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CEP128 is a crucial risk locus for autoimmune thyroid diseases

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Abstract

Autoimmune thyroid disease (AITD) mainly includes Graves' disease (GD) and Hashimoto's thyroiditis (HT), and its pathogenesis is not clearly defined. This study was designed to explore risk loci for AITD. Genome-wide genetic data were analyzed to identify important risk loci for GD, and a case-control study with 845 AITD patients and 694 healthy controls was also conducted. The functional role of possible risk loci for GD was explored by analyzing the correlations of Centrosomal protein 128 (CEP128) expression level with intrathyroidal immune cells and key genes for candidate immune cells in GD thyroid tissues. *CEP128* was identified as an important risk locus for GD in the genome-wide genetic analysis, and it was located near TSHR without obvious linkage disequilibrium with TSHR. Two tag single-nucleotide variants in *CEP128* including a missense variant rs327463 were substantially related to genetic predisposition to GD and HT in the case-control study. *CEP128* rs327463 was substantially related to GD under the allele model (OR = 1.31, 95%CI 1.08-1.59, P = 0.006) and the dominant model (OR = 1.37, 95%CI 1.09-1.72, P = 0.008), and it was related to HT under the recessive model (OR = 1.85, P = 0.031) and the homozygous model (OR = 1.91, P =0.025). Moreover, CEP128 was substantially correlated with the frequencies of T-follicular helper (Tfh) cell and M1 macrophages in GD tissues. Gene set enrichment analysis suggested that CEP128 was related to several common immune pathways involved in GD pathogenesis, such as interferon-γ mediated signaling pathway and toll-like receptor signaling pathway. This study highlight the crucial role of CEP128 in the pathogenesis of GD, and polymorphisms in *CEP128* contribute to genetic predisposition to both GD and HT.

Keywords: Autoimmune thyroids diseases; Graves' disease; Centrosomal protein 128; Polymorphisms; Genetic predisposition

1. Introduction

Autoimmune thyroid disease (AITD) is one of the most prevalent autoimmune disorders and it affects about 2% to 10% of total population worldwide (McLeod and Cooper, 2012; Tomer, 2014). AITD has two principal subtypes including Graves' disease (GD) and Hashimoto's thyroiditis (HT). GD is the main etiology of hyperthyroidism and about 80% of thyrotoxicosis cases is caused by GD (Franklyn and Boelaert, 2012). GD is typically characterized by thyrotoxicosis and thyroid-stimulating hormone receptor antibody (TRAb) which can bind to and stimulate the thyroid-stimulating hormone receptor (TSHR) on the surface of thyroid follicular cells, which leads to excess production of thyroid hormones and thyrotoxicosis (Smith and Hegedus, 2016). HT is the main cause of hypothyroidism, and is typically characterized by a shortage of thyroid hormones, thyroid peroxidase antibody (TPOAb) positivity and thyroglobulin antibody (TGAb) positivity (Ajjan and Weetman, 2015). Despite the different symptoms, both GD and HT are immune-mediated diseases and have abnormal immune responses, but their molecular mechanisms are still largely elusive (Rydzewska et al., 2018; Tomer, 2014). Currently, it has been well accepted that defects in self-tolerance caused by the interactions among genetic factors, epigenetic factors and environmental factors exert crucial roles during the development of AITD (Tomer, 2014; Wang et al., 2017).

Genetic studies have uncovered many genetic factors related to AITD susceptibility (Erdogan et al., 2017; Inaba et al., 2016; Lombardi et al., 2016; Stefan and Faustino, 2017; Vita et al., 2017). Numerous variants in human leukocyte antigen (*HLA*) genes of the major histocompatibility complex (MHC) region have been identified as important genetic factors for AITD (Bernecker et al., 2013; Inaba et al., 2016; Kuang et al., 2010; Okada et al., 2015; Vita et al., 2017). Apart from genes from the MHC region, several non-MHC genes have been identified as crucial risk loci for AITD, such as *TSHR*, protein tyrosine phosphatase nonreceptor 22 (*PTPN22*) and Cytotoxic T-lymphocyte associated antigen 4 (*CTLA4*) (Dultz et al., 2009; Fujii et al., 2017; Pujol-Borrell et al., 2015; Stefan and Faustino, 2017; Yang et al., 2012). Numerous SNPs in *TSHR* were reported to genetic factors related to GD, and many of them were in tight linkage disequilibrium (LD) (Stefan and Faustino, 2017). In

addition, *PTPN22* rs2476601 has been identified as a crucial risk variant for AITD and other autoimmune diseases (Heward et al., 2007; Lopez-Cano et al., 2017). Some variants in other genes have also been reported to be genetic factors related to AITD, such as *IKZF3* and *BCL2L15* (Ban et al., 2016; Li et al., 2018; Lombardi et al., 2016). However, the genetic predisposition of AITD is still not fully defined, and further studies are necessary to provide deeper insights into the genetic predisposition of AITD. Therefore, this study was performed to explore novel risk loci for GD and HT, and to explore the possible functional role of the risk locus in GD. We analyzed data of a genome-wide association study (GWAS) from an online database, and then performed a case-control study to validate the findings from GWAS research. The possible functional role of risk loci for GD was further explored through bioinformatics.

2. Methods

2.1. GWAS study

The Gene ATLAS (http://geneatlas.roslin.ed.ac.uk/) is a large and open access resource of GWAS data using the UK Biobank cohort, and the associations were analyzed utilizing 452,264 White British individuals (Sudlow, Gallacher, Allen et al., 2015). Detailed analysis methods in the UK Biobank cohort have been described in previous literature (Sudlow et al., 2015). Because GD is not a phenotype in the UK Biobank cohort while GD is the main etiology of hyperthyroidism, the phenotype "hyperthyroidism/thyrotoxicosis" in the UK Biobank cohort was used alternatively to identify possible single nucleotide variants related to GD (Smith and Hegedus, 2016). We analyzed those SNPs with substantial associations with hyperthyroidism at the significance level of $P < 1\times10^{-8}$. To display the data above, Manhattan plots were generated using R package 'qqman'. Non-MHC risk loci of interest were selected by comparing with findings from previously published GWAS studies on AITD (Chu et al., 2011). Haploview 4.2 was used to assess the LD of single nucleotide polymorphisms (SNPs) in risk loci of interest through using 1000 Genomes phase3 data, and $r^2 > 0.8$ was deemed to suggest strong LD. Besides, r^2 between 0.50 to 0.80 were deemed to suggest modest LD, and r^2 less than 0.50 suggested weak LD. UK Biobank cohort was approved by the National Health Service National Research

Ethics Service (Sudlow et al., 2015).

2.2. Case-control study and SNP genotyping

845 AITD patients (522 unrelated GD patients, 323 HT patients) and 694 healthy controls from the Chinese Han population were recruited in the case-control validation study. Both GD and HT were diagnosed based on the laboratory examination of thyroid function and thyroid antibodies as previously described (Yang et al., 2012). Controls were randomly selected from individuals receiving physical examination in the same hospital. The Ethics Committee in our hospital approved the study, and all subjects provided written informed consent.

Genomic DNA was extracted from 1 ml peripheral blood for each participant using RelaxGene Blood DNA System (Tiangen Biotech, China). To the quality of extracted DNA, and the purity and concentration of DNA were measured and samples with either lower purity or lower concentration were deleted. Centrosomal protein 128 (*CEP128)* was identified as an important risk locus for GD in the present study, and two tag SNPs in *CEP128* gene including rs327463 and rs12050151 were determined using high throughput-SNP (Hi-SNP) genotyping method with technical support from the Shanghai Biowing Applied Biotechnology company, which was based on three-round multiplex PCR coupled with next generation sequencing (Chen et al., 2016).

2.3. CEP128 expression level and immune cells in GD tissues

To explore the possible function of CEP128 in GD pathogenesis, the correlations of CEP128 expression level with intrathyroidal immune cells in GD thyroid tissues were analyzed by using data from GSE9340 in Gene Expression Omnibus (GEO) database. GSE9340 provided the whole-genome expression profiling of thyroid tissue of GD patients with (n=10) and without (n=8) Graves' ophthalmopathy (GO) (Wescombe et al., 2010). Immune cells in GD tissues were estimated from the gene expression profiles in GSE9340 by CIBERSORT tool (Newman et al., 2015).

2.4. CEP128 expression level and key immune genes

The mRNA expression levels for CEP128 and key immune genes in the GD tissues were extracted from the original data of GSE9340. Correlations of CEP128 expression level with those key immune genes in GD tissues were analyzed. Those

key immune genes included characterized transcription factors or cytokines for candidate immune cells, such as M1 macrophages and $CD4^+$ T cells. Key anti-inflammatory cytokines were also analyzed, such as IL4, IL6, IL10 and TGFB1.

2.5. Functional pathways related to CEP128

Because it is difficult to explore the function of CEP128 through analyzing its correlations with individual gene, gene set enrichment analysis (GSEA) was further performed to identify crucial functional pathways related to CEP128 (Subramanian et al., 2005; Kuleshov et al., 2016). GSEA is well-developed and powerful tool in interpreting genome-wide expression profiling. GSEA focuses on gene sets sharing a common biological function but not single gene, and can thus provide more accurate and more reliable findings than individual gene analysis methods (Subramanian et al., 2005; Kuleshov et al., 2016). GSEA analysis was performed with GSEA v3.0, and predefined genes sets were downloaded from Molecular Signatures Database, which mainly included GO biological process (4,436 genes sets) and KEGG pathway (186 gene sets). Gene sets represented by less than 10 genes were excluded, 1000 permutations were performed. Enrichment score (ES) and nominal P-value were calculated for each gene set, and gene sets with both an ES more than 0.60 and a nominal P value < 0.10 were considered significantly enriched pathways.

2.5. Statistical analysis

Before SNP genotyping, the sample size calculation was performed with an expected OR of 1.40. For the comparison of allele model, a sufficient power over 80% required at least 426 cases and 426 controls to find a significant association of CEP128 rs327463 with AITD. For the dominant model of genotype comparison, a sufficient power over 80% required at least 601 cases and 601 controls to find a significant association. We finally recruited 845 AITD cases and 694 controls, which led to a power of over 95.0% in identifying a significant association. The distribution of alleles and genotypes between cases and controls was compared using chi-square test, and odds ratios (OR) with 95% confidence interval (95%CI) were calculated out by the logistic regression model. Conventionally, four genetic comparison models were analyzed, including the allele model, the dominant model, the recessive model, and the homozygous model. Multivariate logistic regression analysis was also

conducted to adjust for age and gender. Analyses were also stratified by types of AITD. To further assess the roles of CEP128 in GD, the correlations of CEP128 with those genes of interest and intrathyroidal immune cells were analyzed using correlation analysis. Difference in the CEP128 expression between GO patients and non-GO patients was analyzed using unpaired t test. STATA (version 12.0, StataCorp) was used in the statistical analyses and P values <0.05 were considered statistically significant.

3. Results

3.1. Major risk loci for GD in the GWAS study

A total of 10,835 SNPs with P values less than 1×10^{-8} were found to be related to hyperthyroidism in GeneATLA database (Supplementary table 1). As showed in Figure 1, most SNPs were located in the MHC region of Chromosome 6, while the other SNPs were mainly located in Chromosome 14, Chromosome 2 and Chromosome 1 (Figure 1). In Chromosome 1, most of those SNPs were in the *PTPN22* gene which was a well-defined risk locus for GD, and the correlation of *PTPN22* rs2476601 with GD was also verified ($P = 1.16\times10^{-17}$) (Supplementary table 1). In Chromosome 2, most SNPs were from another well-defined risk locus for GD--*CTLA4*, such as rs3087243 ($P = 1.56\times10^{-23}$) and rs231775 ($P = 2.29\times10^{-20}$) (Supplementary table 1). The finds above were consistent with previously published literatures on key risk loci for GD, which proved that it was appropriate for the use of the phenotype "hyperthyroidism/thyrotoxicosis" as a substitute for GD in the UK Biobank cohort.

Apart from Chromosome 6, Chromosome 14 had the second largest SNPs (Figure 1, supplementary table 1). As shown in the Manhattan plot of Chromosome 6 (Figure 2), there were a large number of SNPs with P values less than 1×10^{-8}, and all of them were located in either *CEP128* or *TSHR*. *TSHR* is the most important risk locus for GD, and numerous SNPs in *TSHR* have been identified to be genetic factors related to GD, but *CEP128* is a novel risk locus for GD and no study on its role in autoimmune diseases has been published. The LD between *CEP128* and *TSHR* was then analyzed using 1000 Genomes phase 3 data in Haploview 4.2. As shown in the supplementary

figure 1-A, SNPs in *CEP128* was not in strong LD with SNPs in *TSHR* in Chinese population. Similar findings were also found in both the UK population and African Americans (Supplementary figure 1-B and C). Because *DIO2* is also located close to CEP128 and previous studies reported that several *DIO2* SNPs were associated with AITD (Chistiakov et al., 2004; Panicker et al., 2009; Castagna et al., 2017). However, the outcome for the associations of *DIO2* polymorphisms with GD in the Gene ATLAS database suggested that only some *DIO2* SNPs were modestly associated with GD, and none of those polymorphisms had a P value < 1×10^{-5} (Supplementary figure 2). To analyze the possible LD of *CEP128* with *DIO2*, three common SNPs (rs225014 (Thr92Ala), rs225015 and rs12885300) were added in the LD analysis of *CEP128* with *DIO2*, which were previously reported to be associated with AITD. As shown in Figure 3, there was no obvious LD between *CEP128* and *DIO2* in all three populations (Figure 3).

Though current literatures provided few data on the roles of *CEP128* SNPs in autoimmune diseases including AITD, data from an online supplementary material of a GWAS study from China indicated potential roles of *CEP128* SNPs in genetic predisposition to GD, in which *CEP128* was termed as *C14orf145* (Chu et al., 2011). Another study by Liley et al. reported that SNPs in *CEP128* were likely to contribute to the difference in the genetic basis for GD and HT, but their influence on AITD susceptibility remained unclear (Liley et al., 2017). To explore possible tag SNPs in *CEP128* which were associated with AITD, we selected 7 candidate SNPs in *CEP128* and 8 most significant SNPs in *TSHR* by combining data from the GD GWAS study of China and the GD GWAS study of GeneATLA database. Those 7 *CEP128* SNPs included rs327463, rs162171, rs327434, rs162174, rs7158936, rs2556611 and rs12050151, and rs327463 was the only missense variant in *CEP128* with minor allele frequency (MAF) more than 0.10 (MAF = 0.181) and had not been reported in the previous GD GWAS study from China. As shown in Figure 3, rs327463 is in strong or modest LD with other 6 *CEP128* SNPs, but rs327463 is not in LD with those 8 most significant SNPs in *TSHR* in Chinese population (Figure 3-A). In UK population, rs327463 was in strong or modest LD with other *CEP128* SNPs except for rs12050151 (Figure 3-B), which was similar to that of African Americans.

Therefore, rs327463 and rs12050151 were selected as tag SNPs for validation in the following case-control study, and other SNPs in strong LD with rs327463 were not genotyped.

3.2. Case-control study

The clinical characteristics of those participants are summarized in Supplementary table 2 (Supplementary table 2). Both rs327463 and rs12050151 didn't deviate from HWE in the controls, and the P values of HWE for rs327463 and rs12050151 were 0.71 and 0.91, respectively. As shown in Table 1, there was an obvious difference in the distribution of allele frequencies and genotype for both rs327463 and rs12050151 between AITD cases and controls (Table 1). There was also an obvious difference in the allele frequencies and genotype distribution for both rs327463 and rs12050151 between GD cases and controls (Table 1). The difference in the allele frequencies and genotype distributions of rs327463 and rs12050151 was not statistically significant between HT cases and controls (Table 1).

After adjusting for age and gender, *CEP128* rs327463 was significantly related to genetic predisposition to AITD under all four comparison models (P<0.05) (Table 2). Subgroup analysis by types of AITD suggested that *CEP128* rs327463 was significantly related to GD under the allele model (OR = 1.31, 95%CI 1.08-1.59, P = 0.006) and the dominant model (OR = 1.37, 95%CI 1.09-1.72, P = 0.008), and it was related to HT under the recessive model (OR = 1.85, 95%CI 1.06-3.25, P = 0.031) and the homozygous model (OR = 1.91, 95%CI 1.08-3.38, P =0.025).

After adjusting for age and gender, *CEP128* rs12050151 was significantly related to genetic predisposition to AITD under the allele model, the dominant model, and the homozygous model (P<0.05) (Table 2). Subgroup analysis by types of AITD suggested that *CEP128* rs12050151 was significantly related to GD under the allele model (OR = 1.46, 95%CI 1.19-1.79, P <0.001) and the dominant model (OR = 1.56, 95%CI 1.23-1.98, P<0.001). However, *CEP128* rs12050151 was not significantly related to genetic predisposition to HT under all four comparison models (P>0.05).

3.3. Correlations of CEP128 with intrathyroidal immune cells

The composition of immune cells in GD tissues was successfully estimated by CIBERSORT tool through using gene expression profiles in GSE9340

(Supplementary table 3). As shown in the Figure 4, the mRNA expression level of CEP128 was significantly correlated with higher proportion of T-follicular helper (Tfh) cell in GD tissues (r = 0.48, P = 0.04), and was marginally correlated with higher proportion of M1 macrophages in GD tissues (r = 0.45, P = 0.058) (Figure 4). Moreover, the mRNA expression level of CEP128 was significantly correlated with lower proportion of resting memory $CD4^+$ T cells in GD tissues (r = -0.52, P = 0.026). Moreover, CEP128 was not differently expressed between GO patients and non-GO patients (Supplementary figure 3).

3.4 Correlations of CEP128 with key immune genes

The mRNA expression level of CEP128 was significantly correlated with the mRNA expression levels of STAT1 (r = 0.82, P < 0.0001), STAT3 (r = 0.57, P = 0.013) and BCL6 (r = 0.62, P = 0.006) in GD tissues, which were characterized transcription factors for either M1 macrophages or Tfh (Figure 5-A). The mRNA expression level of CEP128 was also significantly correlated with the mRNA expression levels of IL21 (r = 0.54, P = 0.019), IL1β (r = 0.75, P = 0.0004) and TNF-α (r = 0.76, P = 0.0003), which were characterized cytokines for either Tfh or M1 macrophages (Figure 5-A). Moreover, mRNA expression level of CEP128 was significantly correlated with IL4 (r = 0.74, P = 0.0005) and IL10 (r = 0.49, P = 0.04), but not with either IL6 (r = 0.01, P = 0.96) or TGFB1 (r = 0.36, P = 0.14) (Figure 5-B).

3.5 Functional pathways related to CEP128 in GD pathogenesis

The main findings in the GSEA analysis were shown in Table 3 (Table 3). Those significantly enriched functional pathways related to CEP128 in GD mainly included several common immune pathways involved in GD pathogenesis, such as interferon-γ (IFN-γ)-mediated signaling pathway, positive regulation of toll-like receptor (TLR) signaling pathway, regulation of interferon-α (IFN-α) production pathway, and MyD88-dependent TLR signaling pathway (Table 3). Supplementary figure 4 showed the enrichment plots for those top 4 enriched gene sets in the GSEA analysis (Supplementary figure 4).

4. Discussion

CEP128 is mainly localized to the microtubule organizing center, but its roles in autoimmune diseases including AITD are unclear (Monnich et al., 2018). In the

present study, we firstly explored the roles of CEP128 in AITD through conducting a GWAS analysis and a case-control validation study, and then further investigated the possible functional roles of CEP128 in through bioinformatics. This study suggests that *CEP128* is an important risk loci for AITD, and polymorphisms in *CEP128* contribute to genetic predisposition to GD and HT. Moreover, CEP128 expression level was significantly associated with the frequencies of Tfh and M1 macrophages and their characterized transcription factors or cytokines in GD tissues. Therefore, this study highlight the crucial role of CEP128 in the pathogenesis of GD, and polymorphisms in *CEP128* contribute to genetic predisposition to GD and HT.

Some studies have also suggested AITD in different ethnic populations is likely caused by distinct risk loci, such as *PTPN22*. For instance, *PTPN22* rs2476601 was substantially related to GD in Caucasian population, but its role in Asians was not found (Dultz et al., 2009; Heward et al., 2007; Ichimura et al., 2008). In the present study, the crucial role of *CEP128* gene in genetic predisposition to GD was firstly revealed in Caucasian population through data analysis of GeneATLA database. A recent study by Liley et al. reported that some SNPs in *CEP128* were likely to contribute to the difference in the genetic basis for GD and HT in Caucasian population, which also suggested that CEP128 may be involved in the pathogenesis of AITD (Liley et al., 2017). Our case-control study suggested *CEP128* was also an important risk locus of GD in Asians. Moreover, our study also confirmed the critical role of *CEP128* polymorphisms in genetic predisposition to HT, which expanded our knowledge on the role of *CEP128* in AITD. However, associations of *CEP128* rs327463 and rs12050151 with HT in Caucasians are still elusive, and need to be explored in future search.

Our study investigated the role of CEP128 in AITD through both genetic and functional perspective. Individuals with the CC genotype of *CEP128* rs327463 displayed increased predisposition AITD including GD and HT, suggesting the C allele was a risk variant in the development of AITD (Table 2). The significant associations of *CEP128* with AITD suggested the CEP128 may have a pathogenic role in AITD. However, we could not completely rule out the possibility of this variant acting as an enhancer by affecting the expression of TSHR because of its

proximity to *TSHR* gene. Several studies have suggested that some genes have interactions in their genetic associations with AITD even when they are not located close to each other or not in LD. For instance, *HLA-A*, *HLA-DRB1* and *HLA-DPB1* had synergistic interactions with *CTLA4* in the susceptibility to GD (Takahashi and Kimura, 2010; Kula et al., 2006). One our recent study also suggested that the existence of gene-gene interactions in the associations of *DNMT1*, *DNMT3A* and *DNMT3B* with GD even though those three genes were not located close to each other (Cai et al., 2016). In the present study, even *CEP128* and *TSHR* were not in LD, because *CEP128* is very close to *TSHR*, there is still possibility for the interaction between *CEP128* and *THSR* in their associations with AITD. However, the possible interaction above was unable to be explored in both our study and the Gene ATLAS database. Further studies are recommended to explore the possible gene-gene interactions between *CEP128* and *TSHR* in the development of AITD.

CEP128 is an essential component of a ciliation modulator circuit, and is a new negative regulator of ciliation (Gupta et al., 2015; Monnich et al., 2018). A recent study by Monnich et al. revealed that CEP128 has a conserved role in regulating transforming growth factor-β (TGF-β)/bone morphogenetic proteins (BMPs) signaling, and CEP128 loss in mammalian cells could result in impaired TGF-β/BMPs signaling (Monnich et al., 2018). BMPs are the largest subgroup of signalling ligands of TGF-β superfamily (Eixarch et al., 2018; Seeger et al., 2015). The TGF-β/BMP signaling is a multifunctional pathway, and it mainly regulates the cell proliferation, differentiation and apoptosis. TGF-β/BMP signaling can regulate the proliferation, differentiation and apoptosis and immune cells, and is an important modulator of the immune system (Chen and Ten Dijke, 2016; Seeger et al., 2015). BMP can bind to bone morphogenetic protein receptor of type 2 (BMPR2), and lead to subsequent changes (Eixarch et al., 2018). It has been well defined that TGF-β signaling has immunosuppressive effects in immune response, and it has been suggested to exert important roles in autoimmune diseases (Eixarch et al., 2018; Hadaschik and Enk, 2015; Postigo et al., 2016; Zhang and Bevan, 2012). Some studies revealed that some members of TGF-β superfamily were aberrantly expressed in AITD patients, implicating that the dysfunction of TGF-β/BMP signaling pathway

was involved in the pathogenesis of GD (Matsumoto et al., 2013; Pousada et al., 2018; Vural et al., 2009). TGF-β had an important immunoregulatory effect in thyroid autoimmunity and could inhibit autoreactivity in GD (Widder et al., 1991). Besides, BMP and TGF-β1 could suppress the expression of TSHR mRNA in thyrocytes and inhibit the growth of thyrocytes, suggesting TGF-β/BMP signaling could be involved in regulating thyrocyte growth and thyroid diseases (Franzen et al., 1999; Suzuki et al., 2005). A recent study proved that CEP128 could regulate the TGF-β/BMP signaling in mammalian cells, suggesting its roles in human diseases may be mediated by the TGF-β/BMP signaling (Monnich et al., 2018). However, it's still unclear whether CEP128 can regulate the immunity and autoimmunity by changing TGF-β/BMP signaling, which need to be elucidated in future studies.

There were obviously correlations of CEP128 expression level with the mRNA levels of both pro-inflammatory genes and several anti-inflammatory genes (Figure 5). Since those pro-inflammatory and anti-inflammatory genes are mainly expressed in immune cells and both pro-inflammatory and anti-inflammatory immune cells can infiltrate into thyroid tissues during the development or progression of GD, the obvious correlations above may be caused by the positive correlation of CEP128 with the number of thyroid-infiltrating immune cells in GD patients. The outcomes above further suggested that CEP128 was intensively related to the immune response in GD pathogenesis, but its molecular function remained elusive. The following GSEA analysis suggested that the possible functional pathways underlying the role of CEP128 in GD pathogenesis mainly included IFN-γ mediated signaling pathway and TLR signaling pathway (Table 3), both of which had been suggested to exert important roles in GD pathogenesis (Antonelli et al., 2015; Peng et al. 2016). Therefore, CEP128 is possibly involved in GD pathogenesis through regulating TLR signaling pathway or IFN-γ mediated signaling pathway.

CEP128 rs12050151 is an intron variant while rs327463 is a missense mutation in *CEP128* gene. *CEP128* rs327463 is a mutation from T to C, which results in a H732P amino acid substitution. By using PolyPhen-2 (a tool for the prediction of functional effects of human nsSNPs), *CEP128* rs327463 was predicted to be probably damaging with a score of 0.996, suggesting that this missense mutation is likely to have a

crucial impact on the function of CEP128 protein. However, the functional mechanisms by which *CEP128* rs327463 triggers the pathophysiological process of AITD are still unclear, which need to be elucidated in future research.

There was obvious difference in the allele frequencies and genotype distributions for both rs327463 and rs12050151 between GD cases and controls, but the difference between HT cases and controls was not statistically significant, which was likely caused by the limited sample size in the subgroup analysis in HT. However, as shown in the Table 2, rs327463 was significantly related to HT susceptibility under both the recessive model and the homozygous model, which proved the crucial role of rs327463 in the genetic predisposition to HT. Nevertheless, more upcoming studies with larger sample size are still needed to further verify the relationship between *CEP128* rs327463 and HT.

The ORs for the associations between *CEP128* rs12050151 and GD in our case-control study were larger than 1.40, which were consistent with that in the GWAS study from China (Chu et al., 2011). The ORs for the associations between *CEP128* rs327463 and GD in our case-control study were larger than 1.30 but not larger than 1.50 (Table 2). The outcomes above suggested that those two SNPs in *CEP128* had statistically obvious associations with GD but they did not display a strong influence on GD susceptibility. Therefore, more researches are needed to further determine whether those two SNPs are causative genetic variants for GD or HT.

According to the published article of GSE9340, the duration of GD until the time of thyroidectomy ranged from 9 months to 4 years with a median time of 18 months (Wescombe et al., 2010). Disease duration might influence the type of intrathyroidal infiltration of lymphocytes in GD patients, which may change the expression levels of candidate genes in GD thyroid tissues (Armengol et al., 2008). Because GSE9340 did not provide information on disease duration for each GD patient, the influence of GD duration on CEP128 expression in GD tissues was unable to be analyzed. Therefore, the findings in the present study of 18 GD patients need to be validated in future studies with more GD patients. Moreover, future studies are recommended to explore the clinical significance of CEP128 in GD patients, such as the correlations of

CEP128 expression with GD severity, disease duration and treatment outcomes.

In summary, this study highlight the crucial role of CEP128 in the pathogenesis of GD, and polymorphisms in *CEP128* contribute to genetic predisposition to GD and HT. More studies with larger sample size are needed to verify the roles of *CEP128* SNPs in genetic predisposition to AITD. In addition, the molecular mechanisms underlying the role of CEP128 in AITD and the function of *CEP128* rs327463 are still unclear, and it's necessary to address them in future studies.

Conflicts of interest: We declare that we have no conflicts of interest.

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Figure legends

Figure 1 Distribution of SNPs related to hyperthyroidism in the chromosomes from the GWAS study of GeneATLA database

Figure 2 Manhattan plot showing the Genome-wide association results for SNPs in the *CEP128-TSHR* region of Chromosome 14

Figure 3 Linkage disequilibrium analyses (shown as r^2) in African Americans, Chinese population and UK population by using 1000 Genomes phase 3 data

Figure 3-A Linkage disequilibrium analyses (shown as r^2) in Chinese population

Figure 3-B Linkage disequilibrium analyses (shown as r^2) in UK population

Figure 3-C Linkage disequilibrium analyses (shown as r^2) in African Americans

Figure 4 Correlations of CEP128 with intrathyroidal immune cells in GD tissues

Figure 5 Correlations of CEP128 with the mRNA expression levels of key immune genes in GD tissues

Figure 5-A Correlations of CEP128 with the mRNA expression levels of characterized transcription factors or cytokines for M1 macrophages or Tfh in GD tissues

Figure 5-B Correlations of CEP128 with the mRNA expression levels of anti-inflammatory genes in GD tissues

Table 1 Allele frequencies and genotype distributions of CEP128 polymorphisms in AITD cases and controls

Gene/SNP	Controls	AITD	P value	GD	P-Value	HT	P value
rs12050151							
T	1125	1289	0.001	787	0.001	502	0.079
C	263	401		257		144	
TT	452	483	0.004	288	0.002	195	0.167
TC	221	323		211		112	
CC	21	39		23		16	
rs327463							
T	1063	1228	0.013	754	0.014	474	0.117
C	325	462		290		172	
TT	401	444	0.032	266	0.042	178	0.058
TC	261	340		222		118	
CC	32	61		34		27	

(AITD, autoimmune diseases; GD, Graves' disease; HT, Hashimoto's thyroiditis)

Table 2 Odds ratios (ORs) of the associations of CEP128 polymorphisms with AITD before and after adjusting for confounders (age and gender)

Comparison models	Unadjusted estimates		Adjusted estimates*	
	OR(95%CI)	P values	OR(95%CI)	P values
rs12050151 and AITD				
Allele model	1.35(1.13-1.62)	0.001	1.38(1.15-1.66)	0.001
Dominant model	1.40(1.14-1.72)	0.001	1.44(1.17-1.78)	0.001
Recessive model	1.55(0.90-2.66)	0.112	1.55(0.89-2.70)	0.118
Homozygous model	1.74(1.01-3.00)	0.047	1.78(1.02-3.11)	0.044
rs12050151 and GD				
Allele model	1.43(1.17-1.75)	0.001	1.46(1.19-1.79)	<0.001
Dominant model	1.52(1.20-1.92)	<0.001	1.56(1.23-1.98)	<0.001
Recessive model	1.48(0.81-2.70)	0.205	1.52(0.83-2.80)	0.176
Homozygous model	1.72(0.93-3.16)	0.082	1.82(0.98-3.38)	0.059
rs12050151 and HT				
Allele model	1.23(0.98-1.56)	0.076	1.22(0.96-1.55)	0.112
Dominant model	1.23(0.93-1.61)	0.142	1.22(0.92-1.63)	0.165
Recessive model	1.67(0.86-3.25)	0.130	1.52(0.76-3.07)	0.239
Homozygous model	1.76(1.02-3.03)	0.041	1.69(0.96-2.99)	0.070
rs327463 and AITD				
Allele model	1.24(1.05-1.46)	0.012	1.28(1.08-1.52)	0.004
Dominant model	1.24(1.01-1.51)	0.040	1.30(1.06-1.60)	0.013
Recessive model	1.61(1.04-2.50)	0.034	1.62(1.03-2.53)	0.036
Homozygous model	1.72(1.10-2.70)	0.018	1.78(1.12-2.81)	0.014
rs327463 and GD				
Allele model	1.27(1.05-1.54)	0.012	1.31(1.08-1.59)	0.006
Dominant model	1.32(1.05-1.66)	0.018	1.37(1.09-1.72)	0.008
Recessive model	1.44(0.88-2.37)	0.149	1.46(0.88-2.40)	0.143
Homozygous model	1.60(0.96-2.66)	0.069	1.66(0.99-2.77)	0.054
rs327463 and HT				
Allele model	1.19(0.96-1.48)	0.116	1.23(0.98-1.55)	0.068
Dominant model	1.11(0.85-1.45)	0.423	1.19(0.90-1.57)	0.230
Recessive model	1.89(1.11-3.21)	0.019	1.85(1.06-3.25)	0.031
Homozygous model	1.90(1.11-3.27)	0.020	1.91(1.08-3.38)	0.025

(AITD, autoimmune diseases; OR, Odds ratio; 95%CI, 95% confidence interval; * Age and gender were adjusted in the multivariate logistic regression analyses.)

Table 3 Summary of significantly enriched pathways in the GSEA analysis

Gene set	ES	Nominal P value	Core genes
Interferon-γ mediated signaling pathway	0.665	0.052	OASL, HLA-A, CD44, PTAFR, STAT1, IRF1, IFNGR1, IFI30, TRIM38, HLA-E, HLA-F, OAS2, ICAM1, HLA-G, HLA-B, TRIM25, HLA-DRB4, IFNG, HLA-DPB1, HLA-DQB2, HLA-DRA, HLA-C, CIITA, IRF9, TRIM21, HCK, HLA-DRB3, IRF5, HLA-DQB1, HLA-DPA1, CAMK2D, SP100, GBP2, CAMK2G, TRIM22, GBP1, JAK2, IRF4, OAS3, VCAM1, OAS1, TRIM68, IRF8
Positive regulation of toll-like receptor signaling pathway	0.702	0.015	TICAM2, NR1H3, TLR5, WDFY1, PIK3AP1, TLR2, TLR3, PELI1, PTPN22, RSAD2, TIRAP, CYBA, TLR9
Regulation of interferon-α production	0.722	0.021	IFIH1, RIPK2, TLR7, HAVCR2, TLR8, DDX58, ZC3HAV1, IRF5, TLR3, TBK1, NMI
MyD88-dependent toll-like receptor signaling pathway	0.672	0.020	TLR5, IRAK2, TLR1, TLR7, CD14, MYD88, LY96, TLR8, TLR2, IRAK1, UBA52, TLR6, TNIP1, TIRAP, MAP3K7, TLR9
Response to type I interferon	0.617	0.074	IFNAR2, OASL, IFIT2, HLA-A, STAT2, PSMB8, STAT1, IRF1, IFI35, TYK2, ADAR, HLA-E, HLA-F, OAS2, HLA-G, IFITM1, HLA-B, HLA-C, SHMT2, IRF9, XAF1, IKBKE, IRF5, TRIM56, MX1, ISG15, SP100, GBP2, IFITM3, RSAD2, IFITM2, IRF4, OAS3, BST2, OAS1
Detection of biotic stimulus	0.669	0.056	TREM2, HLA-A, TLR1, LY96, NOD2, HLA-B, HLA-DRB4, TLR2, HLA-DRB3, TLR6, CD1D, C4B, NLRC4, SCARB1, NLRP3
Detection of other organism	0.708	0.043	HLA-A, TLR1, NOD2, HLA-B, HLA-DRB4, TLR2, HLA-DRB3, TLR6, CD1D, NLRC4
Toll-like receptor-4 signaling pathway	0.721	0.065	TICAM2, RIPK2, ITGAM, CD14, LY96, PIK3AP1, LGALS9, IRAK1, TNIP3, ITGB2, TIRAP
Response to interferon-alpha	0.643	0.0615	IFNAR2, IFIT2, ADAR, IFITM1, GATA3, IFITM3, IFITM2, LAMP3, AXL, BST2, OAS1, MX2, EIF2AK2, IFIT3, STAR, KLHL20, ENTPD2, GAS6

(ES, Enrichment score. Gene sets with both an ES more than 0.60 and a nominal P value < 0.10 were considered significantly enriched pathways)

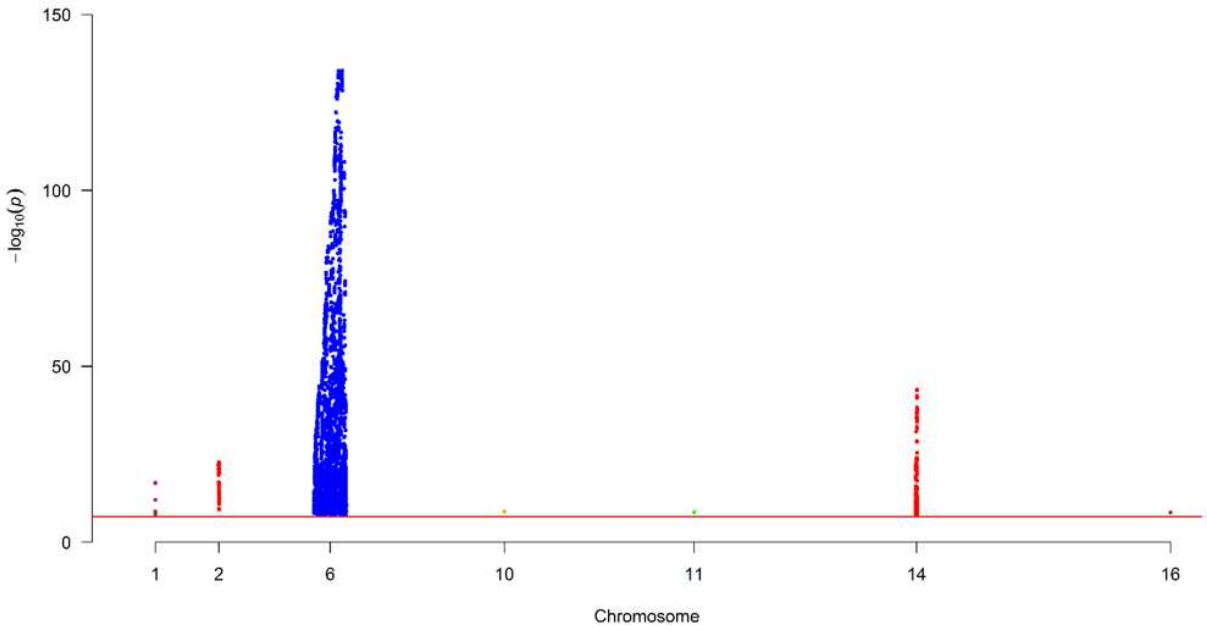

Figure 1 Distribution of SNPs related to hyperthyroidism in the chromosomes from the GWAS study of GeneATLA database

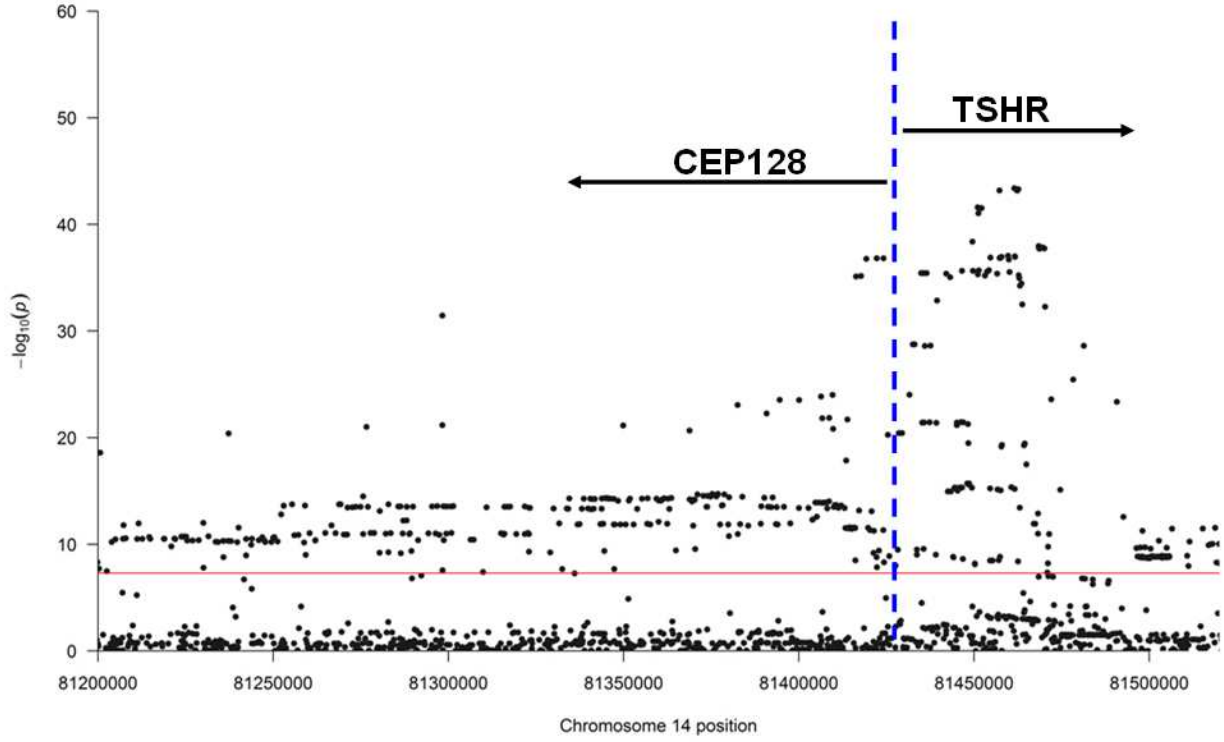

Figure 2 Manhattan plot showing the Genome-wide association results for SNPs in the *CEP128-TSHR* region of Chromosome 14

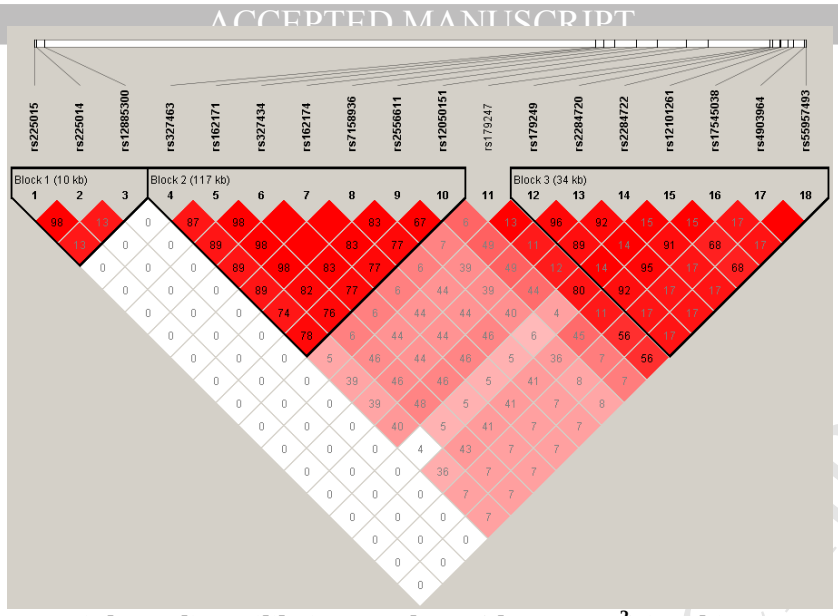

Figure 3-A Linkage disequilibrium analyses (shown as r^2) in Chinese population

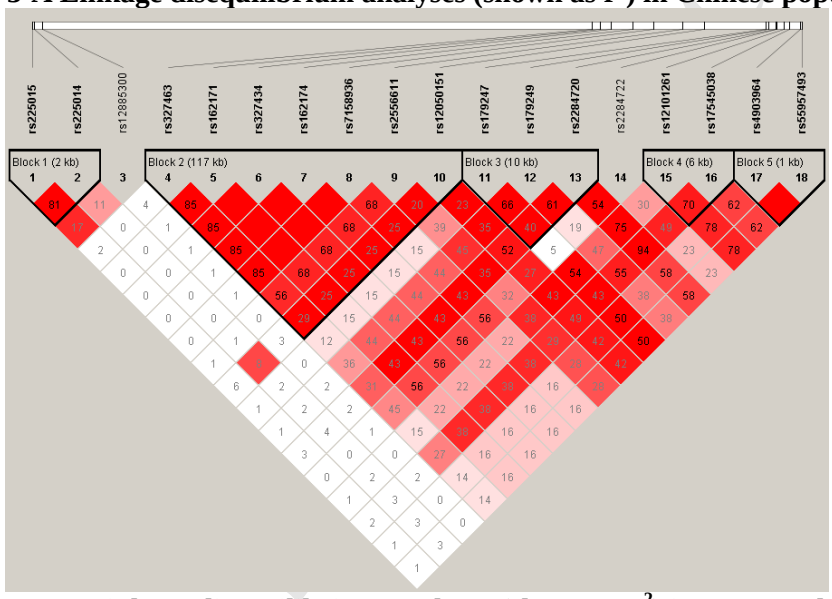

Figure 3-B Linkage disequilibrium analyses (shown as r^2) in UK population

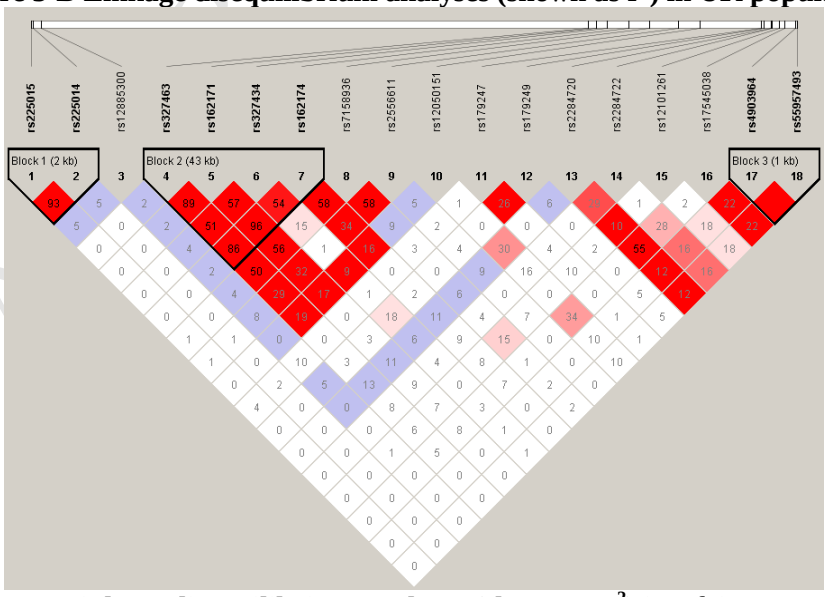

Figure 3-C Linkage disequilibrium analyses (shown as r^2) in African Americans

Figure 3 Linkage disequilibrium analyses (shown as r^2) in African Americans, Chinese population and UK population by using 1000 Genomes phase 3 data

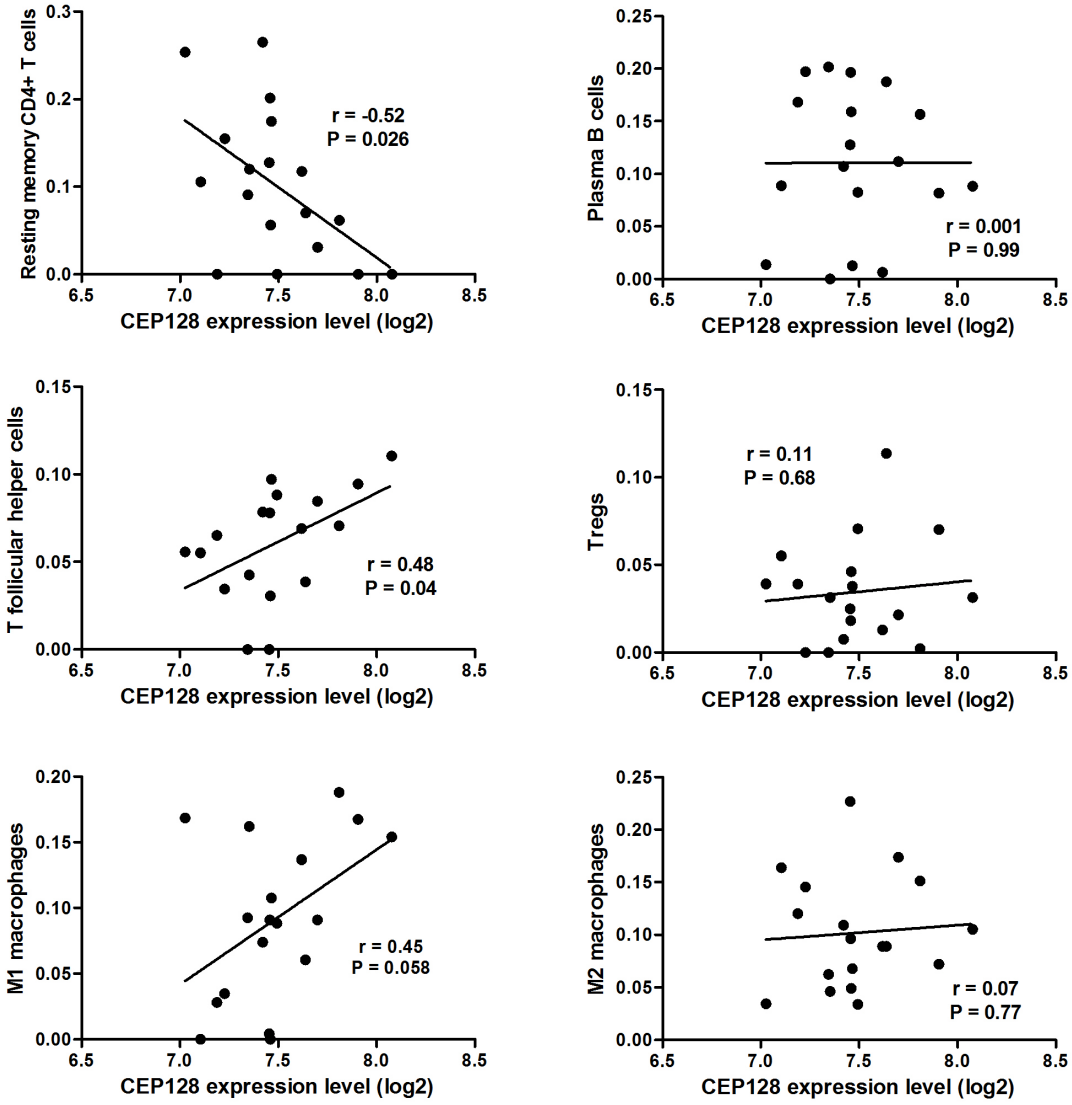

Figure 4 Correlations of CEP128 with intrathyroidal immune cells in GD tissues

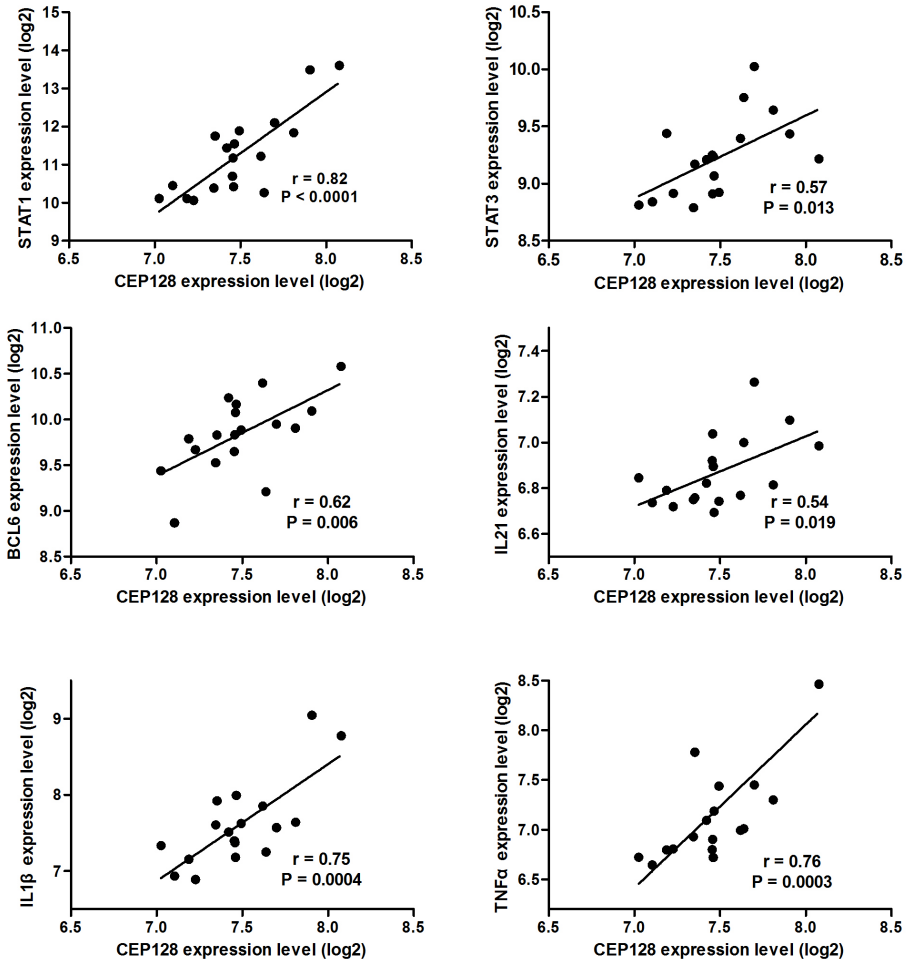

Figure 5-A Correlations of CEP128 with the mRNA expression levels of characterized transcription factors or cytokines for M1 macrophages or Tfh in GD tissues

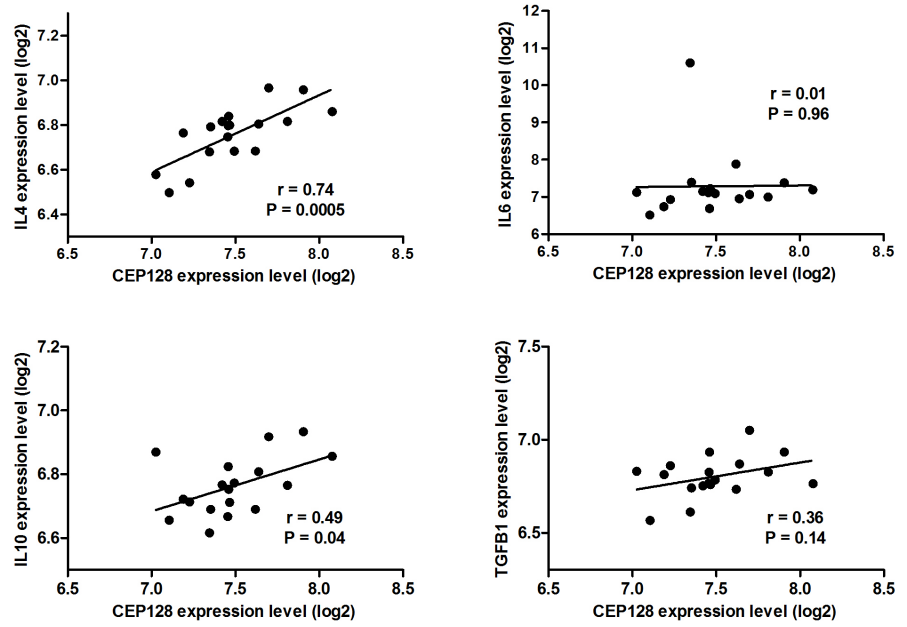

Figure 5-B Correlations of CEP128 with the mRNA expression levels of anti-inflammatory genes in GD tissues

Figure 5 Correlations of CEP128 with the mRNA expression levels of key immune genes in GD tissues

Highlights

1. CEP128 is a crucial risk locus for autoimmune thyroid diseases.

2. rs327463 is significantly related to Graves' disease and Hashimoto's thyroiditis.

3. CEP128 is correlated with Tfh and M1 macrophages in thyroid tissues.