Molecular and Cellular Endocrinology 399 (2015) 244–249

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Variants in *IRAK1-MECP2* region confer susceptibility to autoimmune thyroid diseases

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ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form 10 October 2014
Accepted 14 October 2014
Available online 18 October 2014

Keywords:
Interleukin-1 receptor-associated kinase 1 (*IRAK1*)
Methyl-CpG-binding protein 2 (*MECP2*)
Single nucleotide polymorphism (SNP)
Autoimmune thyroid disease (AITD)
Graves' disease (GD)
Hashimoto's thyroiditis (HT)

ABSTRACT

Our objective was to investigate whether interleukin-1 receptor-associated kinase (*IRAK1*) and methyl-CpG-binding protein 2 (*MECP2*) are associated with autoimmune thyroid diseases (AITDs). We selected four single nucleotide polymorphisms (SNPs), rs3027898, rs1059703 in *IRAK1* and rs2075596, rs2239464 in *MECP2*, for genotyping using PCR-based ligase detection reaction (LDR) method in 1042 AITDs patients and 897 controls. Minor alleles in the four SNPs were strongly associated with AITDs, and similar associations were found in Graves' disease (GD). In Hashimoto's thyroiditis (HT) patients, a significantly increased risk of T allele in rs1059703 was found. There were obvious differences in allele and genotype distributions in female AITDs, GD and HT patients. Moreover, the haplotypes CCAA and ATGG were the associated variants for AITDs and GD. Besides, these two haplotypes showed similar associations with AITDs and GD in female patients. Our results firstly indicated that *IRAK1* and *MECP2* genes are crucial risk factors for AITDs.

1. Introduction

Autoimmune thyroid diseases (AITDs), mainly including Graves' disease (GD) and Hashimoto's thyroiditis (HT), are a group of complex and thyroid-specific autoimmune diseases with up to 2~5% in incidence (Simmonds, 2013). The diseases have a strong preponderance for females, with female-to-male ratio ranging from 5:1 to 10:1 (Effraimidis and Wiersinga, 2014). Although the clinical manifestations of GD and HT are quite different, the two subtypes indeed own a high degree of familial aggregation and share some common features, such as T lymphocytes infiltration in the thyroid and the existence of circulating autoantibodies against thyroid antigens, like antibody against thyroid peroxidase (TPOAb), thyroglobulin (TgAb) and thyroid stimulating hormone receptor (TRAb). Despite the exact etiology of AITDs remaining unclear, a large body of literature supports the vital roles of the genetic, environmental factors and immune dysfunction mechanisms (Weetman, 2003).

Interleukin-1 receptor-associated kinase coding gene (*IRAK1*), 8.4 kbp in length, which is located on X-chromosome, participates in Toll-like receptor (TLR) signaling, up-regulates transcription factor NF-κB and activates innate immune response (Martin and Wesche, 2002). Its neighboring gene, methyl-CpG-binding protein 2 coding gene (*MECP2*), is with 76 kbp in length that characteristically owns 4 exons, 8.5 kbp of 3' untranscripitical region (UTR) and a very large intron 2 (65 kbp). *MECP2* is located on chromosome Xq28 and encodes for a protein which plays a crucial role in epigenetic transcriptional regulation of methylation-sensitive genes and regulation of T-cell immune function (Kimura and Shiota, 2003). Previously, single nucleotide polymorphisms (SNPs) in *IRAK1* and *MECP2* were reported to be independently associated with the risk of several other autoimmune diseases, such as systemic lupus erythematosus (SLE) (Liu et al., 2013; Zhai et al., 2013), rheumatoid arthritis (RA) (Han et al., 2013; Zhang et al., 2013) and systemic sclerosis (SSc) (Carmona et al., 2013; Dieude et al., 2011). Given the fact that various autoimmune diseases share multiple common genetic variations, we hypothesized that the X-chromosome genes *IRAK1* and *MECP2* may be the potential AITDs genetic factors based on these features: AITDs predominance in women, immune dysfunction and abnormal

Abbreviations: AITD, autoimmune thyroid disease; Chr, chromosome; *CI*, confidence interval; GD, Graves' disease; HT, Hashimoto's thyroiditis; HWE, Hardy–Weinberg equilibrium; *IRAK1*, interleukin-1 receptor-associated kinase 1; LD, linkage disequilibrium; LDR, ligase detection reaction; LOD, logarithm of odds; MAF, minor allele frequency; *MECP2*, methyl-CpG-binding protein 2; NC, normal controls; *OR*, odds ratio; Pos, position; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism; SSc, systemic sclerosis; TgAb, antibody against thyroglobulin; TLR, Toll-like receptor; TPO, thyroid peroxidase; TPOAb, antibody against thyroid peroxidase; TRAb, antibody against thyroid stimulating hormone receptor; UTR, untranscripitical region.

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http://dx.doi.org/10.1016/j.mce.2014.10.013
0303-7207/

regulation of methylation-sensitive T-cell genes in AITDs (December, 2014, our unpublished data).

In the current study, by utilizing a candidate gene association approach, we investigated the polymorphisms of *IRAK1* (rs3027898 C/A and rs1059703 C/T, Ser532Leu) and *MECP2* (rs2075596 A/G and rs2239464 A/G) loci in 1042 AITDs patients and 897 matched controls.

2. Subjects and methods

2.1. Subjects

Here, a cohort of 1042 Chinese patients with AITDs and 897 healthy Chinese controls from two regions of China, Shanghai (south of China) and Xi'an (north of China), were available for this current study. All AITDs patients were enrolled from the Out-patient Department of Endocrinology of Jinshan Hospital and the First Affiliated Hospital of Xi'an Jiaotong University; ethnically and geographically matched healthy controls were recruited from the Healthy Check-Up Center of the two hospitals. All the patients and controls gave written informed consent. And sample collection was approved by the ethical committees of Jinshan Hospital and the First Affiliated Hospital of Xi'an Jiaotong University, respectively. Each participant, including patients and controls, was ethnic Chinese Han.

AITDs patients were diagnosed as described in our published papers (Muhali et al., 2013; Song et al., 2014). All GD patients met the diagnosis criteria, which included clinical manifestations and biochemical assessments of hyperthyroidism and the positive circulating TRAb, with or without positive TPOAb or TgAb and diffuse goiter of the thyroid. HT cases were defined based on the high level of autoantibodies (TPOAb or/and TgAb), the presence of an enlarged thyroid, with or without clinical and biochemical hypothyroidism. A few patients with suspicious HT were then confirmed by thyroid fine needle aspiration biopsies. All the control subjects were recruited with negative thyroid peroxidase (TPO) antibody, without personal or family history of thyroid disease and any other autoimmune diseases or immune hypersensitivity diseases. In the current study, immunochemiluminescence method with great specificity and sensitivity was used for detecting TPOAb, TgAb and TRAb, and all of the kits were provided by Roche Company, Shanghai, China.

Demographic data and clinical characteristics of the cases are described in Table 1. Our study investigated a total of 1042 AITDs patients, including 688 GD patients [477 females (69.331%) and 211 males (30.669%), average age of 36.900 (5~77) years old] and 354 HT patients [310 females (87.571%) and 44 males (12.429%), average age of 34.890 (4~78) years old]. There were 161 AITDs patients with disease onset age of ≤18 years old who were teenager patients, with a percentage of 15.451% in the whole AITDs cases. Among the teenager subjects, there were 112 with GD (16.279% in GD group) and 49 with HT (13.842% in HT group). In GD patients, 124 had ophthalmopathy and 142 individuals had a family history (18.023% and 20.640%, respectively). In HT subjects, 6 had ophthalmopathy and 73 individuals had a family history (1.695% and 20.621%, respectively).

Table 1
Demographic data and clinical characteristics of patients with AITDs.

	AITDs (%)	GD (%)	HT (%)
Number	1042	688	354
Gender			
Female	787 (75.528)	477 (69.331)	310 (87.571)
Male	255 (24.472)	211 (30.669)	44 (12.429)
Age	36.220 ± 14.356	36.900 ± 14.594	34.890 ± 13.805
Onset of age	33.330 ± 14.149	33.840 ± 14.471	32.330 ± 13.462
≤18 years	161 (15.451)	112 (16.279)	49 (13.842)
≥19 years	881 (84.549)	576 (83.721)	305 (86.158)
Ophthalmopathy			
(+)	130 (12.476)	124 (18.023)	6 (1.695)
Family history			
(+)	215 (20.633)	142 (20.640)	73 (20.621)

2.2. DNA sample collection

Blood samples were collected from all the participants and genomic DNA was extracted from 2 ml peripheral venous blood using RelaxGene Blood DNA System (Tiangen Biotech, Beijing, China), following the instruction of the kit. The concentration and purity (represented as A260/A280 ratio) of all DNA samples were measured by Nano Drop 2000 Spectro-photometer (Thermo Scientific Company, Waltham, MA, USA). The DNA samples of great quality (high purity and concentration) were prepared for genotyping.

2.3. SNP selection and genotyping

The marker-tagging SNPs were selected from the Hapmap CHB data (http://hapmap.ncbi.nlm.nih.gov/) using the Tagger program of Haploview software 4.2 (Barrett et al., 2005) to meet the following criteria: minor allele frequency (MAF) > 0.05, Hardy–Weinberg equilibrium (HWE) with $P > 0.001$, and logarithm of odds (LOD) > 3.0. Also, these SNPs were chosen because of their strong association with SLE, RA (Han et al., 2013; Liu et al., 2013; Sawalha et al., 2008; Zhang et al., 2013) or their potential causal polymorphisms for AITDs. rs3027898 (C/A) located in 3'-untranslated region (UTR) of *IRAK1*, exonic SNP rs1059703 (C/T, Ser532Leu) and two intronic loci of *MECP2*, rs2075596 (A/G) and rs2239464 (A/G), were undertaken for genotyping by means of ligase detection reaction (LDR) platform.

Moreover, to ensure the quality of the assay, we made double positive controls (duplication of the same DNA samples) and negative controls (blank samples without DNA) in the process of SNP genotyping. Quality control analysis was also performed so that only SNPs and samples which passed the 95% quality control threshold were subjected to further statistical analysis. All genotype call rates were manually recorded and conflicting results were liberally re-genotyped by sequencing. Primers specific for the four SNPs in the region of *IRAK1-MECP2* are shown as follows.

rs3027898 Upper Primer – TAGTAACAAGACCCTGGACG
Lower Primer – ACTTTGTTGCACCGAAAGCC
rs1059703 Upper Primer – ACACGTAGGAGTTCTCCTGC
Lower Primer – AGAAGCTGCAGGCAGTGGTG
rs2075596 Upper Primer – GAAACATGCTTCTTCACCCC
Lower Primer – GGATGGAATAGCTGCGGAAG
rs2239464 Upper Primer – CATTTGACTTTTGAACCTGG
Lower Primer – ATGGCACAAGGAGACATATC

2.4. Genotyping-clinical phenotype analysis

Subphenotype stratification analysis was conducted by a case-only approach, in which basic allelic and genotypic examinations were performed by comparing minor allele and genotype frequency of cases with a specific sub-phenotype to cases without the specific sub-phenotype. The clinical manifestations include: (1) adult- or childhood-onset AITDs patients, according to the age of disease onset (≤18 years versus ≥19 years); (2) presence or absence of ophthalmopathy in GD group (defined as a distinctive disorder characterized by inflammation and swelling of the extraocular muscles, eyelid retraction, periorbital edema, episcleral vascular injection, conjunctive swelling and proptosis); (3) presence or absence of thyroid dysfunction in HT group: euthyroid status or hypothyroidism; (4) presence or absence of AITDs family history (defined as the subjects' first-degree relatives including parents, children and siblings or second-degree relatives such as grand-parents, uncles and aunts who had AITDs occurrence); (5) intractable GD or GD in