Investigation of Intestinal Atresia in a Jersey Sire Family.

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Abstract

Intestinal atresia is a congenital defect resulting in calf mortality within a few days of birth. This study focuses on two half-sibling Jersey sires who were identified as having a high proportion of their progeny exhibiting atresia from a longitudinal study of 39 dairy herds over an 8 year period. The objective of this study was to investigate the genetic cause of intestinal atresia within this family. Phased high-density genotypes of the two half-sibling sires were used to identify if common haplotypes were inherited by all affected animals. Whether or not the paternal haplotype that was inherited by the affected animal at a given locus deviated from the expectation was tested. A total of 16 SNPs were significantly overrepresented, 14 located in one region on chromosome 14 and the remaining 2 located in one region on chromosome 26.

Introduction

Intestinal atresia is a congenital defect, found in a wide variety of species, which results in complete occlusion of the intestinal lumen, occurring in the jejunum, ileum, colon and/or anus (Ducharme et al., 1988). In calves, the blockage inhibits the normal movement of gut contents resulting in failure to pass meconium and faeces, inappetence, abdominal distension, and usually results in death due to autointoxication within a few days of birth (Syed and Shanks, 1992). The inheritance of atresia in cattle is still not completely understood (Constable and Morin, 2000). As part of a larger study, active surveillance of 39 dairy herds over 8 years was used to assess the incidence of intestinal atresia in dairy herds in Ireland. Of interest, two Jersey sires had a high proportion of their progeny exhibiting intestinal atresia; further investigation revealed that these two sires shared a common sire. The objective of this study was to investigate the genetic cause of intestinal atresia within this family.

Materials & Methods

Data collection. A total of 39 dairy herds, including 3 research dairy herds from Teagasc, participated in a longitudinal study over 8 years to assess factors associated with intestinal atresia in dairy herds in Ireland. As part of the study any calf that died within the first 7 days of life was transported to Teagasc Moorepark and subjected to a full post-mortem by a veterinary surgeon. Following post-mortem, a total of 197 cases of intestinal atresia were identified. Additional data on all calves born on each farm during study participation was provided by the Irish Cattle Breeding Federation (ICBF) and consisted of calf ID, date of birth, sex, dam breed, dam ID, dam parity, sire breed, sire ID and birth type (single or twin).

Identification of Sire family. During data exploration, generalized linear models were used in SAS version 9.4 (SAS Institute Inc., Cary, NC) to quantify the association of risk factors associated with atresia. Initial results indicated sire was a significant factor in intestinal atresia among Jersey sired calves. Further investigation, limiting records to sires that had greater than 50 progeny born in at least 5 different herds, indicated an over-representation of affected

progeny for a number of sires. The two most significant sires, which are paternal half-siblings, had 2.5 and 4.1 percent of their progeny exhibiting intestinal atresia

DNA extraction and genotyping. Muscle tissue (> 1g) was collected post-mortem from 28 atresia cases from the two prominent sires, submerged in RNAlater solution (Ambion), left overnight at 4 °C and stored at -20 °C. For DNA extraction approximately 100 mg of tissue was minced in 1.5 mL of digestion buffer (1 M Tris-HCL pH 8.0, 0.5 M EDTA, 10% SDS, 500 µg/mL Proteinase K) and incubated for 4 h at 55 °C with shaking. The sample was then extracted twice with an equal volume of phenol:chloroform and once with an equal volume of chloroform. The DNA was precipitated with 0.1 volumes of 3 M sodium acetate pH 5.2 and 1 volume of isopropanol, washed with 70% ethanol, air dried and resuspended in 100 µL of H_2O. DNA was cleaned using the Genomic DNA Clean and Concentrator kit (Zymo Research) and quality assessed by agarose gel electrophoresis. DNA was genotyped with the Illumina 777K BovineHD beadchip (Illumina Inc, San Diego, CA). Illumina Bovine50k beadchip genotypes from 640 unaffected progeny of either sire were also provided by ICBF as healthy controls and imputed to HD using FImpute (Sargolzaei et al., 2014).

Linkage analysis with Jersey family. High-density genotypes were available on the two sires along with 28 affected progeny; the genotypes were phased using FImpute (Sargolzaei et al., 2014). The phased genotypes of the two sires were used to determine which paternal haplotypes were inherited. Only haplotypes where both sires inherited the same paternal allele and which were heterozygous in both sires were retained. Following edits 46,177 SNPs remained. Whether or not a paternal haplotype that was inherited by the affected progeny at a given locus deviated from the expectation of equal representation was tested. The critical t-statistic was calculated using equation 1.

$$\frac{P-0.5}{\sqrt{\frac{P(1-P)}{n}+0.001}} \quad (1)$$

In equation 1, P is the proportion of progeny with the paternal haplotype inherited from the grandsire and n is the number of grand-progeny. The level of significance was determined using a one-tailed t-test with n-1 degrees of freedom; the mean P-value of a 10-SNP sliding window was used to avoid sporadic single SNP significance.

Bioinformatics. SNPs in close proximity (1Mb) were combined into a single QTL. Genes within 50kb span of the start and end of the QTL were identified using Ensembl 94 on the UMD3.1 bovine genome assembly implemented in BiomaRt in R.

Results

Of the 730,540 autosomal SNPs available from the phased genotypes, 46,177 SNPs remained after confirming the same paternal haplotype was inherited by both sires as well as ensuring both sires were heterozygous. A total of 16 SNPs were significant, 14 located in one region on chromosome 14 and the remaining 2 located in one region on chromosome 26 (Table 1 and Figure 1). The first region between 14: 24,769,617 and 14: 24,939,286 contained 41 SNPs, 14 of which were part of the binomial analysis; this region contained six genes (Table 1). A total of 27 out of 28 affected animals had the same haplotype inherited for 41 of these SNPs with the remaining animal differing at 8 SNPs. The second region on chromosome 26 between 18,028,791 and 18,029,994 contained only the two SNPs that were part of the binomial analysis;

in total 26 of the 28 animals had the same haplotype inherited from the paternal-grandsire. There was one gene located in this region, PIK3AP1.

Table 1. Significant QTLs associated with intestinal atresia in 28 affected Jersey cross Holstein animals originated from a common paternal-grandsire.

Chromosome	Start position	End position	Number of SNPs	P-value	Genes
14	24,769,617	24,939,286	14	1.79E-15	TMEM68, TGS1, LYN, RPS20, U1, MOS
26	17890705	17891907	2	3.04E-08	PIK3AP1

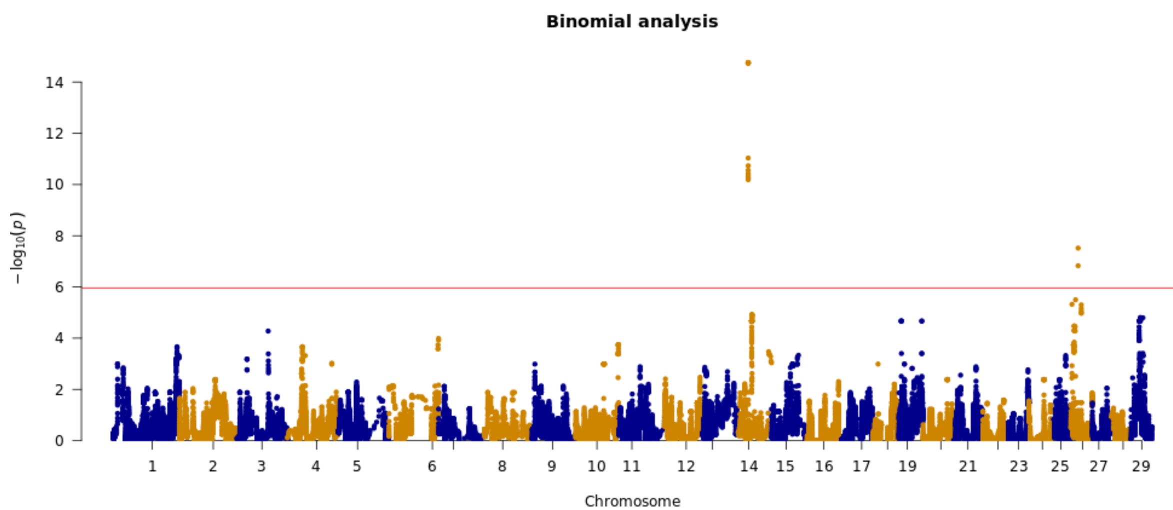

Figure 1. Binomial analysis of 28 affected Jersey cross Holstein animals originated from a common paternal-grandsire.

Discussion

There was a total of 197 animals identified through post-mortem as part of a longitudinal study across 39 herds over 8 years as having intestinal atresia; of these, 28 affected animals originated from two paternal half-sibling Jersey sires. All animals within the study originated from dairy herds with the majority Holstein-Friesian or Holstein-Friesian crosses. Although Holstein-Friesian animals are reported to have a greater risk of atresia compared to other cattle breeds (Constable et al., 1997), our data suggests that the genetic cause of intestinal atresia in these animals originated from the Jersey parent. The overall incidence of atresia in the current dataset was 0.35% whereas the two sires of interest in this study had an incidence among their progeny of 2.5% and 4.1%.

Unfortunately, the genotype of the paternal grandsire of the affected animals was unavailable; however, HD genotypes of both sires were available. Using both sire's genotypes, the haplotype of the paternal grandsire that was inherited by the affected animals was inferred using phased haplotypes. Due to the number of affected animals, traditional GWAS might not be able to provide strong evidence of regions associated with atresia. The alternative method used in the current study aimed to indicate any region that may require further investigation. One regions on chromosome 14 was shown to have the paternal grandsire's haplotype over-represented. In this region, all but one animal had inherited the same 169 kb region. Additionally, this region

was also examined in the healthy controls; in the unaffected animals the region was also significantly over represented with 450 out of 680 animals also inheriting the same haplotype as the affected animals. None of the genes were obvious candidate genes, although mutations in these genes have been associated with multiple health and disease traits.

Conclusion

Intestinal atresia normally results in the death of the calf on-farm. Although it has been reported that bovine intestinal atresia has a genetic aetiology (Syed and Shanks, 1992) no causal mutation has been identified. In the current study we identified a sire-family that has an over-represented incidence of atresia. Although there is strong evidence of association with a region on chromosome 14, the genes within this region have not been previously implicated in intestinal atresia. There are possible benefits to fine mapping this region in affected and unaffected animals to narrow the QTL containing any causal mutation.

References

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