



Brief Communication

Autoimmunity-associated intronic SNP (rs2281808) detected by a simple phenotypic assay: Unique case or broader opportunity?



Sushmita Sinha^a, Pranav S. Renavikar^a, Michael P. Crawford^a, Jake W. Rodgers^a, Eva Tsalikian^b, Michael Tansey^b, Nitin J. Karandikar^{a,*}

^a Departments of Pathology, University of Iowa Health Care, Iowa City, IA 52242, United States

^b Departments of Pediatrics, University of Iowa Health Care, Iowa City, IA 52242, United States

ARTICLE INFO

Keywords:

SNP rs2281808
SIRP γ
Autoimmunity
Diagnostic
Flow cytometry

ABSTRACT

Multiple genome-wide association studies have shown that the single-nucleotide polymorphism (SNP) rs2281808 TT variant, present within the signal regulatory protein gamma (SIRPG) gene, is associated with autoimmune diseases, such as type 1 diabetes. SIRP γ is the only SIRP expressed on T cells. The role of SIRP γ in human T-cells or the effect of the TT variant are poorly understood. In this short report, we demonstrate the rather unusual finding that this intronic SNP is associated with a reduction of SIRP γ expression on T cells, both in healthy subjects as well as patients with type 1 diabetes. Using this information, we propose that a simple flow cytometric detection of SIRP γ could be a potential diagnostic testing approach for the presence of SNP in the appropriate clinical context.

1. Introduction

Signal Regulatory Protein γ (SIRP γ) is the only SIRP expressed by human T-cells and is absent in rodents [1,2]. While its function is poorly understood, SIRP γ is one of the ligands for CD47, expressed on antigen-presenting cells and a potential target for cancer immunotherapy [3]. SIRP γ may also be involved in trans-endothelial migration of human T-cells [4].

rs2281808 is an intronic SNP present between exons 5 and 6 of the SIRPG gene and causes a C/T variant. The TT variant is associated with a greater risk for autoimmunity, particularly type 1 diabetes [5–7]. To gain a functional understanding of this SNP, we recently demonstrated that the rs2281808 TT SNP was associated with heightened effector state in CD8+ T-cells [8].

Interestingly, while conducting phenotypic and functional studies in the context of the CC, CT and TT genotypes, we discovered the rather unusual finding that this *intronic* SNP resulted in changed expression of the protein on the surface of both CD4+ and CD8+ T-cells. In this short report, we evaluated whether the evaluation of protein expression by this simple flow cytometric approach could be “diagnostic” of the CC, CT or TT genotypes.

2. Materials and methods

2.1. Human subjects

2.1.1. Ethics statement

All studies were approved by the University of Iowa IRB according to Declaration of Helsinki principles.

2.1.2. Patients and control subjects

After obtaining informed consent, 19 type 1 diabetes (T1D) patients were recruited at the pediatric endocrinology clinics, University of Iowa. De-identified leukoreduction buffy coat samples from 145 healthy donors (HD) were obtained from the University of Iowa DeGowin Blood Center, Department of Pathology. Age range of HD was 25–76 y (52 ± 15) and T1D subjects was 18–29 y (20 ± 3).

2.1.3. Cell preparation and genotyping

PBMC were isolated from all the samples using Ficoll Hypaque (GE Healthcare Biosciences, Pittsburgh, PA) density gradient. The cells were stored in liquid nitrogen until further use. An aliquot of PBMC were used to extract genomic DNA using Qiagen mini DNA prep kit. For rs2281808 genotyping, allelic discrimination PCR was done using TaqMan assay and probes.

* Corresponding author at: Professor and Chair of Pathology, University of Iowa, 200 Hawkins Dr., Iowa City, IA 52242, United States.

E-mail address: nitin-karandikar@uiowa.edu (N.J. Karandikar).

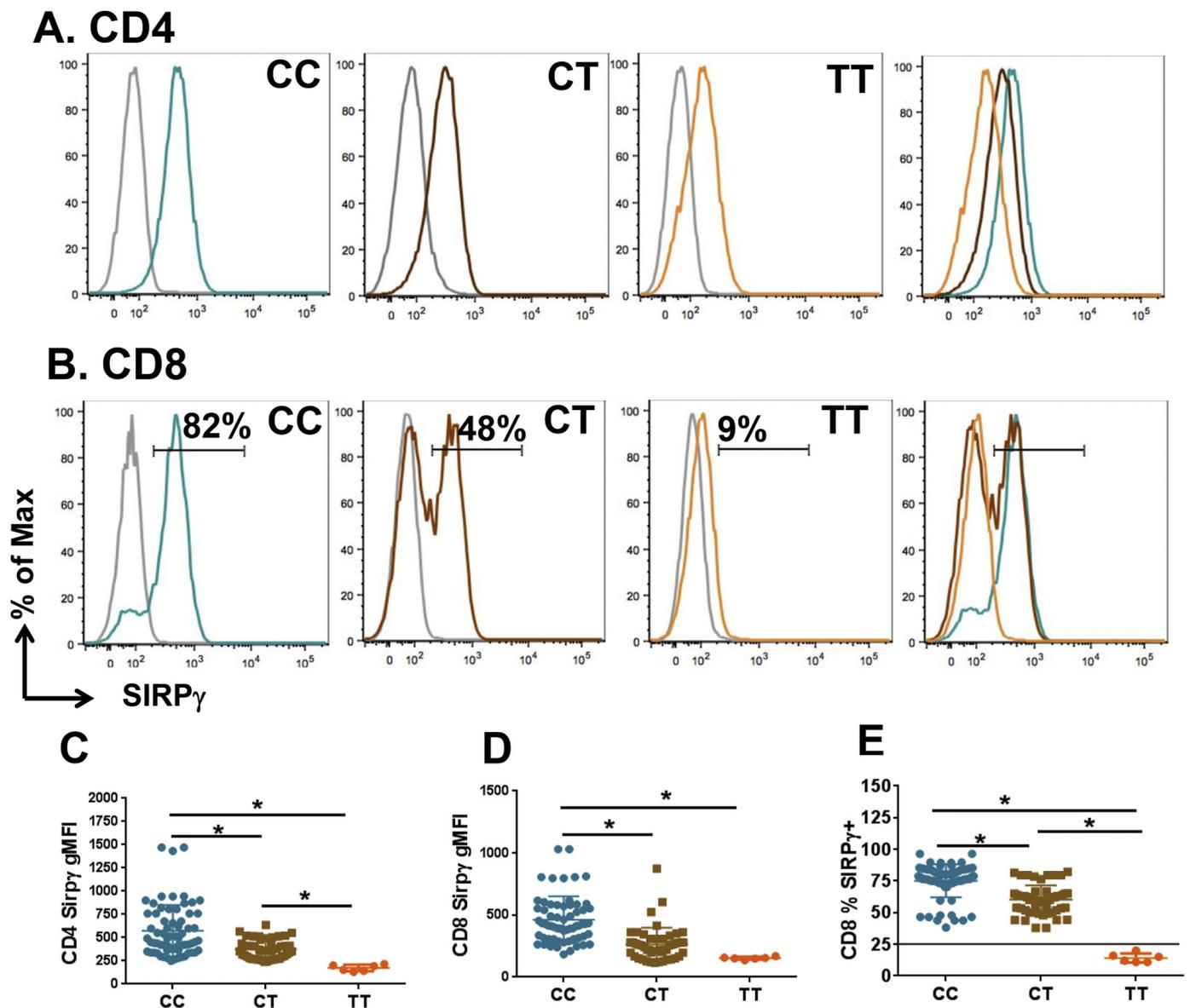


Fig. 1. Low SIRP γ expression is diagnostic of TT genotype in healthy subjects.

PBMC derived from de-identified healthy blood donors were stained for CD3, CD4, CD8 and SIRP γ . Panel A shows representative examples of SIRP γ expression on gated CD4+ T-cells from subjects with a CC, CT or TT genotype, as indicated. The grey histogram depicts isotopic control staining and the rightmost panel shows overlapping histograms from CC, CT and TT, maintaining the same colors, showing decreasing expression of SIRP γ from CC to TT. Panel B shows similar representative examples of SIRP γ expression on gated CD8+ T-cells. The bimodal expression pattern SIRP γ expression on CD8+ T-cells allowed quantification of % SIRP γ + cells within CD8+ T-cells, indicated by the numbers. Panels C-E show cumulative SIRP γ expression data from 79 CC, 60 CT and 6 TT subjects. Panels C and D represent mean fluorescence intensity (MFI) on CD4 and CD8 T-cells, whereas Panel E shows %SIRP γ + CD8+ T-cells. The horizontal black line in Panel E represents a cutoff at 26% expression, based on 3SD interval of the CT expression data, as described in the text. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.1.4. Flow cytometry staining for SIRP γ

PBMC were stained with anti-human CD3-Alexa700, CD4-APC, CD8-BV786 and SIRP γ -PE. All the antibodies were obtained from either BD Biosciences (San Jose, CA), or Biologend (San Diego, CA). Following staining, cells were washed and data was acquired on 4-Laser LSRII using FACSDiva software (Becton Dickinson). Data were analyzed using Flow Jo (TreeStar, Ashland, OR).

2.1.5. Statistical analysis

Data between the groups was analyzed with 2-way ANOVA with Tukey's posthoc test and $p < 0.05$ was considered significant. Chi-Square test was used to compare the HD vs. T1D genotyping data and $p < 0.05$ was considered significant.

3. Results

3.1. Low SIRP γ expression is diagnostic of TT genotype in healthy subjects

PBMC samples from 145 healthy donors were subjected to rs2281808 genotyping and flow cytometry staining to study the surface expression of SIRP γ expression on CD4 and CD8 T-cells. As reported earlier and now confirmed in greater number of samples, this *intronic* SNP resulted in changed expression of the protein on the surface of both CD4+ and CD8+ T-cells (Fig. 1A–D). Thus, individuals with the CC genotype showed the highest expression of SIRP γ , the CT genotype corresponded to medium-level expression, whereas the TT genotype showed the lowest expression.

While the mean fluorescence intensity (MFI) was statistically

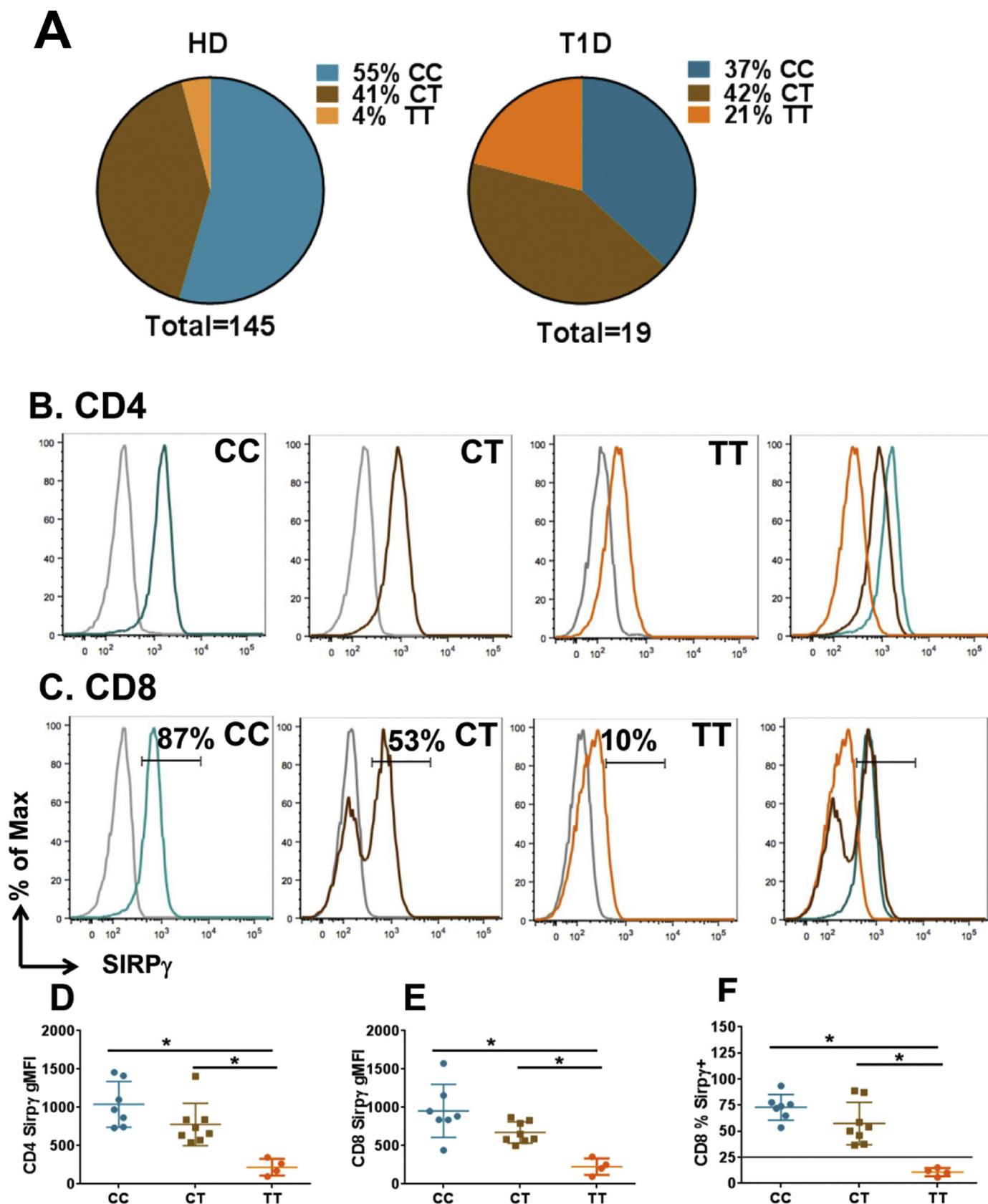


Fig. 2. Low SIRP γ expression is diagnostic of TT genotype in T1D. Panel A shows the distribution of CC, CT and TT genotypes amongst 145 healthy donors and 19 patients with type 1 diabetes (T1D). Panels B–F are displayed in a pattern similar to Panels A–E of Fig. 1, showing SIRP γ expression on CD4 and CD8 T-cells from T1D subjects. Fig. 2F uses the same cutoff (black line) of 26% from Fig. 1E, showing that all T1D subjects with the TT genotype had %SIRP γ + CD8+ T-cells lower than the cutoff.

different amongst the three groups, both on CD4 and CD8 T-cells (Fig. 1C–D), this readout was not amenable to a clean cutoff due to overlap at the lower ends, particularly on CD8+ T-cells (Fig. 1C–D). However, we observed that the bimodal expression of this molecule on CD8+ T-cells allowed us to quantify the percentage of cells that were positive/high vs. negative/low for this marker (Fig. 1B). Specifically, looking at % SIRP γ -positive CD8+ T-cells, we observed that CC individuals showed a mean of 74% with a 2SD interval of 45–102 and a 3SD interval of 31–117 (Fig. 1E). The CT genotype showed a mean of 61% SIRP γ +CD8+ T-cells with a 2SD interval of 38–84 and a 3SD interval of 26–96. Again, while this difference between CC and CT was statistically significant, it showed overlap between the two groups, with no clear cutoff.

Interestingly, the TT genotype, which is associated with autoimmunity, was prominent in having a significantly lower proportion of % SIRP γ +CD8+ T-cells, with a mean of 14%, a 2SD interval of 7–22 and 3SD interval of 4–25. This allowed for a clean cutoff of 26%, based on the lower end of 3SD interval of the CT group, where 100% of the non-TT cohorts were above the cutoff and vice versa, as shown by the horizontal black line in Fig. 1E.

3.2. Low SIRP γ expression is diagnostic of TT genotype in T1D

The rs2281808 SNP is known to be over-represented in autoimmune diseases, particularly in type 1 diabetes (T1D). Therefore, we recruited a cohort of patients with T1D ($n = 19$) to test the whether this “diagnostic cutoff” would also work in the context of autoimmune disease, where there could potentially be a larger spread of SIRP γ expression.

As anticipated, we found a significantly higher proportion of T1D patients expressing the TT genotype (4/19, 21%), compared to healthy subjects (6/145, 4%, $p < 0.05$) (Fig. 2A). Once again, similar to healthy subjects, CD4 and CD8 T-cells from T1D patients also showed statistically different levels of SIRP γ expression between CC, CT and TT individuals (Fig. 2B–E). While expression in CC and CT subjects was statistically different in terms of MFI expression as well as % SIRP γ +CD8+ T-cells, the data were not amenable to a diagnostic cutoff between these two groups due to significant overlap. However, once again, we were able to use the cutoff for % SIRP γ +CD8+ T-cells determined from 3SD intervals of healthy subjects (26%, Fig. 1E) to “diagnose” the TT genotype with 100% sensitivity and specificity, as shown in Fig. 2F.

4. Discussion

In this Brief Communication, we present this interesting example of a simple phenotypic assay of protein expression tightly correlating with an intronic SNP. While we are aware of examples of certain point mutations in coding sequences being detected by changed proteins (e.g., sickle hemoglobin) and also SNPs resulting in different isoforms, to our knowledge, this is the first example of a SNP (especially an intronic SNP) resulting in a dramatic change in protein expression, to the extent of potentially being used as a robust “diagnostic/prognostic test,” in the appropriate clinical context.

Despite significant age differences between the HD and T1D groups, SIRP γ expression on T-cells from T1D subjects with the TT genotype was significantly lower than those with CC genotype, demonstrating that neither the younger age range nor the autoimmune disease status of these subjects alter this association. In corroboration of this finding, we also did not observe a correlation between age and SIRP γ expression within HD. In the same vein, we have also observed the same tight correlation between the TT genotype and low SIRP γ expression in the setting on multiple sclerosis, a different immune-mediated disease in a patient population with age range similar to our HD cohort (data not shown).

At this point, we are not sure how this intronic SNP causes reduction in protein expression. It might create an alternative splice variant of

SIRP γ resulting in unstable RNA or loss of protein. It has been suggested that in the thymus, this SNP is located within the predicted enhancer [9], raising the possibility that its presence could potentially alter the transcription of SIRP γ . This can be a focus of future investigation.

In addition, the bimodal expression of this protein on CD8 T-cells (but not CD4 T-cells), particularly in CT individuals, also suggests distinct regulatory mechanisms. While the mechanism of this bimodal expression is not understood, it does provide the ability to create an expression cutoff that can be used to predict the TT genotype. As shown recently [8], in CC individuals, the vast majority (> 90%) of SIRP γ ^{low} CD8 T-cells were present in memory/terminally-differentiated fraction and not in the naïve T-cell fraction, indicating an acquired SIRP γ ^{low} phenotype post-antigenic exposure. However, in CT as well as TT subjects, a significant proportion of naïve CD8 T-cells show low SIRP γ expression, indicating the genotypic influence even as these cells emerge from the thymus. It will be important to understand the intrinsic regulation of expression in naïve T-cells versus one that happens post-antigenic response.

Our finding could also spur investigation of correlation between such SNPs and protein expression to see if there is a broader applicability of this principle, both in the biology of genotype-phenotype conversion and for potential diagnostic testing.

Funding

These studies were supported, in part, by grants to NJK from the National Institutes of Health (R01AI092106) and the National Multiple Sclerosis Society.

Author contributions statement

S.S. and N.J.K. were involved in study design, interpretation of results, and preparation of the manuscript; S.S., P.S.R., M.P.C. and J.R. performed the experiments, M.P.C., E.T., and M.T. were involved in recruitment of subjects. All authors reviewed the manuscript and provided input in manuscript preparation and approved the final draft for submission.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

All relevant datasets generated for this study are included in the manuscript.

References

- [1] A.N. Barclay, M.H. Brown, The SIRP family of receptors and immune regulation, *Nat. Rev. Immunol.* (6) (2006) 457–464.
- [2] L. Piccio, W. Vermi, K.S. Boles, A. Fuchs, C.A. Strader, F. Facchetti, M. Cella, M. Colonna, Adhesion of human T cells to antigen-presenting cells through SIRPbeta2-CD47 interaction costimulates T-cell proliferation, *Blood* 105 (2005) 2421–2427.
- [3] Y. Huang, Y. Ma, P. Gao, Z. Yao, Targeting CD47: the achievements and concerns of current studies on cancer immunotherapy, *J. Thorac. Dis.* 9 (2017) E168–E174.
- [4] M. Stefanidakis, G. Newton, W.Y. Lee, C.A. Parkos, F.W. Lusinskas, Endothelial CD47 interaction with SIRPgamma is required for human T-cell transendothelial migration under shear flow conditions in vitro, *Blood* 112 (2008) 1280–1289.
- [5] J.C. Barrett, D.G. Clayton, P. Concannon, B. Akolkar, J.D. Cooper, H.A. Erlich, C. Julier, G. Morahan, J. Nerup, C. Nierras, V. Plagnol, F. Pociot, H. Schuilenburg, D.J. Smyth, H. Stevens, J.A. Todd, N.M. Walker, S.S. Rich, C. Type 1 Diabetes Genetics, Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes, *Nat. Genet.* 41 (2009) 703–707.
- [6] A.K. Kiani, P. John, A. Bhatti, A. Zia, G. Shahid, P. Akhtar, X. Wang, F.Y. Demirci, M.I. Kamboh, Association of 32 type 1 diabetes risk loci in Pakistani patients,

- Diabetes Res. Clin. Pract. 108 (2015) 137–142.
- [7] M.V. Reddy, H. Wang, S. Liu, B. Bode, J.C. Reed, R.D. Steed, S.W. Anderson, L. Steed, D. Hopkins, J.X. She, Association between type 1 diabetes and GWAS SNPs in the southeast US Caucasian population, *Genes Immun.* 12 (2011) 208–212.
- [8] S. Sinha, N. Borchering, P.S. Renavikar, M.P. Crawford, E. Tsalikian, M. Tansey, E.T. Shivapour, F. Bittner, J. Kamholz, H. Olalde, E. Gibson, N.J. Karandikar, An autoimmune disease risk SNP, rs2281808, in SIRPG is associated with reduced expression of SIRPgamma and heightened effector state in human CD8 T-cells, *Sci. Rep.* 8 (2018) 15440.
- [9] I.S. Gabrielsen, S.S. Amundsen, H. Helgeland, S.T. Flam, N. Hatinoor, K. Holm, M.K. Viken, B.A. Lie, Genetic risk variants for autoimmune diseases that influence gene expression in thymus, *Hum. Mol. Genet.* 25 (2016) 3117–3124.