***REN147D07***	0.0001
14	25.1	REN141B14	0.0014
14	36.3	FH2658	0.0106
14	47.4	FH2763	0.0438
15	45.8	REN193M22	0.0035
15	66.7	FH3939	0.0423
16	20.0	REN85N14	0.0500
18	40.1	FH3815	0.0130
18	47.3	FH3824	0.0022
19	34.9	REN297N10	0.0154
19	52.8	FH3746	0.0031
20	9.5	REN55P21	0.0087
20	50.4	REN122G10	0.0332
20	82.8	FH2887	0.0499

Total Population			
CFA	Location (Mb)	Marker	p-value
14	47.4	FH2763	0.0428
15	45.8	REN193M22	0.0031
15	66.7	FH3939	0.0388
16	20.0	REN85N14	0.0165
16	32.0	C16.147	0.0095
16	45.4	***REN292n24***	0.0000
18	5.0	FH2356	0.0203
18	21.0	REN54P11	0.0328
18	40.1	FH3815	0.0127
18	46.5	WilmsTF	0.0309
18	47.3	FH3824	0.0023
18	56.7	LEI1E3	0.0064
19	34.0	REN243I20	0.0459
19	52.8	FH3746	0.0038
20	9.5	REN55P21	0.0101
20	46.7	FH2158	0.0152
20	50.4	REN122G10	0.0314
21	3.1	FH2312	0.0026
22	4.0	REN49F22	0.0341
22	39.7	FH3411	0.0273
22	47.0	C22.468	0.0210
23	#	REN113M13	0.0076
23	11.3	AHTk253	0.0304
23	12.1	FH3078	0.0186
23	19.2	FH2626	0.0346
24	3.6	***FH3750***	0.0003
24	33.8	FH3083	0.0045
24	50.4	REN241J01	0.0247
26	26.2	DGN10	0.0451
27	5.0	FH2289	0.0268
27	7.7	REN208N23	0.0331
27	10.3	PEZ16	0.0103
27	22.8	REN277K09	0.0184
27	35.8	C27.671	0.0398
27	47.6	REN72K15	0.0442
27	48.9	C27.436	0.0305
28	3.0	FH2759	0.0171
28	26.0	REN74M16	0.0391
28	26.5	FH2785	0.0026
30	6.2	**FH3489**	0.0015
30	11.0	REN292B07	0.0339
30	33.2	Fh3632	0.0081
31	9.7	**REN43H24**	0.0014
34	#	REN53L08	0.0091

Stratified Population			
CFA	Location (Mb)	Marker	p-value
21	3.1	FH2312	0.0025
22	39.7	FH3411	0.0265
23	11.3	AHTk253	0.0467
23	12.1	FH3078	0.0162
23	19.2	FH2626	0.0328
23	#	REN113M13	0.0083
24	3.6	***FH3750***	0.0002
24	33.8	FH3083	0.0034
24	39.8	FH2079	0.0331
24	50.4	REN241J01	0.0244
25	#	AHT140	0.0193
26	26.2	DGN10	0.0459
26	#	REN48E01	0.0235
27	5.0	FH2289	0.0355
27	#	2246	0.0277
28	26.5	FH2785	0.0301
30	6.2	**FH3489**	0.0005
30	12.0	REN51C16	0.0073
30	33.2	Fh3632	0.0082
31	6.6	RVC11	0.0258
31	9.7	REN43H24	0.0129
31	37.5	FH2712	0.0307
34	#	REN53L08	0.0099
35	22.8	REN157J09	0.0287
36	9.0	REN106107	0.0121
37	29.4	AHTh174	0.0206
37	32.1	FH3449	0.0020
38	22.7	REN164E17	0.0325
Unmapped		PEZ08	0.0218

Total Population			
CFA	Location (Mb)	Marker	p-value
34	22.0	FH3060	0.0186
34	30.5	2609	0.0468
36	9.0	REN106107	0.0119
36	22.0	AHTh130	0.0183
37	32.1	FH3449	0.0036

Bold: significant based on FDR
Italics: significant based on Bonferroni
Shaded: significant markers in common in both analyses
location not published

Table 3. Partial list of positional candidate genes based on proximity to statistically significant markers. These genes were selected based on their potential role in etiopathogenesis of CCLR.

CFA#	Gene
3	Fibroblast growth factor-binding protein
3	Insulin-like growth factor-1 receptor
3	Chondroitin sulfate proteoglycan 2 (Versican)
3	Proteoglycan link protein 2 precursor
3	Aggrecan core protein precursor (Aggrecan)
3	Carbohydrate (chondroitin) synthase 1
10	Fibroblast growth factor receptor substrate 2 (FRS2)
10	Transforming growth factor alpha (TGFalpha)
10	Bone morphogenetic protein 10 precursor (BMP10)
10	Interleukin 2 receptor, beta (IL2RB)
10	Interleukin-1 receptor type I
10	Interleukin-1 receptor type II
23	Transforming growth factor beta type II receptor (TGFB2R)

Table 4: Qualitative information regarding 435 MSATs from UC-Davis genome scan.

Number of markers	435,
Number monomorphic markers	8
Number polymorphic markers	427
Average number of alleles/marker	4
Range of alleles/marker	1-7
Chromosome coverage	1-38, not X or Y

Table 5. Pearson Chi-square probability results for positional candidate genes.

Gene	Status	1,1 genotype (no.) animals	1,2 genotype (no.) animals	2,2 genotype (no.) animals	Chi-square value, prob	1 allele (no.)	2 allele (no.)	Chi-square value, prob	Chrom
BMP-10	normal	11	22	11	7.27,	44	44	7.07, **0.01**	10
	affected	3	20	20	**0.03**	26	60		
FRS2	normal	0	9	33	3.50,	9	75	3.23, 0.07	10
	affected	0	3	39	0.06	3	81		
TGFalpha	normal	22	5	0	3.58,	49	5	1.50, 0.22	10
HpyCH4IV	affected	20	3	3	0.17	43	9		
TGFalpha HhaI	normal	14	17	10	0.36,	45	37	0.33, 0.56	10
digest	affected	14	16	7	0.84	44	30		
Versican MwoI	normal	23	10	11	0.92,	56	32	0.58, 0.75	3
digest	affected	24	6	9	0.82	54	24		
Versican NlaIII	normal	20	18	3	2.32,	58	24	2.05, 0.36	3
digest	affected	27	15	1	0.51	69	17		
Versican BsaWI	normal	3	27	9	3.38,	33	45	0.007, 0.997	3
digest	affected	7	21	14	0.34	35	49		
IL2RB BsaI	normal	1	12	19	1.89,	14	50	1.74, 0.42	10
digest	affected	3	17	16	0.596	23	49		
IL2RB Bsu361	normal	23	12	1	0.77,	58	14	0.51, 0.78	10
digest	affected	20	16	1	0.86	56	18		
IL2RB HaeIII	normal	14	18	9	7.23,	46	36	8.37, **0.02**	10
digest	affected	6	14	19	0.07	26	52		
Aggrecan	normal	33	10	0	2.16,	76	10	1.95, 0.38	3
SNP236	affected	39	5	0	0.34	83	5		
Aggrecan	normal	22	18	3	7.8,	62	24	7.57, **0.02**	3
SNP432	affected	35	8	1	**0.05**	78	10		
TgfB2R	normal	26	15	2	6.29,	67	19	6.47, **0.04**	23
SNP358	affected	17	15	9	0.1	49	33		
TgfB2R	normal	2	15	26	4.74,	19	67	4.96, 0.08	23
SNP456	affected	7	17	17	0.19	31	51		
TgfB2R	normal	31	11	1	5.06,	73	13	3.57, 0.17	23
SNP305	affected	37	3	1	0.17	77	5		

p values in bold are significant

II. FINANCIAL SECTION

Date	Expenditure	Amount
4/01/06	Genome Scan	$15,340.50
4/01/06	Research Assistant Salary	$29,005.77
4/01/06	Fringe Benefits 34.5%	$9,624.48
4/01/06	Candidate gene lab supplies: PCR reagents, primers, DNA sequencing, gel electrophoresis	$33,365.86
4/01/06	Mailing DNA for genome scans	$55.41
4/01/06	Travel to collect DNA, meeting to present data	$1,859.98
4/01/06	Indirect costs	$7,140
4/01/06	Total	$96,392

Budget explanation

1. Budget adjustment for fringe benefits: Due to a university policy change that increased the percentage of fringe benefits that are paid based on an individual's salary, the amount listed in fringe benefits is more than was initially allocated in the original budget.
2. Final genotyping subcontract fees were less than anticipated due to contractual changes made between ISU and UC-Davis for their services.
3. Travel expenses – were informed that these are not generally allowed but an ok was given after last report. The investigators regret this oversight.

III. REFERENCES

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