Immunogenetics (2012) 64:201–208
DOI 10.1007/s00251-011-0573-1

ORIGINAL PAPER

The same ELA class II risk factors confer equine insect bite hypersensitivity in two distinct populations

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Received: 8 June 2011 / Accepted: 9 September 2011 / Published online: 23 September 2011

Abstract Insect bite hypersensitivity (IBH) is a chronic allergic dermatitis common in horses. Affected horses mainly react against antigens present in the saliva from the biting midges, *Culicoides* ssp, and occasionally black flies, *Simulium* ssp. Because of this insect dependency, the disease is clearly seasonal and prevalence varies between geographical locations. For two distinct horse breeds, we genotyped four microsatellite markers positioned within the MHC class II region and sequenced the highly polymorphic exons two from *DRA* and *DRB3*, respectively. Initially, 94 IBH-affected and 93 unaffected Swedish born Icelandic horses were tested for genetic association. These horses had previously been genotyped on the Illumina Equine SNP50 BeadChip, which made it possible to ensure that our study did not suffer from the effects of stratification. The second population consisted of 106 unaffected and 80 IBH-affected Exmoor ponies. We show that variants in the MHC class II region are associated with disease susceptibility ($p_{raw}= 2.34\times10^{-5}$), with the same allele (*COR112:*274) associated in two separate populations. In addition, we combined microsatellite and sequencing data in order to investigate the pattern of homozygosity and show that homozygosity across the entire MHC class II region is associated with a higher risk of developing IBH ($p=0.0013$). To our knowledge this is the first time in any atopic dermatitis suffering species, including man, where the same risk allele has been identified in two distinct populations.

Keywords Insect bite hypersensitivity · Summer eczema · ELA · MHC · Horse

Electronic supplementary material The online version of this article (doi:10.1007/s00251-011-0573-1) contains supplementary material, which is available to authorized users.

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Introduction

Insect bite hypersensitivity (IBH; also known as "sweet itch") is a chronic allergic dermatitis that is common in horses. A veterinary handbook from the Tang Dynasty in China contains the first known record of the disease (Li and Ji 1959). Since then, numerous reports describing the disease in a wide range of horse breeds and across most continents have been published (Henry and Bory 1937; Datta 1939; McCaig 1973). The association to biting midges of the *Culicoides* species was established in the 1950s (Riek 1953; Nakamura et al. 1956; Ishihara 1957). Horses with IBH mainly react against protein antigens present in the saliva of biting midges, *Culicoides* ssp, and occasionally black flies, *Simulium* ssp (Mellor and McCraig 1974; Hellberg et al. 2006; Hellberg et al. 2009). Because of this dependency on insects, the disease is clearly seasonal and prevalence varies between geographical locations (Riek 1953; Brostrom et al. 1987).

The acute stage of the disease involves an IgE-mediated type I hypersensitivity reaction (Wagner et al. 2006). However, other immunological reactions, such as delayed type IV hypersensitivity, cannot be excluded during the chronic stages of the disease (Fadok and Greiner 1990). Skin sections reveal infiltration of eosinophils and mast cells as well as increased numbers of IgE-bearing cells, an increased total number of T cells and expression of T helper cells (Th2) (Van der Haegen et al. 2001; Heimann et al. 2011). Clinical signs include intensely pruritic lesions, urticaria, oedema and papules (Baker and Quinn 1978; Halldordsottir and Larsen 1991). These are usually most prominent along the dorsal midline, in particular the tail base, mane, head, withers and rump. Horses suffering from IBH that have been left unattended can easily be recognized by the almost complete loss of hair from the mane and tail and continuous scratching may cause open wounds. Secondary symptoms include lichenification, crusts and scaling.

In Sweden, the Icelandic Horse is one of the breeds most frequently affected by IBH. In this breed, the prevalence among horses born in Sweden is approximately 8%, whilst it is as high as 26–35% for horses born in Iceland and imported to Sweden (Brostrom et al. 1987; Bjornsdottir et al. 2006). *Culicoides* ssp are not known to exist in Iceland, and the difference in disease prevalence may be due to native horses not being exposed to bites in early life (Marti et al. 2008). This conclusion is supported by the fact that imported horses often experience a more severe form of IBH than their Swedish-born counterparts (Brostrom et al. 1987).

The present study examines both the Icelandic Horse and a second breed, the Exmoor Pony. IBH is also a significant problem in terms of numbers affected and disease severity in this old, native British pony (personal communications, Exmoor Pony Society). The Icelandic horses and Exmoor ponies are clearly two separate populations. This has been demonstrated in several phylogenetic studies where microsatellite markers have been utilized to calculate genetic distances (Juras et al. 2003; Bömcke et al. 2011).

IBH is partly under genetic control and the heritability in Icelandic horses has been estimated at 0.3 (Eriksson et al. 2008). Two studies have shown serologically that certain horse major histocompatibility complexes (MHC), i.e. equine leukocyte antigen (ELA) class II specificities, are linked to IBH susceptibility. In an examination of 303 exported Icelandic horses, for 25 different leukocyte antigens specificities, the allele frequency for Be8 was significantly different in affected horses versus unaffected controls (Halldorsdottir et al. 1991). However, the relatedness of the horses was not described in that study and potential stratification may have been missed. In another study, the ELA class II specificity W23 was shown to segregate with IBH susceptibility in a family-based study consisting 24 typed Swiss warm-blooded horses (Marti et al. 1992; Lazary et al. 1994).

In humans, human leukocyte antigen (HLA) class II plays a role in the pathogenesis of several allergic phenotypes, for example, pollen, peanut and cow milk allergies as well as gluten intolerance (Zwollo et al. 1989; Petronzelli et al. 1997; Camponeschi et al. 1997; Howell et al. 1998). Certain HLA variants are also associated with an increased risk of human atopic dermatitis, a disease that partially resembles equine IBH (Saeki et al. 1995; Kiyohara et al. 2008).

The ELA class II region is positioned on horse chromosome 20q and harbours three *DQA* loci, two *DQB* loci and three *DRB* loci (Tseng et al. 2010). Only one copy of the *DRA* locus is present in the horse genome; however, contrary to many other species, this gene is polymorphic in the domestic horse, with at least four different alleles identified (Bailey 1994; Albright-Fraser et al. 1996; Brown et al. 2004). In the present study, the first to use DNA genotyping as a substitute for ELA serology, we show that the ELA class II region in horses is associated with IBH susceptibility. In addition, we show that the same genetic risk factors are present in two distinct breeds.

Materials and methods

Animal resources

We initially used 94 IBH-affected and 93 unaffected Icelandic horses to test for associations between ELA class II markers and disease status. All horses were born in Sweden except for one (an unaffected horse exported from

Iceland) and certified by the Icelandic Horse breed registry. The horses consisted of paternal half-siblings sired by 42 different stallions. The number of offspring per stallion ranged from 1 to 24 (median 3, average 4.45). The stallions were represented by an equal number of affected and unaffected offspring in order to avoid stratification but were not themselves included. We were unable to find matching controls for five horses and so we compensated by adding unrelated controls. All horses were unrelated on the maternal side for at least two generations. None of the controls were from areas with a known low *Culicoides* ssp pressure such as coastlines. The average age of affected horses was 11.5 years (median 11, range 4–21), whilst for control horses this was 11.0 (median 10, range 4–27).

We also investigated a second population consisting of 106 unaffected and 80 IBH-affected Exmoor ponies. These 24 stallions, 51 geldings and 111 mares were all ponies living in the UK and registered with the Exmoor Pony Society. Family relationships were not considered in the selection of these ponies. The average age of the affected ponies was 10.6 years (median 8, range 1–42) and of the controls was 11.5 years (median 10, range 1–37). The controls were not selected based on any parameter other than disease status.

The study was approved by the Ethics Committee for Animal Experiments in Uppsala, Sweden, for the Icelandic horses and the Animal Health Trust Clinical Research Ethics Committee in Newmarket, UK, for the Exmoor ponies. Signed consent was obtained from all horse owners.

Phenotype assessment

Icelandic horses were scored as affected or unaffected based on a questionnaire sent to owners (Eriksson et al. 2008). Inclusion criteria for an affected horse were defined as clearly seasonal signs of IBH for a minimum of the two preceding grazing seasons before the questionnaire was filled out and returned. Only horses with no history of pruritic skin problems were selected as unaffected controls. The Exmoor ponies were classed as either affected or unaffected based on written owner reports of significant IBH signs at any time during the lifetime of the pony.

Genotyping and sequencing

In a separate study, all Icelandic horses were genotyped on the Illumina Equine SNP50 BeadChip (unpublished data). These data were used to assess the population for stratification. A paucity of SNPs covering the MHC II region (average distance between SNPs is 76 kb) led us to more densely cover this area with both microsatellite markers and direct sequencing of two exons.

Six microsatellite markers were interrogated (*COR112*, *COR113*, *UM011*, *COR114*, *AHT004* and *Autosomal1*; Online resource 1). *AHT004* and *Autosomal1* were only used for LD calculations and had nine and two alleles, respectively. The latter was developed from the horse reference genome in the same way as described earlier (Andersson et al. 2008). An M13 tail was added to the forward primers to facilitate labelling with FAM or VIC. PCR reactions were carried out as described previously (Andersson et al. 2008). Amplified fragments were multiplexed and separated using a MegaBACE™ 1000 instrument (GE Healthcare, Sweden) according to the manufacturer's recommendations, prior to analysis with Genetic Profiler version 2.2 (GE Healthcare, Sweden).

PCR-amplified exon 2 from each of the MHC class II genes *DRA* and *DRB3* were sequenced by traditional Sanger methodology. Primer design and reactions were carried out as described previously (Andersson et al. 2008). Results were analysed in CodonCode Aligner version 3.0.1 (CodonCode Corporation, USA). Because of the high density of SNPs, we could easily designate *DRB3* and *DRA* alleles manually. All genotypes were carefully checked for inheritance error, which would indicate the existence of dropouts (alleles that do not amplify), mistakes in phasing or non-specific amplification. All sequences have been deposited to GenBank; accession numbers can be found in Online resources 2 and 3. The *DRA* alleles were named according to previously established nomenclature (Janova et al. 2009). This was not possible for *DRB3* alleles due to the lack of distinction between different loci and because the polymorphic 5′ end of exon 2 is missing in most published sequences. However, a BLAST search (www.ncbi.nlm.nih.gov) was performed in order to find similar *DRB3* alleles and we refer to the names established previously (Diaz et al. 2001). A schematic representation of the genes and markers in horse ELA class II region is available in Online resource 4.

Data analysis

PLINK (Purcell et al. 2007) was used to examine the SNP data for genomic inflation factor. For microsatellites, the average number of alleles and private allelic richness were calculated in HP-RARE v1.0 (Kalinowski 2005). Pair-wise linkage disequilibrium (LD) was calculated using the metric $x^{2'}$, a standardized chi-square statistic suitable for use with multi-allelic markers (Zhao et al. 2005) where, for biallelic markers, $x^{2'}=r^2$. Two-marker haplotype frequency estimations and LD calculations were performed as reported previously (Meadows et al. 2008). Non-syntenic LD was estimated from the Icelandic Horse population using three microsatellite markers located on chromosome 6, 20 and 24, respectively (Online resources 1). R (Team 2008) was used for most statistical calculations. Alleles with frequencies less than 0.10 and 0.075 for microsatellites and exon 2 sequences (*DRA* and

DRB3), respectively, were pooled. Each marker was then analysed in a $2 \times N$ contingency table and Fisher exact tests were used to generate *p*-values and odds ratios for differences in allele frequencies in a 2×2 table (one allele vs. all other). Cochran–Mantel–Haenszel tests for differences in allele frequencies were performed in the SAS package (SAS 2010). Bonferroni correction was used to adjust for multiple testing in the initial scan for association; this was over-conservative as the tests were not independent. FastPHASE was used for phasing combined microsatellite data and exon 2 sequences (Scheet and Stephens 2006).

Results

Test for stratification

Approximately 44,200 SNPs were used to evaluate the Icelandic horse sample set for potential population stratification. The result from an allelic case/control test was compared to the expected distribution of *p*-values. The genomic inflation factor was 1.00 (mean 0.90), which implies a low risk of false positives due to population stratification. No genomewide data were available for the Exmoor ponies.

Genotyping of MHC class II microsatellites

In total, we genotyped four microsatellite markers, positioned within the MHC class II region, in 185 Icelandic horses (Table 1) and 186 Exmoor ponies (Table 2). The average number of alleles (A_N) was higher in the Icelandic horses (A_N=9.50) compared to the Exmoor ponies (A_N=7.75). The Icelandic horses also had more private alleles, measured by private allele richness (pA_R=2.25), compared to the Exmoor ponies (pA_R=0.50). For the Icelandic horses, the degree of LD between markers ranged from 0.31 to 0.59 (Online resource 5). This was considerably higher than the non-syntenic LD, which was estimated at 0.067.

Since the Icelandic horses did not appear to be stratified, we first tested these for association between genotypes and IBH status. Marker *COR112* was clearly associated with IBH status (p_{raw}=0.001, p_{corr}=0.006). Allele 274 was enriched in cases (*f*=0.22) compared to controls (*f*=0.06) with an odds ratio of 4.19 (95% CI=2.00–9.44, $p=2.34\times10^{-5}$). None of the remaining microsatellite markers were significantly associated with IBH when analysed with the appropriate degrees of freedom (Table 1). However, when performing a Fisher exact test, analysing only the allele with the highest frequency difference between IBH affected horses and unaffected controls against all other alleles, the two microsatellite markers closest to *COR112* were also associated, p_{raw}=0.037 for *COR113* (allele 282) and p_{raw}=0.033 for *UM011* (allele 180). This association did not hold under multiple testing and may have been driven by LD to *COR112* (*COR113–COR112*, $x^{2'}$=0.34; *UM011–COR112*, $x^{2'}$=0.31).

All four MHC class II microsatellites were subsequently genotyped in the Exmoor ponies (Table 2). In this breed, *COR112* was the only marker in which the same allele (274) was associated with disease as in the Icelandic Horse. The frequency of allele 274 in the affected horses was 0.60 compared to 0.50 in healthy ponies (p_{raw}=0.043). Further, *UM011* allele 186 was associated with disease when tested independently in the Exmoor Pony. In Icelandic horses, the allele frequency of *UM011* allele 186 was 0.26 in both affected horses and controls (data not shown).

Sequencing of *DRA* and *DRB3* genes

The highly polymorphic exon 2 was sequenced in two genes within the Equine MHC class II region. The *DRA* gene was selected because, unlike in many species, it shows variation in the horse. *DRA* exists as a single copy, so sequencing this particular gene reduced the possibility of

Table 1 Association results between ELA markers and IBH in Icelandic horses

					IBH		Healthy				
Marker	Position (bp)	*p*-value ($2\times N$)	df	A_d	Number, *n*	*p* (A_d)	Number, *n*	*p* (A_d)	*p*-values (2×2)	OR	95% OR
DRA	32690939	0.025	2	DRA*0201	94	0.17	91	0.08	0.012	2.28	1.15–4.71
COR112	33282436	0.001	5	274	86	0.22	87	0.06	2.34×10^{-5}	4.19	2.00–9.44
DRB3	33364480	0.008	3	1.2	91	0.13	91	0.04	0.004	3.60	1.45–10.2
COR113	33480825	0.271	3	282	89	0.14	86	0.07	0.037	2.17	1.01–4.93
UM011	33510120	0.151	4	180	90	0.13	86	0.06	0.033	2.25	1.02–5.26
COR114	33516304	0.354	4	259	89	0.13	89	0.08	0.229	1.61	0.77–3.45

df degrees of freedom, A_d allele most strongly associated with disease, *p* (A_d) frequency of A_d, *OR* odds ratios

Table 2 Association results between ELA markers and IBH in Exmoor ponies

					IBH		Healthy				
Marker	Position (bp)	*p*-value (2×N)	df	A_d	Number, *n*	*p* (A_d)	Number, *n*	*p* (A_d)	*p*-values (2×2)	OR	95% OR
DRA	32690939	0.332	1	DRA*0201	79	0.23	99	0.28	0.332	0.78	0.46–1.29
COR112	33282436	0.111	2	274	77	0.60	99	0.50	0.043[a]	1.48	0.97–2.27
DRB3	33364480	0.843	4	1.2	80	0.19	104	0.15	0.886	1.07	0.58–1.95
COR113	33480825	0.644	1	284	79	0.52	103	0.50	0.673	1.10	0.71–1.70
UM011	33510120	0.116	2	186	79	0.46	101	0.35	0.039	1.58	1.01–2.48
COR114	33516304	0.322	3	261	80	0.46	103	0.39	0.201	1.33	0.86–2.06

df degrees of freedom, A_d allele most associated with disease, *p* (A_d) frequency of A_d, *OR* odds ratios

[a] One-tailed

paralog gene amplification. The *DRB3* gene was selected as it is one of the most polymorphic genes in the genome and can be specifically amplified (personal communications). However, since the *DRB* gene exists in three copies, the alleles inherited from each stallion were carefully checked for Mendelian errors.

In the *DRB3* gene, we found 30 SNPs and two indels, which after phasing constitute eight different alleles (Table 3; Online resource 2). Our alleles are defined by a combination of coding and non-coding sequences. One indel (g.33364687insT) is a thymine insertion positioned approximately 100 bp upstream of the exon 2 start site. The other indel (g.33364942delCGGCGGAGCGCCGAG) resides within the coding region and is a 15-bp deletion. Since this indel corresponds to exactly five amino acids, the reading frame of the protein remains intact. Only five of the eight alleles were found in the Exmoor ponies, with the most common allele present at a frequency of 0.66. In summary, 19 SNPs introduced an amino acid change and produced in total five different polypeptide variants (Online resource 6). Apart from allele 5, these alleles have been described previously (Fraser and Bailey 1996). The first amino acids are lacking in the published *DRB* sequences; however, we provide additional novel sequence 5′ of the coding region, thus extending previously published sequences. Alleles 1, 1.2 and 2 further distinguish the allele designated as ELA-DRB*11. It is the very start of the sequences, not included in published *DRB* sequences, which differentiates allele 2 from alleles 1 and 1.2. The coding sequences of alleles 3, 3.1 and 3.2 are identical and correspond to ELA-DRB*6, while allele 4 corresponds to ELA-DRB*9.

Table 3 *DRB3* alleles defined by a combination of coding and non-coding sequences

Allele	Freq (Icelandic's)	Freq (Exmoor's)	g.33364593C>T	g.33364687insT	g.33364692A>C	g.33364694T>A	g.33364763T>C	g.33364791A>T	H35R	F42S	S47F	Q51E	L54R	H57D	Y59L	L73V	R84del	P85R	D86S	A87del	E88del	E103K	V107Y	G113A	Q121P
1	0.440	0.661	C		C	T	T	A	H	F	S	Q	L	H	L	L	R	P	D	A	E	E	V	G	Q
1.2	0.082	0.115	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	0.016	0.000	.		.	.	.	.	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	0.206	0.000	T		A	.	C	T	.	.	.	.	.	.	.	.	.	R	S	.	.	.	.	.	P
3.1	0.003	0.000	T		A	.	C	.	.	.	.	.	.	.	.	.	.	R	S	.	.	.	.	.	P
3.2	0.003	0.083	T		A	A	C	.	.	.	.	.	.	.	.	.	.	R	S	.	.	.	.	.	P
4	0.187	0.083	.		A	.	.	.	.	S	F	E	R	D	Y	V	.	R	S	.	.	K	Y	A	.
5	0.063	0.057	.		A	.	.	.	.	S	F	E	R	D	Y	V						K	Y	.	.

The sequence for allele 1 is shown as reference. Nucleotides and amino acids that are identical to the reference are depicted with a dot. Black boxes indicate an indel. The grey area indicates variation located within the coding sequence

The allele frequency of allele 1.2 was three times higher in affected Icelandic horses compared to controls and was significantly associated with IBH status (p_{raw}=0.004, OR=3.60). Interestingly, we also found that allele 1 was possibly protective (p_{raw}=0.015) in Icelandic horses (data not shown). It was present at a frequency of 0.51 in healthy horses but only 0.37 in affected horses, giving an odds ratio of 0.58 (95% CI=0.38–0.91). Hence, the associations that these alleles display are probably not due to their phenotypic effect but rather due to LD with causal variants.

In the *DRA* gene, we detected three SNPs, all positioned in the coding region. Two of these were non-synonymous (Online resource 3). The SNPs define three alleles, which correspond to three different polypeptide chains (Online resource 7). At the protein level, the alleles were identical to ELA-Eqca-DRA*0201 (Albright et al. 1991), ELA-Eqca-DRA*0301 (Albright-Fraser et al. 1996) and ELA-DRA*Hld105 (accession ACN66628). Only two of these were found in the Exmoor Pony. We saw no association between IBH status and *DRA* alleles in Icelandic horses or Exmoor ponies.

The degree of LD between microsatellites, *DRB3* and *DRA* alleles was estimated for Icelandic horses and Exmoor ponies separately (Online resources 5). The average degree of LD was 0.37 in the Icelandic horses and 0.49 in the Exmoor ponies. In an effort to find extended haplotypes associated with disease, fastPHASE was used to phase both microsatellite and exon 2 sequences. Unfortunately, too few common haplotypes left the experiment underpowered and so the potential of homozygosity mapping was explored.

Homozygosity

We combined microsatellite and sequencing data in order to investigate the pattern of homozygosity throughout the MHC class II region. In Icelandic horses, 0.13 of affected individuals were homozygous, compared to only 0.04 of the control horses. Homozygous horses were even more prevalent in the Exmoor Pony population, with frequencies of 0.30 and 0.15, respectively. A Cochran–Mantel–Haenszel test, which combines data from both breeds, showed that homozygosity in the region confers a higher risk of developing a disease, with an odds ratio of 2.67 (p=0.0013, 95% CI=1.22–4.66).

Discussion

In this study, we showed that genetic variants in the MHC class II gene clusters are associated with IBH susceptibility. We tested over 350 horses and found the same allele (274 from *COR112*) associated with disease in two separate populations. To our knowledge, this is the first time in any atopic dermatitis-suffering species, including man, where the same risk allele has been identified in two distinct populations.

This is also the first equine study where direct DNA genotyping, as an alternative to serology, has been used in an association study to IBH. In a previous investigation of the horse MHC class II region, Tseng et al. (2010) showed that multiple microsatellite haplotypes could be associated with a single serotype. DNA genotyping therefore provides better resolution than earlier methodologies. In this particular study, phasing all markers (microsatellites, *DRB3* and *DRA* alleles) dramatically decreased the power to detect associations. We used the extent of LD in our two populations to examine why we did not observe common long haplotypes associated with allele 274 from *COR112*. For example, in the Icelandic horses, the marker *DRB3* is in highest LD with *COR112*, with LD estimated at 0.37 at a genomic distance of 82 kb. This LD estimate is similar to estimates in Icelandic horses published previously where LD values, as measured in r^2, between SNPs were found to be just below 0.20 for markers positioned approximately 100 kb apart (Wade et al. 2009). This low level of LD indicates that recombination has broken the risk haplotype between these markers. Further genotyping of densely spaced markers, or direct sequencing, in a larger horse material will be required to build informative haplotypes.

Candidate gene studies may suffer from undetected stratification which increases the risk of false-positive associations (Cardon and Palmer 2003). However, much effort was taken to avoid stratification in the Icelandic horses used. First, we inspected the pedigree of each horse carefully and selected matched paternal half-siblings that were unrelated (or distantly related) on the maternal side. Second, all horses were genotyped using the Illumina EquineSNP50 BeadChip. This provided an excellent opportunity to test for stratification because genomewide data provide a more reliable estimate of stratification compared to a small number of neutral markers. The genomes of these horses have thus been well characterized and the relationship between cases and controls carefully analysed. Because of the vast amount of genotypic data available on the horses in this study, there is a low risk of false-positives due to population substructure.

We demonstrated that being homozygous across the MHC class II region influenced IBH susceptibility in both the Icelandic Horse and in the Exmoor Pony population. The level of homozygosity differs greatly between these two horse breeds, with the latter having more homozygous individuals. This is not surprising as the Icelandic Horse is an old breed with over 200,000 registered horses worldwide. Even though the Exmoor Pony is also a very old breed, there have always been fewer representatives (fewer than 3,000 registered ponies today) and these have mainly

been localized in the southwest of England. In addition, the Exmoor Pony endured a severe genetic bottleneck at the time of the Second World War, when the population fell to only 53 animals. A recent analysis of the studbook revealed that only 24 mares and three stallions contributed significantly to the present-day population, which constitutes around 500 breeding animals (Baker 2008). The different population structures and histories were also evident in the average numbers of alleles (7.75 and 9.50 for Exmoor ponies and Icelandic horses, respectively) as well as the average degree of LD (0.49 and 0.37, respectively).

IBH is a complex disease that is likely controlled by a combination of multiple genes and many environmental factors. In a threshold sire model, we have previously estimated the heritability for IBH in Icelandic horses born in Sweden at 0.33 (s.d.<0.19) on an underlying, continuous scale (Eriksson et al. 2008). Similar estimates have been reported for Shetland ponies and Friesian horses with heritability values at 0.24 (SE=0.06) and 0.29, respectively, on the underlying scale (Ruyter 2005; Schurink et al. 2009). Interestingly, a study performed on 330 Icelandic horses exported from Iceland to Germany, Denmark and Sweden found no heritability for the disease (Bjornsdottir et al. 2006). This may have been due to the focus on exported horses. As discussed previously, horses exported from Iceland have a higher prevalence of IBH compared to horses born outside of Iceland. These two categories of horses (born in versus outside of Iceland) are of similar genetic background, but the greater environmental pressures on exported horses likely cause the difference in IBH prevalence. Increased environmental influences act to dilute the impact of genetic variation and consequently the power to dissect the genetic predisposition. In order to keep the environmental variation to a minimum, our study focused on Icelandic horses born in Sweden. Our hypothesis is that among horses that develop IBH, those born in Sweden have on average a stronger genetic predisposition, compared to their counterparts imported from Iceland.

It is still unclear what the exact MHC class II causal variants are. The different *DRB3* isoforms have a direct physiological role in B-cell antigen presentation to T-helper cells. This process ultimately leads to the proliferation of new B cells and production of antibodies. Because risk allele 1.2 produces the same polypeptide as the protective allele 1, there are most likely other variations that are causative in this region. Deep sequencing of the entire MHC class II region in targeted horses would be a logical approach towards the identification of candidate genes and ultimately causative quantitative trait nucleotides.

In conclusion, we have shown that variants in the MHC class II region are associated with IBH susceptibility, with the same allele associated in two separate populations. In addition, we have also shown that homozygosity across the entire MHC class II region is associated with a higher risk of developing IBH.

Acknowledgements We would like to thank all anonymous horse owners for providing samples for this study. Financial support was obtained from The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS) as well as the Swedish Foundation for Equine Research (SHF) to GL. We also thank Donald Miller and Doug Antczak for valuable advice about the MHC class II region. The authors declare that they have no conflict of interest.

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