Table 3: Results of TDT and PDT by genotypig tag SNPs in SORCS3

rs number	allele	Trans*	Not trans**	TDT P value (corrected)	PDT P value (corrected)
rs768731	C	47	35	0.1843 (0.98)	0.2878 (0.99)
rs790640	C	21	37	0.03446 (0.50)	0.05183 (0.65)
rs790726	G	47	31	0.06905 (0.76)	0.2346 (0.99)
rs791123	G	71	50	0.05563 (0.68)	0.1291 (0.93)
rs971527	G	44	48	0.6766 (1)	0.5258 (1)
rs1472050	T	42	43	0.9136 (1)	0.9178 (1)
rs1490173	G	60	47	0.2083 (0.99)	0.2522 (0.99)
rs1565415	G	50	50	1 (1)	0.9164 (1)
rs1953071	C	52	45	0.4771 (1)	0.3538 (0.99)
rs2491388	A	38	34	0.6373 (1)	0.5675 (1)
rs3011669	A	35	49	0.1257 (0.93)	0.2793 (0.99)
rs4532962	T	51	55	0.6976 (1)	0.6633 (1)
rs7084834	C	19	27	0.237 (0.99)	0.1829 (0.98)
rs7096635	T	53	37	0.09083 (0.85)	0.05368 (0.66)
rs7895087	G	67	29	8.42E-05 (0.0017)	0.0003507 (0.007)
rs9943297	G	44	60	0.1159 (0.91)	0.2017 (0.98)
rs10509784	C	16	39	0.001635 (0.03)	0.007646 (0.14)
rs10509785	C	40	48	0.3934 (1)	0.4579 (1)
rs10884049	G	62	45	0.09957 (0.88)	0.1014 (0.88)
rs11192320	G	41	18	0.002409 (0.047)	0.007646 (0.14)

* Number of alleles transmitted to the affected children. ** Number of alleles not transmitted to the affected children

Examining SNPs, instead of microsatellite markers for linkage is unlikely to yield different results because it has been reported that SNP-based genome-wide linkage study has the potential to be as powerful as traditional microsatellite-based analysis and offers good identification of specific locations for further fine-mapping association analysis [13]. We performed a genome-wide linkage study with 5861 SNP markers, although previously performed

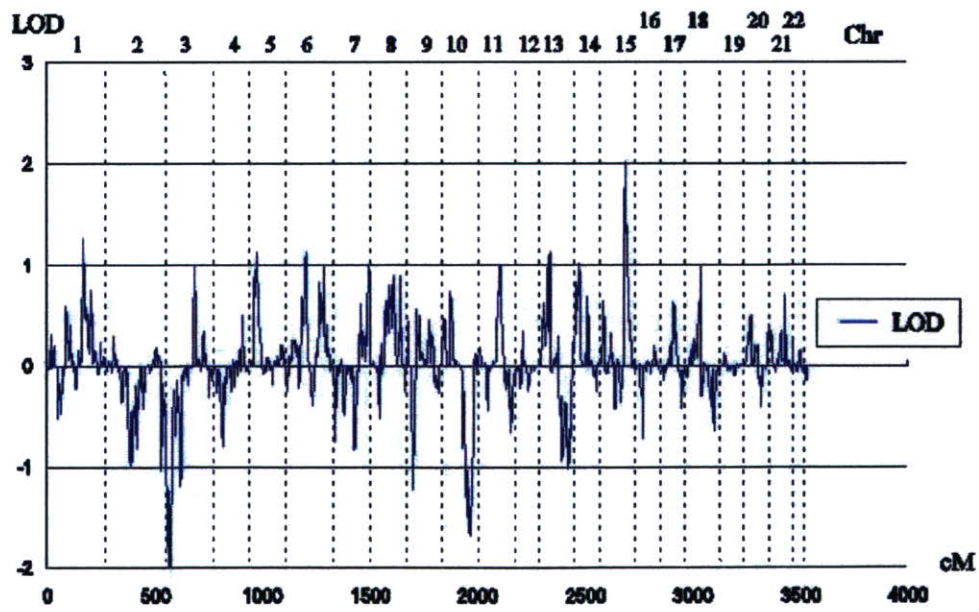

Figure 1
Multipoint nonparametric LOD score of genome-wide scan for ATOD in the Japanese.

Table 4: Whole genome linkage studies for ATOD

Year	Authors	Population	Genotyping	No. of markers	No. of families No. of ASP*	Phenotype	Linkage regions
2007	Present study	Japanese	SNPs	5861	77 111	ATOD	1q24 15q21
2004	Haagerup	Danish	Microsatellite	446	23 N/A	ATOD and specific IgE	3q26-3q24 4q15-4q14 18q11-18q12
2002	Bradley	Swedish	Microsatellite	367	109 206	ATOD ATOD and specific IgE ATOD and severity score	3p24-22 18q21 3q14 13a14 15q14 17q21
2001	Cookson	British	Microsatellite	385	148 213	ATOD ATOD and asthma	1q21 17q25 20p
2000	Lee	European	Microsatellite	380	199 N/A	ATOD	3q21

*ASP; affected sib-pairs

ATOD genoeme-wide scans have been done with smaller numbers of microsatellite markers. SNPs are distributed more abundantly and uniformly along the human genome than are microsatellite markers are more reliably typed, and require a smaller sample of DNA. Genome-wide linkage mapping of genes with fixed SNP panels, such as our Golden Gate assay, is a cost-effective and time-saving technology [24]. Several recent studies have found that SNP panels provide higher data quality, more accurate genotyping results and higher information content, and they may also have higher power to detect linkage than do traditionally used panels of microsatellite markers [25,26]. Because LD between closely spaced SNPs can falsely inflate linkage statistics, we remove LD from the marker sets in an automated fashion.

The 15q21 linkage region has not been reported previously as a region associated with ATOD. However, linkage of 15q21 to several other inflammatory diseases including osteoarthritis [8] and macular degeneration [27], has been reported. The 15q21 region contains candidate genes for ATOD such as Mothers against decapentaplegic homolog of 3 (SMAD3). SMAD proteins are involved in biologic responses to TGF-beta and related ligands. Smad3-knockout mice show accelerated cutaneous wound healing with complete reepithelialization, and Smad3-deficient keratinocytes show altered patterns of growth and migration [28].

The 1q24 linkage region includes candidate genes such as T-cell receptor zeta chain isoform 2 precursor (*CD3Z*) and chemokine ligand 2 (*XCL2*). CD3Z plays an important role to recognize the coupling antigen to several intracellur signal-transduction pathways [29]. Antigen recognition is one of the most important events in the pathology of ATOD, especially in the memory T cells that encounter their specific antigen, generating an allergen response, which then activates leukocytes leading to production of several cytokines and atopic skin inflammation [3]. Chemokines have fundamental roles in regulation of several types of T cells, development, homeostasis, and function of the immune systems, especially in leukocyte trafficking. During the multistep process of leukocyte trafficking, chemokine ligand-receptor interactions mediate the firm adhesion of leukocytes to the endothelium and initiate transendothelial migration from the blood vessel into perivascular pockets [30]. From perivascular spaces, matrix-bound sustained chemokine gradients direct skin-infiltrating leukocyte subsets to subepidermal or intraepidermal locations. In ATOD regions, that caused by chemokines recruit pathogenic leukocytes to skin in response to mechanical injury such as scratching [31].

Our linkage region on chromosome 1 was located near 1q21, which was previously reported as a linkage region in a British population [10]. It was reported that the skin barrier is impaired in patients with ATOD [32], and recent studies showed that loss-of-function mutations in *FLG* on 1q21 were associated with ATOD in 2 independent populations [8]. FLG is involved in aggregation of the keratin cytoskeleton, which causes collapse of granular cells into flattened anuclear squames. The condensed cytoskeleton is crosslinked by transglutaminases during formation of the cornified cell envelope, the outermost barrier layer of

the skin [33], which prevents water loss and impedes the entry of allergens and infectious agents. 1q21, which has been linked to both ATOD and psoriasis [10], houses a cluster of genes known as the epidermal differentiation complex that encode proteins involved in keratinocyte terminal differentiation [34]. Several genes in this region have been reported to be associated with skin diseases such as psoriasis [35]. Because it is possible that the true disease susceptibility gene is located further away from the actual linkage peak, the 1q21 region may include one or more ATOD susceptibility genes for our Japanese population.

Several candidate genes for ATOD were identified by PDT analysis (Table 3). CD200 and its receptor CD200R are both type I membrane glycoproteins that contain two immunoglobulin-like domains. CD200-CD200R interaction has been shown to be important for regulation of the macrophage lineage. In CD200-deficient mice, there were increased numbers of macrophages in the spleen and the mesenteric lymph nodes, and these macrophages show increased activation [36]. In chronic lichenified lesions of ATOD skin, there is an increased number of Langerhans' cells in the epidermis, and macrophages dominate the dermal mononuclear cell infiltrate, and macrophages are important source of cytokines that cause inflammation of the skin [37]. Another candidate is laminin alpha 4 chain (LAMA4). Laminins are a large family of heterotrimeric extracellular matrix glycoproteins in the basement membrane that promote cell adhesion, migration, differentiation, proliferation, and angiogenesis. Lama4-deficient mice showed deterioration of microvessel growth [38], and LAMA4 are located in the basement membrane zone of capillary vessels and in an area adjacent to fibroblast-like cells [39]. SORCS3 is one of the VSP10 domain-containing receptor, that shares the greatest homology with SORCS1. The function of SORCS3 remains unclear, but several SNPs in *SORCS3* showed association with ATOD by PDT analysis (Tables 2 and 3). Although not in the linkage region, the results of family-based association study suggest that these genes may be associated with the pathogenesis of ATOD.

In conclusion, we performed the first genome-wide linkage study for ATOD in an Asian population, and identified 2 linkage regions, one on 15q21 and one on 1q24. A recent review suggested that there was no substantial overlap between the genetic architecture of ATOD and that of other atopic diseases, such as asthma, but there is a greater degree of similarity between ATOD and psoriasis [7]. Our linkage region on 15q21 overlaps with regions linked to other inflammatory diseases, suggesting that common inflammatory genes may be located in this region. The results of our genome-wide linkage study may lead to identification of novel genes for ATOD, which would improve our understanding of the pathogenesis of ATOD.

Conclusion

We report the first genome-wide linkage analysis for ATOD in an Asian population and identified novel loci on chromosomes 15q21 and 1q24 linked to ATOD. The results of our genome-wide linkage study may lead to identification of novel genes for ATOD, which would improve our understanding of the pathogenesis of ATOD.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

HE carried out molecular genetic study, participated in the study design and coordination and wrote the draft of the manuscript. SI, TT, KH, MI, TK, TA, YS, MK, MT, TS, and FO carried out molecular genetic studies. EN and TA participated in the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

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