### Title

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# Alopecia areata: updates from the mouse perspective

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## Abstract

Alopecia areata (AA) is a cell mediated autoimmune disease that targets actively growing hair follicles in mammals, including humans and mice. Development of the C3H/HeJ spontaneous mouse model AA nearly 20 years ago provided a much needed tool to test hypotheses and ultimately serve as a preclinical model for drug testing. Discoveries in both human AA patients and the mouse model supported each other and lead to discoveries on the incredibly complex genetic basis of this disease. The discovery that A/J, MRL/MpJ, SJL/J, and SWR/J strains also develop AA now allows genome wide association mapping studies to expand the list of genes underlying this disease. Potential new targets for unraveling the pathogenesis of AA include the role of retinoic acid metabolism in the severity of disease and hair shaft proteins that may be either the inciting antigen or ultimate target of the immune reaction leading to breakage of the shaft causing clinical alopecia. Comparing these model systems with human and mouse clinical disease, for both discovery and validation of the discoveries, continues to resolve the complex questions surrounding AA.

### Keywords

vitamin A; C3H/HeJ mice; genetics; mouse models

Alopecia areata (AA) is a cell mediated autoimmune disease that targets actively growing, anagen stage, hair follicles. This is a disease of mammals (McElwee *et al.*, 1998b), not just human beings, so much can be learned by studying AA in all species affected. While AA has been reported in rats, dogs, horses, cattle, nonhuman primates, and other species (McElwee *et al.*, 1998b), the spontaneous (Sundberg *et al.*, 1994) and graft induced (McElwee *et al.*, 1998a) C3H/HeJ mouse AA models remain the most heavily used based on their availability and high degree of homology at clinical, molecular, and genetic levels

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(King *et al.*, 2008). As reported at the 2012 Alopecia Areata Summit, this mouse model is useful for validating discoveries in human genome wide association studies and is being actively used for a wide variety of preclinical trials. In fact, discoveries in the mouse, verified a decade later in human studies, provided justification for some current drug trials (Sundberg *et al.*, 2011b).

While human gene association studies, screening small groups of families for linkage with specific genes, demonstrated underlying genetic correlations with human AA, the C3H/HeJ mouse model was used in quantitative trait locus (QTL) analyses to demonstrate at least 4 QTLs associated with AA, each containing multiple genes that affect onset and progression of disease (Sundberg *et al.*, 2004).

Disease surveillance studies identified other strains of mice that spontaneously develop an AA-like disease. Essentially all of the commonly available C3H substrains were found to develop AA spontaneously, including both C3H/HeJ and C3H/HeN which were toll-like receptor 4 (*Tlr4*) null or wildtype, respectively. In addition, A/J were found to develop AA (McElwee *et al.*, 1999). More recently, large scale aging studies (Sundberg *et al.*, 2011a) revealed three additional strains that develop AA, the MRL/MpJ, SJL/J, and SWR/J strains. With 5 strains known to develop AA spontaneously, it is now possible to perform genome-wide association mapping studies, similar to those on human populations (Petukhova *et al.*, 2010), to find additional candidate genes involved in AA. The first pass genome-wide association mapping studies for AA in a 31 inbred strain survey, which included all 5 strains that develop AA, yielded candidate genes within three of the four QTLs identified in the original C3H/HeJ X C57BL/6J cross. Additional candidate genes in many other chromosomes were identified demonstrating that no one genetic approach in mice or humans will find all the genetic variants associated with AA. Combined, the mouse and human genetic studies are just beginning to demonstrate the incredible complexity of the genetics underlying AA.

Shotgun proteomic studies of hair shafts obtained from many different inbred strains of mice and a selected group that also had single gene mutations known to cause major structural defects in hair shafts demonstrated that strains could be distinguished using this technology and that normal inbred strains could be separated from those with mutations. Furthermore, allelic series, in which different mutations in the same gene occurred on different inbred strain backgrounds, could be both identified, differentiated from other normal or mutant mouse hairs, and the alleles could be differentiated from each other, presumably due to different affects of the mutations or strain specific background affects. An interesting finding was that hair proteins from clinically normal, young, C3H/HeJ mice were more distinct from other inbred strains but not as different than those with mutations causing structural defects in the hair shafts (Rice *et al.*, 2012). These findings suggest that C3H/HeJ mice may have inherent abnormalities or protein differences that predispose them to developing AA later in life, either as novel target proteins for the immune system or structural weaknesses that might exacerbate the effects of the cell mediated autoimmune response.

Screening gene array studies of C3H/HeJ mice with spontaneous AA or progressive, cross sectional studies of graft–induced AA revealed molecular pathways not previously considered important in the pathogenesis of this disease. Retinoic acid metabolism was down-regulated in the direction of storage and degradation but up-regulated in the direction of transport into the nucleus to initiate transcription of hundreds of genes. To determine if dietary levels of vitamin A affected the pathogenesis or severity of AA in the C3H/HeJ mouse model, cohorts of graft-induced mice were placed on synthetic diets with various levels of vitamin A. The moderate effect observed indicates that non-genetic factors can influence AA (Duncan *et al.*, 2013). Other pathways affecting interferon gamma, such as *Cxcr3* and its ligands, *Cxcl9*, *Cxcl10*, and *Cxcl11*, were also found to affect the pathogenesis of AA in the C3H/HeJ mouse model (McPhee *et al.*, 2012). Many other novel pathways are also affected in AA.

While the C3H/HeJ strain is susceptible to AA, C57BL/6J mice are not under natural conditions. Using a clonal $CD8^+$ T lymphocyte-mediated cell transfer model into C57BL/6J mice carrying the recombinase activating gene 1 (*Rag1*$^{tm1Mom}$) mutation, which causes T and B cell deficiency, an AA-like disease was induced. This approach was the first identification of an epitope that initiates an AA-like disease in any species, the significance of which is that it is not a hair keratin, melanin, or melanin associated protein, all of which have been considered the prime candidates in the past.

Since the first AA workshop in 1990, great progress has occurred in developing mouse models and to a lesser extent models in other species. When combined with the human studies, these demonstrate the extreme complexity of the genetic predisposition to AA, complicated pathogenesis, and elusive but now potentially identifiable effective treatments.

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## Abbreviations

| | |
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| **AA** | alopecia areata |
| **QTLs** | quantitative trait loci |
| **Cxcr3** | *Cxcl9*, *Cxcl10*, *Cxcl11* (genes/transcripts) |
| **chemokine (C-X-C motif) receptor 3** | chemokine (C-X-C motif) ligand 9, 10, and 11, respectively |
| **Rag1$^{tm1Mom}$** | recombinase activating gene 1 knockout mouse |
| ***Tlr4* (gene)** | Toll-like receptor 4 |

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