Published in final edited form as:
J Autoimmun. 2013 August ; 44: 8–12. doi:10.1016/j.jaut.2013.06.001.

The autoimmune disease-associated SNP rs917997 of *IL18RAP* controls IFNγ production by PBMC

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Abstract

Type 1 Diabetes (T1D) is an autoimmune disorder characterized by aberrant T cell responses. Innate immune activation defects may facilitate a T helper 1 (Th1) phenotype. The cytokine IL-18 synergizes with IL-12 to induce IFNγ production and Th1 differentiation. The IL-18R subunit *(IL18RAP)* SNP rs917997 has been linked to decreased *IL18RAP* gene expression. Prior reports link rs917997 allele A with protection from T1D, and conversely with susceptibility to Celiac disease. However, few studies have investigated the IL-18 pathway in T1D. In this study, we analyzed responsiveness to IL-18 in T1D, and the effect of rs917997 genotype on *IL18RAP* gene expression post-activation. Upon IL-12 and IL-18 treatment, peripheral blood mononuclear cells from subjects carrying susceptibility alleles at rs917997 produced higher levels of IFNγ than those with protective genotypes. Additionally, the SNP modified IL18RAP surface protein expression by NK cells and gene expression in activated T cells. Taken together, these data suggest that the disease-associated rs917997 allele G permits hyperresponsiveness to IL-18, providing a novel target for therapeutic intervention in T1D.

1. Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease with a rising incidence worldwide [1, 2]. Disease onset often occurs in childhood, resulting from a T cell mediated destruction of the insulin-producing pancreatic β cells and a subsequent lifelong requirement for exogenous insulin therapy [3]. Although several recent, large-scale clinical trials aimed at preventing or curing the disease have been conducted, few have yielded promising results [4, 5]. Therefore, it is critical that the mechanisms underlying the autoimmune activation and β cell destruction be further elucidated, both to improve biomarker monitoring in clinical trials as well as to develop therapeutic interventions to overcome pathway defects associated with defective immunoregulation [6].

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Conflict of Interest Disclosure
The authors declare no conflicts of interest related to this study.

IL-18, a member of the IL-1 superfamily of cytokines, is a monomeric cytokine primarily produced by macrophages and dendritic cells (DC). Like IL-1β, it is produced as an inactive precursor, pro-IL-18, then cleaved by caspase-1 into its active form. IL-18 synergizes with IL-12 to promote the production of IFNγ by natural killer (NK) cells, T cells, and macrophages, and is customarily associated with a Th1 response [7]. In addition, it can augment Th2 and Th17 responses [8, 9], and may also potentiate Treg activity [10]. The pleiotropic nature of IL-18 has been demonstrated in the murine non-obese diabetic (NOD) model of T1D, where early treatment with IL-18 enhances diabetes and late administration is preventative [11, 12]. However, despite the importance of IL-18 in regulating IFNγ production and Th1 skewing, studies investigating the IL-18 pathway in human T1D are limited.

The heterodimeric IL-18R, composed of IL-18R1 and IL-18R accessory protein (IL-18RAP), is constitutively expressed on innate immune system cells, while IL-18R1 is expressed on T cells. IL-18RAP is required for signaling and is upregulated during activation, particularly in the presence of IL-12 [13]. Recently, genome-wide association studies (GWAS) have identified *IL18RAP* SNP rs917997, located on chromosome 2, as protective in T1D [14]. The SNP, a G to A substitution in the 3′ UTR of *IL18RAP,* has been linked to decreased gene expression in peripheral blood [15]. However, as with many SNPs identified in GWAS, a functional explanation for the disease association has yet to be investigated. In this study, we determined the effect of rs917997 genotype on stimulated IL18RAP expression, as well as on functional responses to IL-18.

2. Materials and Methods

2.1 Subjects

All study participants were consented in accordance with the institutional review boards of the University of Florida and Nemours Children's Hospital-Orlando. Subjects did not have any overt illnesses at the time of sample collection; control subjects did not have any chronic diseases. Peripheral blood samples were collected in EDTA or heparin coated vacuum tubes (BD Biosciences); basal gene expression was analyzed from peripheral blood using PAXgene Blood RNA tubes (Qiagen).

2.2 PBMC stimulation

PBMC were isolated from heparinized blood by density gradient centrifugation (Ficoll; GE Healthcare) within 20–28 h of collection. 5×10^5 cells/mL were cultured in 96 well round-bottom plates (Costar) in RPMI complete media with 10% FBS (Mediatech, Inc). PBMC were treated with 10 ng/mL IL-18, 1 ng/mL IL-12, or the combination; supernatants were harvested at 24 h.

2.3 T cell differentiation

Naïve T cells ($CD4^+CD45RA^+$) were isolated by negative selection from PBMC using the naïve T Cell Isolation Kit II (Miltenyi Biotec); monocytes were enriched by selective density centrifugation (RosetteSep; Stemcell). Naive T cells and monocytes were cultured at a 4:1 ratio in CTL media (Cellular Technology Ltd.) supplemented with HEPES, L-glutamine, antibiotics, and 2-mercaptoethanol at a final concentration of 6.25×10^5/mL in a 96-well plate. Cells were stimulated (5 μg/mL soluble anti-CD3, 2.5 μg/mL soluble anti-CD28, and 5 ng/mL IL-2) or in the presence of Th1 (5 μg/mL anti-IL-4 and 2.5 ng/mL IL-12p70) or Th17 polarizing cytokines (10 ng/mL IL-1β, 25 ng/mL IL-6, and 5 ng/mL TGFβ) (eBioscience). RNA was isolated from total cocultured cells after 72 h (RNAqueous Micro; Life Technologies).

2.4 ELISA

IFNγ concentration was determined by a Ready-Set-Go! ELISA kit (eBioscience). Samples were analyzed on SOFTmax PRO (Molecular Devices).

2.5 Genotype determination

DNA was isolated from EDTA-treated peripheral blood (QIAamp DNA Blood Mini; Qiagen). rs917997 genotype was determined by TaqMan probe set (sequences proprietary; Life Sciences) on a LightCycler 480 (Roche Diagnostics) as per manufacturer guidelines.

2.6 Quantitative PCR

Custom primers were designed utilizing the Universal ProbeLibrary (Roche Diagnostics) and NetPrimer (Premier Biosoft) (Table 2). All primers were validated by melt curve analysis and gel electrophoresis. Total RNA was isolated from cultured cells (described above) or PAXgene Blood RNA Tubes (Qiagen). RNA samples were treated with DNAse prior to cDNA generation from 250–500 ng RNA (SuperScript III First-Strand Synthesis SuperMix; Life Technologies), and qPCR performed on a LightCycler 480 using PerfeCTa SYBR Green FastMix (Quanta Biosciences). A comparative threshold cycle (Ct) method with a cutoff of 35 cycles was used to determine mRNA copy number relative to GAPDH. Relative gene expression data are shown as $2^{\Delta Ct} \times 10^3$, where $\Delta Ct = (Ct^{GAPDH} - Ct^{TARGET\ GENE})$.

2.7 Intracellular Cytokine Staining

PBMC were cultured in the presence of IL-12 and IL-18 for 24 h, with Golgistop (BD Biosciences) added for the last 6 h of culture. Cells were harvested, fixed, permeabilized, blocked with Fc block (eBioscience), and stained for expression of CD56, CD3, CD14 and IFNγ (Biolegend). For determination of IL18RAP expression, frozen PBMCs were thawed and cultured as above, stained for expression of CD3, CD56, IFNγ (Biolegend), and IL18RAP (R&D Systems). Samples were read on an LSRII Fortessa (BD Biosciences) and data analyzed with FlowJo (Tree Star).

2.8 Statistics

Data analyses were performed in GraphPad Prism 5.1 (GraphPad). Normally distributed data sets were analyzed by a 2-tailed student's t test or one-way ANOVA; the Mann-Whitney U test was otherwise applied. Spearman correlations were used to determine covariable associations. In all cases, a $P < 0.05$ was deemed significant.

3. Results

3.1 rs917997 genotype modifies IL18RAP gene expression

In a preliminary study, we analyzed gene expression in healthy controls and subjects with T1D for 22 genes associated with Th1 and Th17 T effector cell function, in peripheral blood and stimulated cell culture (Table 1). In a subset of these subjects, DNA was available to retrospectively determine the effect of rs917997 genotype on *IL18RAP* gene expression. In peripheral blood, *IL18RAP* gene expression was significantly lower in patients with T1D (n=21, mean age 14.24, mean disease duration 6.51 years, 67% G/G, 28% G/A, 5% A/A) compared to controls (n=22, mean age 17.21, 50% G/G, 41% G/A, 9% A/A, $P = 0.0198$; Figure 1A). In both groups, the rs917997 G/G genotype corresponded to higher levels of *IL18RAP* expression than the G/A genotype ($P < 0.05$; Figure 1B). The rare nature of the A/A genotype precluded its analysis, but trends continue to support a gene dose effect. We next retrospectively determined the effect of rs917997 genotype on naive T cell/monocyte co-cultures incubated either with TCR stimulus alone, or in the presence of Th1 or Th17

polarizing cytokines. Subjects with T1D (n = 9, mean age 19.56, mean disease duration 8.38 years, 78% G/G, 11% G/A. 11% A/A) and controls (n = 10, mean age 19.8, 50% G/G, 40% G/A. 10% A/A) were combined to determine the effect of the SNP on *IL18RAP* irrespective of disease state. Under TCR stimulus or Th17 polarizing conditions, the G/G genotype (n = 12) was associated with significantly higher levels of *IL18RAP* gene expression than the G/A genotype ($P < 0.05$; Figure 1C).

rs917997 allele A has previously been associated with decreased peripheral blood *IL18RAP* gene expression in a large cohort of controls as well as a cohort of subjects with celiac disease [15, 16]. Observing a similar trend in T1D supports that this effect is not significantly altered by disease state. The SNP effect on gene expression may be due to decreased RNA stability, for instance, by affecting a miRNA binding site. However, lower basal *IL18RAP* expression in T1D, irrespective of genotype, has not been previously reported. This discrepancy was not due to fewer subjects with the permissive G/G genotype, as G/G was slightly enriched in the T1D cohort (67% G/G) compared to controls (50% G/G). Though conditions which upregulate *IL18RAP* have been characterized (e.g., IL-12 stimulation), there is little information regarding negative regulation of the protein. Further study is needed to determine if lower *IL18RAP* gene expression is caused by metabolic factors or altered immunoregulatory mechanisms in T1D.

3.2 Elevated production of IFNγ in response to IL-12 and IL-18 treatment associates with rs917997 genotype

To characterize IL-18 responsiveness in T1D, we tested PBMCs with IL-18 *in vitro.* Cytokine dose titrations demonstrated IL-18 synergized with low levels of IL-12 (1 ng/mL) in PBMC, leading to robust IFNγ production within 24 h (Figure 2A). Total PBMC from controls (closed symbols, n = 53, mean age 33.62, 47% G/G, 40% G/A, 13% A/A) and subjects with T1D (open symbols, 5 new onset indicated by **x**) n = 44, mean age 14.84, mean disease duration 5.21 years), 52% G/G, 43% G/A, 5% A/A) were cultured for 24 h with IL-12 (1 ng/ml) and IL-18 (10 ng/ml) (Figure 2B). There was no significant difference in IFNγ production in culture supernatants between T1D and control, and IFNγ production in the T1D cohort did not correlate with disease duration (data not shown).

We next sought to determine if rs917997 genotype had any effect on IL-12/IL-18-induced IFNγ production. Analyzing the combined T1D and control cohorts segregated by SNP genotype showed higher IFNγ production in individuals with G/G compared to G/A or A/A (Figure 2B). Subjects with homozygous susceptibility exhibited increased IFNγ production (G/G, n = 48, mean ± SD = 10317 ± 12513 pg/ml) as compared to G/A (n = 40, 5687 ± 4564 pg/ml) and A/A (n = 9, 3636 ± 2272 pg/ml) subjects (P = 0.0296, One-way ANOVA). Under these conditions, rs917997 G/G was more permissive to IL-12/IL-18-induced IFNγ production, though the majority of samples in both G/G and G/A individuals produced moderate levels of IFNγ.

It is intriguing that the production of IFNγ occurred in the absence of antigen or TCR stimulus, as previously reported [17]. We sought to identify the cellular subset in PBMC responsible for the observed robust IFNγ production. Utilizing intracellular FACS, we were able to identify that $CD3^{-}CD56^{+}$ NK cells were a major source of IFNγ (Fig. 2C). Further analysis of frozen PBMCs of subjects homozygous at rs917997 revealed that NK cells from AA individuals expressed significantly reduced IL18RAP in terms of both frequency (Fig. 2D, left, AA, n = 5, mean ± SD = 2.01 ± 0.75, G/G, n = 5, mean ± SD = 3.02 ± 0.52, p=0.03) and intensity (Fig. 2D, middle, AA, mean ± SD = 47.14 ± 10.53, G/G, mean ± SD = 60.44± 4.68, p=0.04), with a trend toward lesser NK cell involvement in cytokine production for GG subjects (Fig. 2D, right). Notably, prior studies have reported a deficiency of NK cells in T1D subjects [18]. This raises the question of whether the

genotype-controlled influence noted here would impact stimulated T cells over longer activation periods, and represents an avenue of further investigation.

4. Discussion

These data support a novel functional effect of IL18RAP (rs917997), and a potential mechanism for the protective effect of the minor (A) allele in T1D. While the (A) allele has a minor protective influence in T1D (OR 0.87), it appears to confer increased risk for coeliac disease [14, 19]. This opposing action may result from alternate disease mechanisms, opposing tissue-specific influences, or the combined influence of additional immune modulating loci. As one example of the pleiotropic nature of this cytokine, IL-18 has been reported to enhance murine Treg differentiation *in vitro* by DCs, and increased *in vivo* tolerance in mice challenged with a pathogenic bacterium [10]. The potential for opposing functions of IL-18 in these diseases necessitates careful consideration when investigating IL-18 pathways as possible therapeutic targets.

A potential enhanced NK cell response to IL-12 and/or IL-18 raises an intriguing possibility whereby early production of IFNγ by NK cells, prior to activation of the adaptive immune system, may push the differentiation toward a Th1 phenotype. The prospect of dysregulated NK cells in T1D is particularly interesting given the large body of research investigating viral infections as environmental triggers of disease [20]. In T1D, heightened responsiveness to IL-18 in individuals with the rs917997 allele G may be one factor leading to the break in tolerance. This is particularly true where other susceptibility alleles may intersect with IL-18 (e.g., IL-12/STAT4), to augment Th1 responses. Though such alleles are weakly associated with disease (i.e., low OR), multiple susceptibility alleles may synergize to skew the immune system towards a proinflammatory phenotype, creating an environment permissive to disease pathogenesis. Such combinatorial synergism was recently described in high-risk children who went on to develop T1D. In this analysis, the IL18RAP susceptible SNP rs917997 was highly represented (92%) [21]. Determining the functional effect of disease-associated SNPs will allow us to better understand, and potentially modify, the pathogenesis of T1D.

Acknowledgments

This study was supported, in part, by funding from the National Institutes of Health (AI42288), the Juvenile Diabetes Research Foundation, including a Cord Blood Center grant to MAA and a Career Development Award to TMB (2-2012-280), and the Brehm Coalition for Diabetes Research. The authors wish to thank the study participants, as well as the clinical support staff including Cynthia Kemp, Ann Powell, Jessica Ferguson, Roberta Cook, and Annie Abraham who make these studies possible.

References

1. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet. 2001; 358:221–9. [PubMed: 11476858]
2. Patterson CC, Dahquist GG, Gyurus E, Green A, Soltesz G, Grp ES. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. Lancet. 2009; 373:2027–33. [PubMed: 19481249]
3. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature. 2010; 464:1293–300. [PubMed: 20432533]
4. Greenbaum C, Atkinson MA. Persistence is the Twin Sister of Excellence An Important Lesson for Attempts to Prevent and Reverse Type 1 Diabetes. Diabetes. 2011; 60:693–4. [PubMed: 21357468]
5. Van Belle TL, Coppieters KT, Von Herrath MG. Type 1 Diabetes: Etiology, Immunology, and Therapeutic Strategies. Physiol Rev. 2011; 91:79–118. [PubMed: 21248163]

6. Skyler JS, Ricordi C. Stopping Type 1 Diabetes: Attempts to Prevent or Cure Type 1 Diabetes in Man. Diabetes. 2011; 60:1–8. [PubMed: 21193733]
7. Dinarello CA. IL-18: A T-H1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immun. 1999; 103:11–24. [PubMed: 9893178]
8. Hoeve MA, Savage NDL, de Boer T, Langenberg DML, Malefyt RD, Ottenhoff THM, et al. Divergent effects of IL-12 and IL-23 on the production of IL-17 by human T cells. European Journal of Immunology. 2006; 36:661–70. [PubMed: 16482511]
9. Leite-De-Moraes MC, Hameg A, Pacilio M, Koezuka Y, Taniguchi M, Van Kaer L, et al. IL-18 enhances IL-4 production by ligand-activated NKT lymphocytes: A pro-Th2 effect of IL-18 exerted through NKT cells. Journal of Immunology. 2001; 166:945–51.
10. Oertli M, Sundquist M, Hitzler I, Engler DB, Arnold IC, Reuter S, et al. DC-derived IL-18 drives Treg differentiation, murine Helicobacter pylori-specific immune tolerance, and asthma protection. Journal of Clinical Investigation. 2012; 122:1082–96. [PubMed: 22307326]
11. Rothe H, Hausmann A, Casteels K, Okamura H, Kurimoto M, Burkart V, et al. IL-18 inhibits diabetes development in nonobese diabetic mice by counterregulation of Th1-dependent destructive insulitis. Journal of Immunology. 1999; 163:1230–6.
12. Oikawa Y, Shimada A, Kasuga A, Morimoto J, Osaki T, Tahara H, et al. Systemic administration of IL-18 promotes diabetes development in young nonobese diabetic mice. Journal of Immunology. 2003; 171:5865–75.
13. Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, et al. IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: Synergism with IL-18 for IFN-gamma production. Journal of Immunology. 1998; 161:3400–7.
14. Todd JA, Smyth DJ, Plagnol V, Walker NM, Cooper JD, Downes K, et al. Shared and Distinct Genetic Variants in Type 1 Diabetes and Celiac Disease. New England Journal of Medicine. 2008; 359:2767–77. [PubMed: 19073967]
15. Zhernakova A, Elbers CC, Ferwerda B, Romanos J, Trynka G, Dubois PC, et al. Evolutionary and Functional Analysis of Celiac Risk Loci Reveals SH2B3 as a Protective Factor against Bacterial Infection. American Journal of Human Genetics. 2010; 86:970–7. [PubMed: 20560212]
16. Hunt KA, Zhernakova A, Turner G, Heap GA, Franke L, Bruinenberg M, et al. Newly identified genetic risk variants for celiac disease related to the immune response. Nat Genet. 2008; 40:395–402. [PubMed: 18311140]
17. Yang JF, Murphy TL, Ouyang WJ, Murphy KM. Induction of interferon-gamma production in Th1 CD4(+) T cells: evidence for two distinct pathways for promoter activation. European Journal of Immunology. 1999; 29:548–55. [PubMed: 10064070]
18. Qin HL, Lee IF, Panagiotopoulos C, Wang XX, Chu AD, Utz PJ, et al. Natural Killer Cells From Children With Type 1 Diabetes Have Defects in NKG2D-Dependent Function and Signaling. Diabetes. 2011; 60:857–66. [PubMed: 21270236]
19. Wang K, Baldassano R, Zhang H, Qu HQ, Imielinski M, Kugathasan S, et al. Comparative genetic analysis of inflammatory bowel disease and type 1 diabetes implicates multiple loci with opposite effects. Hum Mol Genet. 2010; 19:2059–67. [PubMed: 20176734]
20. van der Werf N, Kroese FGM, Rozing J, Hillebrands JL. Viral infections as potential triggers of type 1 diabetes. Diabetes-Metab Res. 2007; 23:169–83.
21. Winkler C, Krumsiek J, Lempainen J, Achenbach P, Grallert H, Giannopoulou E, et al. A strategy for combining minor genetic susceptibility genes to improve prediction of disease in type 1 diabetes. Genes and immunity. 2012; 13:549–55. [PubMed: 22932816]

Research Highlights

- We characterized novel phenotypes associated with IL18RAP SNP rs917997
- The T1D-associated protective allele results in lower production of IFNγ by PBMC
- IL18RAP SNP rs917997 influences both NK and T cell activity
- These findings have implications for the pathogenesis of T1D and Celiac Disease

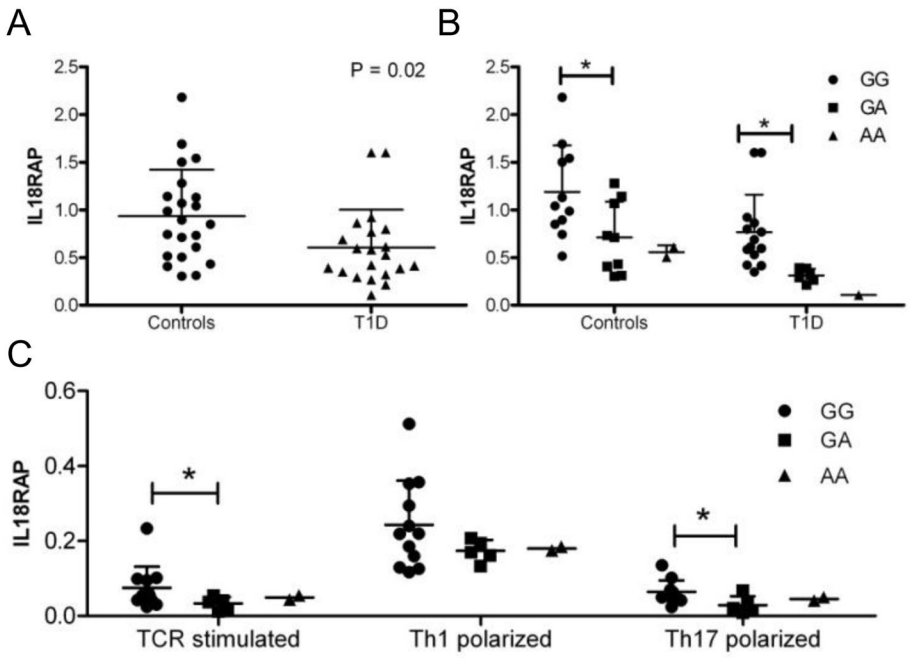

Figure 1.

IL18RAP gene expression is associated with rs917997 genotype. (**A**) Peripheral blood *IL18RAP* gene expression was determined by qPCR in patients with T1D ($n = 21$) and controls ($n = 22$) ($P = 0.0198$, Student's t test), and (**B**) broken down by rs917997 genotype. (**C**) Analysis of *IL18RAP* gene expression of stimulated naïve T cell:monocyte co-cultures at 72 h in a combined T1D and control cohort ($n = 19$). Data were normalized to *GAPDH* expression $\times 10^3$, presented as mean ± SD. $*P < 0.05$, Mann-Whitney U test; A/A genotype excluded from analyses.

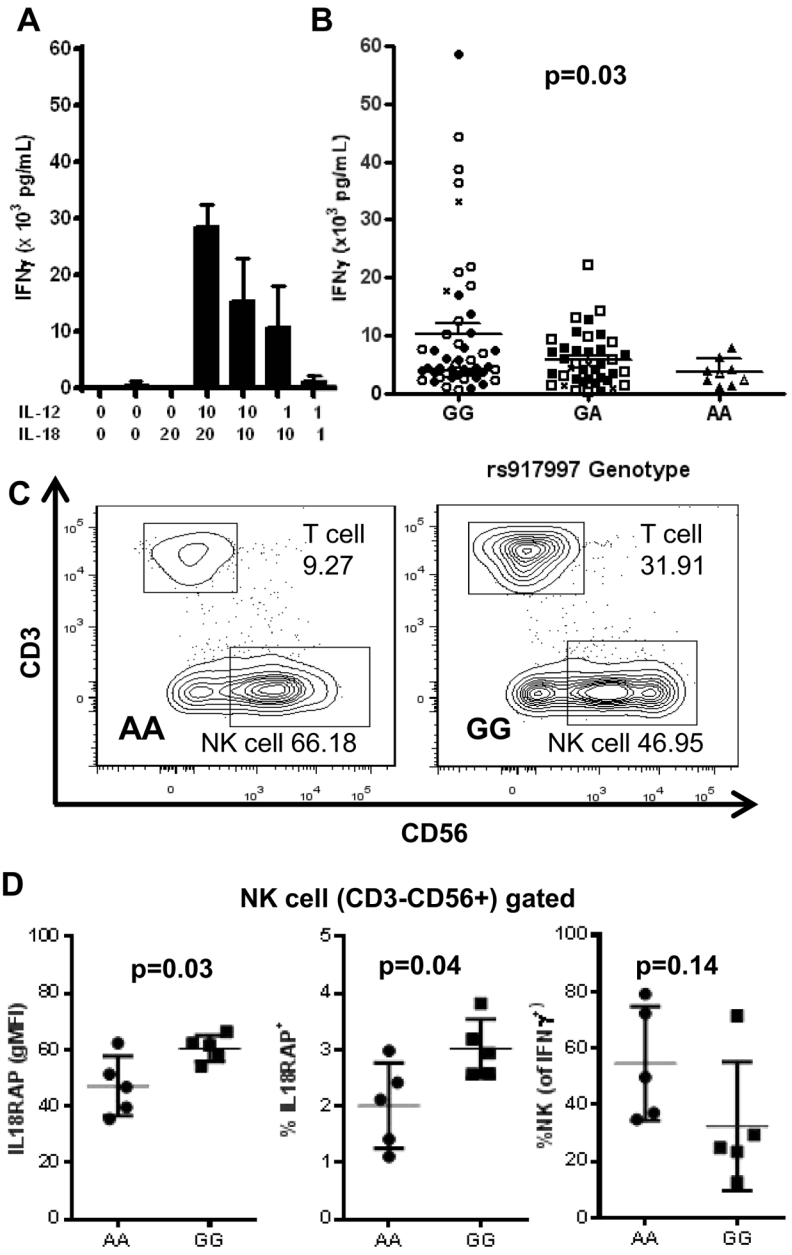

Figure 2.

IFNγ produced by NK cells in PBMC culture following IL-12 and IL-18 stimulation is controlled by the IL18RAP SNP rs917997. (**A**) PBMC IFNγ production following stimulation by titrated doses of IL-12 and IL-18, measured by ELISA (*n* = 2). Treatment concentrations (*bottom*) expressed in ng/mL. (**B**) rs917997 genotype-stratified analysis of PBMC stimulation assays from T1D (*n* = 44, open symbols, 5 new-onset T1D indicated by **x**) and controls (*n* = 53, closed symbols). Cells were treated with 1 ng/mL IL-12 and 10 ng/mL IL-18, and IFNγ production measured at 24 h. (*P* = 0.0296, One-way ANOVA).(**C**) Representative flow plots of stimulated PBMC from individuals with AA (*n*=2) or GG (*n*=2) genotype after treatment with IL-12 and IL-18, gated on IFNγ positive cells. (**D**). Analysis of frozen PBMCs for IL18RAP expression showed AA individuals exhibited lesser frequency (*left*, *P*=0.03, t-test) and intensity (*middle*, *P* =0.04, t-test) of IL18RAP expression

on $CD3^{-}CD56^{+}$ gated NK cells, with a trend toward lesser NK cell involvement in cytokine production for GG subjects (*right*).

Table 1

Fold change in gene expression between subjects with T1D and controls

Gene	Peripheral Blood	TCR Stimulated	Th1 Polarized	Th17 Polarized
EBI3	0.91 (0.5260)	1.09 (0.7621)	0.90 (0.7341)	0.96 (0.9674)
GATA3	*ND*	1.12 (0.7623)	1.14 (0.6385)	0.82 (0.1978)
IL12A	1.08 (0.5441)	0.95 (0.2701)	0.74 (0.6830)	0.61 (0.9048)
IL12B	**1.67 (0.0049)**	0.85 (0.7197)	0.61 (0.1722)	0.78 (0.7802)
IL12RB1	**0.83 (0.0098)**	1.22 (0.6842)	1.05 (0.8940)	1.17 (0.4114)
IL12RB2	1.09 (0.3439)	1.31 (0.4359)	0.95 (0.7548)	1.44 (0.2715)
IL17A	**1.86 (0.0010)**	*ND*	*ND*	*ND*
IL18	1.37 (0.0736)	0.57 (0.0103)	0.52 (0.0151)	0.75 (0.2647)
IL18R1	1.10 (0.427)	1.23 (0.1387)	1.10 (0.4527)	1.45 (0.0048)
IL18RAP	**0.64 (0.0068)**	2.28 (0.0233)	1.63 (0.0119)	1.81 (0.0751)
IL1B	0.88 (0.3157)	0.83 (0.2428)	0.75 (0.2883)	0.67 (0.3416)
IL23A	1.31 (0.2081)	0.82 (0.683)	0.74 (0.3761)	0.89 (0.6522)
IL23R	1.43 (0.0409)	1.92 (0.2263)	1.35 (0.3763)	2.84 (0.0358)
IL27A	1.61 (0.0249)	*ND*	*ND*	*ND*
IL27RA	1.17 (0.1398)	0.96 (0.4813)	0.81 (0.6254)	0.79 (0.4553)
IL6	**1.67 (0.0017)**	1.12 (0.7708)	0.85 (0.7197)	0.84 (0.8421)
IL6R	1.24 (0.1980)	*ND*	*ND*	*ND*
IL6ST	**1.49 (0.0121)**	1.33 (0.8534)	0.99 (0.6842)	0.95 (0.8818)
RORA4	1.11 (0.3433)	1.1 (0.7335)	1.03 (0.7335)	0.90 (0.4904)
RORC	1.17 (0.1699)	1.31 (0.2475)	0.93 (0.4359)	0.99 (0.7394)
TBX21	1.21 (0.1200)	1.43 (0.1109)	1.24 (0.0868)	1.59 (0.1296)

Data presented as fold change (P value). Peripheral blood (Paxgene) analysis cohort consisted of 28 subjects with T1D and 23 controls. All stimulation conditions were on a cohort of 10 subjects with T1D and 10 controls. Bold values are significant by Student's *t* test after correction for multiple testing (Benjamini-Hochberg algorithm). *ND* = not determined. Fold change determined by dividing mean gene expression in T1D by mean gene expression in controls.

Table 2

Quantitative PCR primers

Gene	Forward primer (5′ – 3′)	Reverse primer (5′ – 3′)	Amplicon length
EBI3	TGTTCTCCATGGCTCCCTAC	GCTCCCTGACGCTTGTAAC	158
GAPDH	ACAGTCAGCCGCATCTTCTT	AATGAAGGGGTCATTGATGG	149
GATA3	GAACCGGCCCCTCATTAAG	ATTTTTCGGTTTCTGGTCTGGAT	197
IL12A	GAATGCAAAGCTTCTGATGGA	TGGCACAGTCTCACTGTTGAA	112
IL12B	AGATGGTATCACCTGGACCTTG	TCCTTTGTGACAGGTGTACTGG	114
IL12RB1	TGACCCTGCAGCTCTACAAC	GCCAACTTGGACACCTTGAT	70
IL12RB2	GATCTTCGTTGGTGTTGCTCCAGA	CACAGTCCCCTGTTCTCCCTTCTGT	73
IL17A	TCATTGGTGTCACTGCTACTGCTGC	TCGTGGGATTGTGATTCCTGCC	68
IL18	CCTCCTGGCTGCCAACTCT	GAAGCGATCTGGAAGGTCTGAG	100
IL18R1	AGTTATGCATATTTGAAAGGGATGT	TGAGTGGATTTCATCAACAACA	62
IL18RAP	CAGATATTCTGGATCCTGTCGAG	TGCTTTGCAGCTAATAGTTAAAGG	74
IL1B	GCTGAGGAAGATGCTGGTTC	GTGATCGTACAGGTGCATCG	145
IL23A	ATGATGTTCCCCATATCCAGTGTGG	GCAAGCAGAACTGACTGTTGTCCCT	75
IL23R	CTGAAACAGTTCCCCAGGTCACATC	GCAACTGTTAGCCCAGAATTCCATG	70
IL27A[a]	-	-	123
IL27RA	GAGTTGGACCCTTGGGCGACTT	CGGTACTTTTGGCTCTGGAGGTGTA	94
IL6	CAGCAAAGAGGCACTGGCAGAA	GGCAAGTCTCCTCATTGAATCCAGA	95
IL6R[a]	-	-	150
IL6ST	TGCAACATTCTTACATTCGGACAGC	TTTTCTGGAGGCAAGCCTGAAATTA	77
RORA4	TGTGATCGCAGCGATGAAAG	ACAGTTCTTCTGACGAGGACAGG	149
RORC	TGACAGAGATAGAGCACCTGGTGCA	AAGATGTTGGAGCGCTGCCG	100
TBX21	TGTGACCCAGATGATTGTGCTC	AGTAAAGATATGCGTGTTGGAAGC	121

Amplicon length in base pairs.

[a]Primers purchased from SABiosciences; proprietary sequences.