Expression quantitative loci for TLR6 which leads cytokine secretion important in pathogenesis of AD

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1. Introduction

Atopic dermatitis (AD) is a recurrent, chronic skin inflammation disease that results in itchy, red, and dry skin. According to an Epidemiological research on 2018, 15-30% of children and 2-10% of adults are suffering from the disease [1]. Through plenty of studies, the abnormal secretion of Th2 cytokines which orchestrate the allergic immune response has been suggested as a major reason so far [2]. In production of Th2 cytokines, TLR6 expression acts as the initiation by triggering various immune-pathways leading Th2 cell differentiation and cytokine secretion. Therefore, study of TLR6 is needed to understand the pathogenesis of AD. Expression of certain gene is highly affected by the alteration of nucleotides (SNPs). This study aimed to find significant SNPs that might regulate TLR6 expression.

2. Methods and Materials

2.1 Materials

This study was conducted with the TLR6 mRNA expression data from 373 Europeans' Lymphoblastoid cell lines which were examined by Geuvadis RNA-sequencing project. Phenotype data contains gene expression levels calculated with reads per kilobase per millions mapped reads (RPKM) from 1000 Genomes Project. This data excludes sex chromosome (chromosome 23) and is based on Genome Reference Sequence(GRCh37/hg19). Genotype data contains individuals' SNPs information and genotypes information. 5,913,563 SNPs with Minor allele frequency (MAF) over 5% and Hardy Weinberg Equilibrium (HWE) over 1×10^{-6} were used.

2.2 Methods

Using PLINK ver. 1.90, we performed Linear regression analysis between allele and gene expression applying additive model and p-value threshold 5×10^{-8} to find significant SNPs. With Haploview (Broad Institute), we constructed Linkage Disequilibrium blocks (LD blocks). R programming helped us to web-crawl dbSNP, reapply proper model for linear regression analysis by F-test and T-test and visualize various results. For identifying function of SNPs, UCSC GeneHancer, HaploReg ver. 4.1, chIA-PET data were used. From UCSC GeneHancer, we could get the location of SNP and the interaction between genes. HaploReg ver. 4.1 showed us possible chromatin states and regulatory functions of SNPs especially in Lynphoblastoid cells. With chIA-PET data, we checked if our SNPs are in promoter or enhancer region. Finally, Gene Cards, NCBI, UNIPROT were used for searching function of genes where SNPs are located and STRING for searching interaction between proteins.

3. Results

As a result of Linear regression analysis with PLINK ver. 1.90, we got 142 SNPs closely related with

TLR6 expression (p-value < 5 x 10^{-8}) (Figure.1, Supplementary Table.1). Except 2 SNPs, rs10081995 on chromosome 1 and rs188472507 on chromosome 10, all SNPs were on chromosome 4 within 100,000 bp from TLR6 gene. These SNPs were on TLR1, TLR6, TLR10, FAM114A1, LOC105374412 or not. 140 SNPs constructed 5 LD blocks and left 2 single SNPs (Supplementary Figure.2). Among SNPs on each LD blocks, we picked 5 representative SNPs regarding its p-value, location, and function. Including the single SNPs, 7 SNPs were selected as captain SNPs that will be studied (Table.1). F.test and T.test were done between selected SNPs and TLR6 expression datasets. 6 SNPs met the standard of additive model linear regression, but rs55963023 turned out to be proper to apply dominant model which gave higher beta value (Supplementary Figure.1). Due to UCSC and chIA-PET data, 4 SNPs out of 7 were located on the enhancer, promoter which targets TLR6 or on TLR6. Some of them were reported to have epigenetic markers related to transcription level(Supplementary Table.2). From Haploreg, we could find the regulatory chromatin state markers in the SNP region and the change of protein affinity depending on the shift of nucleotide. Other 3 SNPs turned out not to be on the part that is strongly associated with transcription. However, except one SNP, rs73236625, that has no information, other 2 were reported on Haploreg to have regulatory chromatin state and change the protein affinity.

Table.1 Representative SNPs selected for study. Single SNPs are highlighted in gray.

LD	Chr	SNPs	Genes	Bp	Ref > Minor	Beta-value	P-value
1	4	rs116781354	LOC105374412	38753751	G>T	2.728	1.19E-08
2	4	rs10023850	NA	38762072	G>A	3.083	3.71E-09
3	4	rs5743566	TLR1 Non Coding Transcript Variant	38804321	G>C	3.124	1.29E-14
4	4	rs67206233	TLR1 5 Prime UTR Variant	38806232	T>C	3.859	3.13E-14
5	4	rs4833096	TLR1 2KB Upstream Variant	38807011	T>C	3.579	2.30E-13
6	4	rs73236625	NA	38808303	G>A	3.886	4.10E-14
7	4	rs55963023	TLR6 Intron Variant	38855125	T>C	3.514	1.45E-12

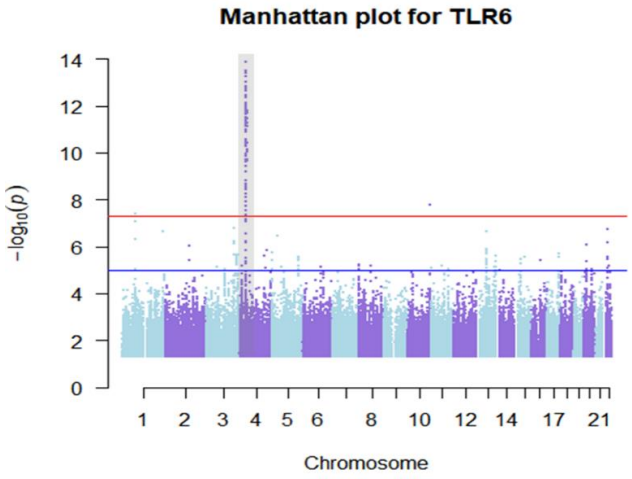

Figure.1 Manhattan plot for Genome-wide association of SNPs with mRNA expression of TLR6. P-values were log-scaled and the red line indicates p-value threshold 5 x 10^{-8}.

4. Discussion

In this study, we found 140 cis-acting SNPs that are closely related to the upregulation of TLR6. With these SNPs, we picked 7 representative SNPs from 5 LD blocks and 2 single SNPs. 7 representative SNPs were on TLR1 intron, exon site, TLR6 intron site, LOC105374412 intron site or none of the genes.

rs67206233, rs5743566 are on GH04J038803 promoter. GH04J038803 promoter targets TLR6 first exon meaning that this sequence will regulate transcription of TLR6. On rs5743566, H3K27Ac mark which indicates higher activation of transcription was found in lymphoblastoid cell line. Moreover, this site was DNase hypersensitive site which shows this region is not densely packed. From HaploReg, we could find the alteration of nucleotide G>C increased the affinity of RREB-1 protein which promotes the promoter activity and gene transcription [3]. With this information, we could presume that rs5743566 minor allele will up-regulate gene TLR6. In case of rs67206233, H3k27Ac mark also existed showing regulatory function of the SNP. Especially, this region was found to be a part of the IKZF1 transcription factor binding site. IKZF1 is a Zinc finger protein that regulates immune cell development in early B cells and CD4+ T cells and differentiate function of individual T helper cells [4]. Therefore, we could take that this region not only affects gene expression but also regulate immune cells.

rs10023850 is on enhancer GH04J038759. This enhancer also targets TLR6 first exon indicating that transcription of TLR6 gene might be regulated by this sequence. According to Haploreg ChIP-seq data, this site might have H3K27Ac mark and is highly sensitive with DNase activation. These both mean that transcription factors can bind here easily. Since this site acts as a regulatory site, this SNP is presumed to higher the transcription level of TLR6. rs55963023 is on the TLR6 promoter right anchor which is near the promoter due to chIA-PET data. To initiate transcription, activator on enhancer and RNA polymerase on promoter should interact via mediator protein. The way to make this possible is forming a loop between the strands that contains enhancer and promoter respectively [5]. Locating on right anchor means that it takes role on connecting proteins and start transcription. Likewise, rs55963023 is thought to regulate expression of TLR6.

When TLR6 is expressed and complexed with TLR2, it initiates Toll like receptor signaling pathway. This Toll like receptor signaling stimulates intracellular NF-κB signaling, which is well known to induce pro-inflammatory genes and activates innate immune responses [11,12]. It is known that blocking NF-κB signals is one of the ways to ameliorate AD symptoms by down regulating Th2 responses [13]. This inversely means that higher the signals transmitted, higher Th2 responses will occur resulting in worsened AD symptoms. Therefore, up-regulated TLR6 will over activates NF-κB signaling and over activated NF-κB signaling will promote Th2 responses, eventually worsening the symptoms. Besides, over expressed NF-κB signals induces expression of IL-33 and TSLP which participate in Th2 cytokine production [14,15]. IL-33 is a pro-Th2 cytokine which induce Th2 cytokine production and TSLP is a novel IL-7-like cytokine which induce T cell differentiation to Th2 and leads secretion of cytokines [8,9]. These two cytokines are also reported to have a high positive correlation with TLR6 [6,7]. Accordingly, we could know TLR6 upregulation will lead to Th2 cytokine production via NF-κB signaling. Since 4 SNPs above are responsible for TLR6 over expression, we considered rs5743566, rs67206233, rs10023850 and rs55963023 to be a significant eQTLs that might give an impact to AD pathogenesis though more researches for the mechanism should be done (Figure.2).

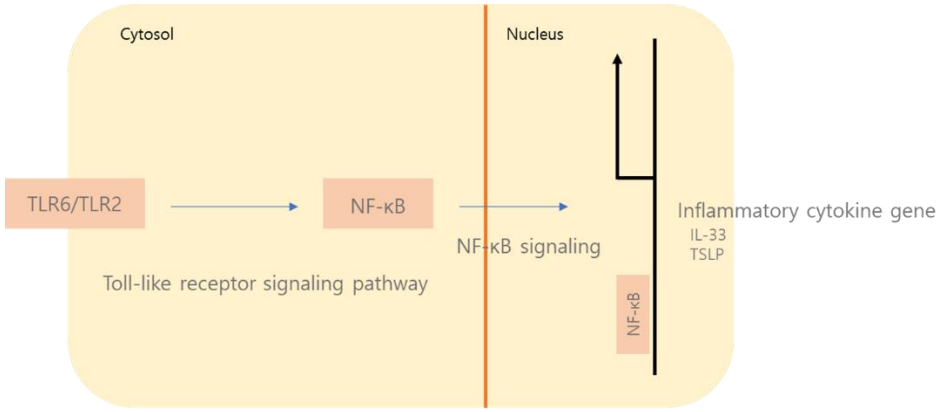

Figure.2 Simple schematic of cytokine producing pathway including TLR6 and NF-κB transcription factor.

rs116781354 is assigned as LOC105374412 500bp Downstream Variant. LOC105374412 is known as RNA gene affiliated with the ncRNA class but the function has not been revealed. Through databases, we could only find epigenetic marker from lymphoblastoid. With lack of information, it was hard to determine the clear association of SNP and gene expression. rs4833096 is located 11bp far, which is very near, from the GH04J038803 promoter targeting TLR6. At dbSNP, it is assigned as TLR1 2kb Upstream Variant. According to STRING, TLR1 and TLR6 are co-expressed when pathogen invades our body [10]. Even so, the information could not elucidate how rs4833096 affects TLR6. PLINK also selected rs73236625 as SNP associated with TLR6 expression. However, we even could not find information about rs73236625 itself, so it was impossible to check relation between them. To unveil the association between 3 SNPs andTLR6 expression, further studies should be performed.

5. Conclusion

To wrap up, we discovered 142 SNPs affecting TLR6 expression and picked 7 captain SNPs for study. Among those, 4 SNPs seemed to have explicit evidences of altering expression level of TLR6 and assumed to give an impact on pathogenesis of AD. Rest of the SNPs still affects TLR6 expression but did not have them. Yet, every SNPs need more experimental researches to make the mechanism of regulating gene expression and relation with pathogenesis of AD clear.

5. Reference

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