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Polymorphisms in *ERAP1* and *ERAP2* are shared by *Caninae* and segregate within and between random- and pure-breeds of dogs

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ABSTRACT

Specific polymorphisms in the endoplasmic reticulum amino peptidase genes *ERAP1* and *ERAP2*, when present with certain MHC class receptor types, have been associated with increased risk for specific cancers, infectious diseases and autoimmune disorders in humans. This increased risk has been linked to distinct polymorphisms in both ERAPs and MHC class I receptors that affect the way cell-generated peptides are screened for antigenicity. The incidence of cancer, infectious disease and autoimmune disorders differ greatly among pure breeds of dogs as it does in humans and it is possible that this heightened susceptibility is also due to specific polymorphisms in *ERAP1* and *ERAP2*. In order to determine if such polymorphisms exist, the ERAP1 and ERAP2 genes of 10 dogs of nine diverse breeds were sequenced and SNPs causing synonymous or non-synonymous amino acid changes, deletions or insertions were identified. Eight ERAP1 and 10 ERAP2 SNPs were used to create a Sequenom MassARRAY iPLEX based test panel which defined 24 ERAP1, 36 ERAP2 and 128 ERAP1/2 haplotypes. The prevalence of these haplotypes was then measured among dog, wolf, coyote, jackal and red fox populations. Some haplotypes were species specific, while others were shared across species, especially between dog, wolf, coyote and jackal. The prevalence of these haplotypes was then compared among various canid populations, and in particular between various populations of random- and pure-bred dogs. Human-directed positive selection has led to loss of *ERAP* diversity and segregation of certain haplotypes among various dog breeds. A phylogenetic tree generated from 45 of the most common ERAP1/2 haplotypes demonstrated three distinct clades, all of which were rooted with haplotypes either shared among species or specific to contemporary dogs, coyote and wolf.

1. Introduction

Misfolded and aggregated proteins (Ruggiano et al., 2014), proteins generated by autophagy (Kobayashi, 2015), and foreign cellular proteins are all subjected to proteolytic digestion and the resulting peptides continuously screened for antigenicity by the major histocompatibility complex (MHC) class I receptors. The endoplasmic reticulum aminopeptidases 1 and 2 (*ERAP1, ERAP2*) are a unique class of proteases found on the luminal side of the endoplasmic reticulum that play a critical role not only in protein cleavage but in preparing the resulting peptides for proper receptor presentation (Hattori and Tsujimoto, 2013). Trimmed peptides resulting from the action of *ERAP1* and *ERAP2* are formed into a peptide-loading complex and transported to the MHC class I receptors by the action of the "transporter associated with antigen processing" genes *TAP1* and *TAP2* (Seyffer and Tampé, 2015). If the peptide is not recognized by any of the receptors, or binds with weak affinity, it will be further degraded and the amino acids recycled. However, if the peptide binds with sufficient affinity, the receptor will activate two processes that will end ultimately in death of the offending cell. The first process involves apoptosis, while the second process involves specific targeting of the cells for destruction by NK cells (innate immunity) or specific $CD8^+$ T cells (adaptive immunity). The affinity with which a particular peptide binds to the MHC class I receptor groove is ultimately dependent on the firmness of ligand/receptor binding. The final structure of both peptide ligand and MHC class I receptor is affected by a large number of nucleotide polymorphisms. Therefore, self/non-self (antigen) recognition is dependent on the makeup of both the peptide and the groove in which the peptide sits and different *ERAP1 and ERAP2*

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polymorphisms can alter that selection of the peptide repertoire within the same MHC class I allele (Reeves et al., 2014).

ERAP1 and *ERAP2* have been implicated in a number of disease processes involving MHC class I, including: 1) cancers, 2) infectious diseases, and 3) autoimmunity (Cifaldi et al., 2011). *ERAP1* and *ERAP2* and MHC class I have received increasing attention in cancer immunity. *ERAP1* and *ERAP2* are universally expressed in human cancers, and the level of MHC class I expression on the tumor cell surface correlates with the level of *ERAP1*, but not *ERAP2*, expression in the cells (Fruci et al., 2006). This suggests that *ERAP* has a role in the generation of the MHC class I repertoire. The expression of *ERAP1* and *ERAP2* varies according to the tumor type and is generally lower in neoplastic cells than normal tissues (Fruci et al., 2008). Down-regulation occurs in ovarian, mammary and lung carcinomas, whereas up-regulation has been observed in colon and thyroid carcinomas (Fruci et al., 2008). Interestingly, expression of ERAPs and MHC class I genes was enhanced by IFN-γ treatment, suggesting that they are both under the control of existing regulatory pathways. Variations in *ERAP1* (Mehta et al., 2009) and MHC class I (Mehta et al., 2008) have been associated with cervical tumor cell survival indicating a role of *ERAP1* and MHC class I for evasion of immune surveillance. This has been supported by experimental studies; treating ERAP1 in T-cell lymphoma lines with small interfering RNA interfered with *ERAP1* expression and enhanced tumor rejection in syngeneic animals by boosting NK- and T-cell-mediated cytolysis (Cifaldi et al., 2011).

Evidence for the role of ERAPs in immunity to infectious agents has come mainly from *ERAP1* deficient (*ERAP1*$^{-/-}$) transgenic mice. *ERAP1*$^{-/-}$ mice are not able to process the immunodominant HF10 decapeptide of *Toxoplasma gondii* and die following experimental infection (Blanchard et al., 2008). The hierarchy of T-cell responses to different immunodominant LCMV epitopes was also markedly changed in *ERAP1*$^{-/-}$ compared to wild-type mice (York et al., 2006). Transgenic mice demonstrate significantly different cytotoxic T cell (CTL) responses to the HLA-B27 associated immunodominant epitope of the influenza virus nucleoprotein depending on whether or not they have deleted or wild-type ERAP and HLA-B27 vs. HLA-B7 haplotypes (Akram et al., 2014). Nucleotide polymorphisms in *ERAP1* have also been associated with susceptibility to HIV-1. Draenert et al. (2004) showed that HIV isolated from individuals with the HLA-B57 MHC class I haplotype possessed a specific mutation in HIV-1*gag* that prevented terminal cleavage by *ERAP1* and diminished CTL responses. Some variants in *ERAP2* have been associated with resistance to HIV-1 infection possibly via the presentation of a distinctive peptide repertoire to CD8^{+} T cells (Cagliani et al., 2010).

Recent genome-wide association studies (GWAS) have linked ERAP1 and ERAP2 nucleotide polymorphisms, along with MHC class I, with autoimmune disease in humans (Fierabracci et al., 2012). A large multinational meta-analysis attributed 26% of the overall incidence for ankylosing spondylitis in a largely Northern European population to specific nucleotide polymorphisms in *ERAP1* (Burton et al., 2007). An interaction between *ERAP1* and *HLA-B27* affecting peptide handling was later shown to be involved in ankylosing spondylitis (Evans et al., 2011). A different ERAP1 variant was associated with the *HLA-B*2705* subtype in Hungarian patients with ankylosing spondylitis (Pazar et al., 2010). Two other recent GWAS identified *ERAP1* and implicated interactions between HLA-C and *ERAP1* in psoriasis (Strange et al., 2010; Sun et al., 2010). A recent meta-analysis of six Crohn's disease GWAS identified *ERAP2* as one of the most interesting candidate genes, bringing the total number of genetic risk factors for the disease to 71 (Franke et al., 2010). An epistatic association between *HLA-B*51* and *ERAP1* has been identified by GWAS for Behçet's syndrome, an arteritis that is often manifested by oral ulcers, retinitis and lesions in other organs (Kirino et al., 2013). Birdshot chorioretinopthy is strongly associated with HLA-A29 (OR = 157.5) and two nucleotide polymorphisms in *ERAP2* (Bakker et al., 2014; Kuiper et al., 2014).

Pure breed dogs have been recognized as excellent models for heritable disorders of humans, whether simple or complex in nature (Karlsson and Lindblad-Toh, 2008; Ostrander and Wayne, 2005). Two of the areas of particular interest to veterinary researchers and those modeling human disease are cancer and autoimmune disease. Increased susceptibility to certain infectious diseases does occur among dog breeds but has not been yet exploited to the degree of cancer and autoimmune disease.

It has been estimated that one-fourth of all dogs in the US will die of cancer with small dogs less susceptible than large dogs (Fleming et al., 2011). However, similar to human families and groups, there is a wide range in the cancer frequency depending on the breed, with the Bernese mountain dog and Golden Retriever being highest (54.6% and 49.9%), and the Pomeranian and Pekinese being the lowest (7.9%) (Fleming et al., 2011). There are also breed-associated differences in the types of cancers. Two of the most common cancers of Golden Retrievers, B-cell lymphoma and hemangiosarcoma, are of particular relevance to humans. Tonomura et al. (2015) conducted a genome wide association study (GWAS) in the breed and identified two loci that appeared to predispose to around 20% of the risk for these particular cancers. These germ-line mutations in B-cell lymphoma and hemangiosarcoma affected pathways involved in T-cell mediated immune response in the tumor, suggesting that an interaction between the immune system and malignant cells plays a common role in the tumorigenesis of these relatively different cancers.

Autoimmune disorders identical to those observed in humans also occur in dogs, and the incidence and breadth of disease tend to be much higher in purebred dogs, which have been associated with small founder size, artificial genetic bottlenecks, inbreeding and loss of heterozygosity (Pedersen et al., 2012a,b Pedersen et al., 2015a,b). The relationship of the canine MHC or dog leukocyte antigen (DLA) complex with autoimmune disease has received a great deal of study over the last two decades. The predisposition for autoimmune diseases of dogs appear mainly to be of an ancestral origin as for humans, and are increased through inadvertent human directed positive selection for certain phenotypic traits and associated inbreeding (Pedersen et al., 2012a,b Pedersen et al., 2015a,b). Many of the autoimmune disorders recognized in pure breeds of dogs have been associated with specific DLA class I and II haplotypes, but these associations have varied in strength and are not always apparent. However, DLA class I and II haplotype diversity has been greatly affected by pure breeding and certain haplotypes predominate in each breed studied (Pedersen et al., 2015a,b). It is possible, therefore, that the tendency of certain DLA class I and II polymorphisms to associate with autoimmune disease is also dependent on the ERAP1 and ERAP2 haplotypes that have been co-inherited by descent during breed development.

There are breed differences in susceptibility to infectious diseases, although these potentially heritable differences have not been as extensively investigated as cancer and autoimmune disease. A significant association was found between certain STR alleles in the DLA class II region and disseminated demodectic mange (It et al., 2010). Resistance to canine leishmaniasis has been linked to two loci on canine autosomes 1 and 2 associated with pathways involved in T helper cell function and macrophage signaling (Utsunomiya et al., 2015). There are other potentially genetic susceptibilities to infectious agents that remain to be determined. Rottweilers, Doberman Pinschers, Labrador Retrievers, American Staffordshire Terriers and German shepherd dogs develop more severe canine parvovirus associated enteritis with a higher mortality (Day, 1999; Glickman et al., 1985; Goddard and Leisewitz,