Predicting *KIR3DL1/S1* copy number from ImmunoChip signals in large scale type 1 diabetes association study

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Abstract

Killer Immunoglobulin-like Receptors (KIR) are important surface receptors of Natural Killer cells. KIRs mediate the fate of target cells based on the composite inhibiting/activating signal generated on binding to their corresponding HLA (Human Leukocyte Antigen) class I ligands. Type 1 diabetes (T1D) is an autoimmune disease strongly associated with genetic variation in the HLA region, primarily with Class II genes, but also with HLA Class I loci including HLA-Bw4, an epitope grouping both HLA-A and HLA-B alleles into HLA-Bw4-80I and HLA-Bw4-80T subgroups. Amongst the 17 known genes of the KIR region, characterised by alternative haplotypes of variable gene copy numbers, *KIR3DL1* is the only one proven to bind in-vitro with both HLA-Bw4 epitopes. Its activating counterpart, *KIR3DS1*, putatively binds the HLA-Bw4-80I epitope only. *KIR3DL1/S1* is therefore an interesting T1D candidate gene but since the KIR region is poorly characterised, genotyping with conventional SNP arrays is unreliable. So far, insufficiently powered PCR-based genotyping studies of less than 600 individuals, have failed to detect convincing association of T1D with presence or absence of *KIR3DL1/S1*. For the first time, we are able to test for association in a sample size of 6,744 cases and 5,362 controls, twenty-fold larger than any previous study, by using *KIR3DL1/S1* copy number calls from a recently developed KIR qPCR assay in order to impute copy number in samples assayed only with ImmunoChip, a custom Illumina genotyping chip with probes for 30 SNPs in the target region.

The association of *KIR3DL1/S1* copy number with T1D was tested in all samples and conditional on the corresponding HLA-Bw4 ligand subsets. The interaction effect between *KIR3DL1/S1* and HLA-Bw4 was also tested with a case-only analysis. We can confirm from our study with a substantially increased sample size, that copy number variation in *KIR3DL1/S1* is not an important modifier of T1D risk ($0.9 < \text{OR} < 1.1$ at 95% confidence for the common genotype), either alone or dependent on HLA-Bw4 epitope, and that furthermore, there is no evidence of statistical interaction effect between HLA-Bw4 and *KIR3DL1/S1* copy number in T1D. *(340 words)*

Introduction

Type 1 diabetes (T1D) is an auto-immune disease in which pancreatic islets of insulin producing β-cells are progressively destroyed primarily through autoreactive T cells (Todd, 2010). Genome wide association studies (GWAS) have confirmed strong association of T1D within the Human Leukocyte Antigen (HLA) loci (chr6p21) as well as 40 other loci including *INS* (chr11p15), *CTLA4* (chr2q33), *PTPN22* (chr1p13), *IL2RA* (chr10p15) and *IFIH1* (chr2q24) (Wellcome Trust Case Control Consortium, 2007; Barrett et al, 2009).

Within the HLA region, the strongest effect comes from HLA Class II loci, HLA-DRB1 ($P = 6.0 \times 10^{-17}$) and HLA-DQB1 ($P = 8.8 \times 10^{-13}$), but there is also an independent effect from HLA Class I loci, HLA-A and HLA-B (Howson et al, 2009). One HLA Class I loci of biological interest, associated with T1D conditional on *HLA-DQB1* and *HLA-DRB1* ($P=6.57 \times 10^{-6}$) (Nejentsev et al, 2007), is the HLA-Bw4 epitope which classifies *HLA-A* and *HLA-B* alleles as either HLA-Bw4-80I or HLA-Bw4-80T, depending on whether the amino acid at position 80 in the heavy alpha chain of the HLA Class I protein is an isoleucine or a threonine (Gumperz et al, 1997; Martin et al, 2002). The HLA-Bw4 class of proteins is known to biologically interact with the KIR3DL1 protein (Vivian et al, 2011) and the protein encoded by the closely related gene *KIR3DS1*, sharing 97% sequence similarity with *KIR3DL1*, is thought to bind the HLA-Bw4-80I subclass of proteins (Martin et al, 2007). *KIR3DL1* and *KIR3DS1* are two of the 17 known Killer Immunoglobulin-like Receptors (KIR), transmembrane glycoproteins expressed by natural killer cells and subsets of T cells. *KIR3DL1/S1* lies in the 150 Kb KIR gene cluster, a region of great haplotype diversity and copy number variation, located within the 1 Mb Leukocyte Receptor Complex on chr19q13.4. The 17 polymorphic and highly homologous KIR genes are named according to the number of extra-cellular Immunoglobulin domains (2D and 3D) they encode and whether the cytoplasmic tail of the protein is short (S) or long (L). The *KIR3DS1* gene is thought to be implicated in viral and autoimmune diseases but there is no substantial evidence of association with T1D (Körner and Altfeld, 2012). However studies to date have been small, and evidence for its association has not yet been addressed in large, well powered studies.

Since the KIR region is poorly mapped, KIR genes can not be genotyped using conventional SNP arrays. More specialised genotyping is required and lately Jiang et al (2012) have designed quantitative PCR assays to study copy number variations in KIR which have led to the discovery of many rare haplotypes.

So far, case-control studies using qPCR in many different ethnicities have looked at whether

the presence or absence of KIR genes but not the copy number are associated with T1D (van der Slik et al, 2003, 2007; Nikitina-Zake et al, 2004; Santin et al, 2006; Middleton et al, 2006; Park et al, 2006; Mogami et al, 2007; Shastry et al, 2008; Jobim et al, 2010; Zhi et al, 2011). These, however, represent an incomplete version of the KIR genotype because, as shown by Jiang et al (2012), a lot of the diversity in the KIR haplotypes arises from copy number variation. Moreover these studies have been limited by small sample sizes which barely exceed three hundred cases and controls due to the limiting time and cost constraints of qPCR technology. These studies are not sufficiently powered to detect an odds ratio of 1.2 or less, which requires sample sizes at least ten-fold larger (Jo's original power calculation?).

Here we present a method whereby using qPCR copy number calls in 1,474 samples as a training set, we are able to impute copy number in a further 12,106 samples from raw genotyping signals in SNP array probes targeting the KIR region, even where those SNPs fail standard quality control due to non specific binding or variable copy numbers.

We applied our method firstly, to test association of *KIR3DL1/S1* copy number with T1D, and secondly, to test for possible interaction effect between *KIR3DL1/S1* copy number and HLA-Bw4 genotype. To the best of our knowledge, this is the largest *KIR3DL1/S1* association study to date in T1D and the first to test for T1D association with copy number variation in *KIR3DL1/S1* instead of just presence/absence.

Methods

Subjects

We analysed 12,106 individuals, 6,744 cases (age of onset less than 16) from the Genetic Resource Investigating Diabetes cohort, and 5,362 controls from the British 1958 Birth Cohort (1958BC). All subjects were of white European ancestry with written informed consent and Ethics Committee/Institutional Review Board approval. The DNA for the cases and controls was prepared using the same protocols in Cambridge and in Bristol respectively. All samples were cell-line derived.

KIR3DL1/S1 Data

qPCR Data

A subset of 1,629 samples, 816 cases and 813 controls, were analysed with quantitative Polymerase Chain Reaction (qPCR) on the LightCycler 480 Real-Time PCR Instrument. Primers were designed to determine copy numbers of *KIR3DL1* and *KIR3DS1*. The housekeeping gene *STAT6*, known to always be present in two copies, was used as a reference. The forward/reverse primers and probe sequences for *KIR3DL1*, *KIR3DS1* and *STAT6* are summarised in Table S**??**. The qPCR assays were developed in collaboration with Jiang et al (2012).

For each qPCR reaction, 2ul of DNA at 5ng/ul were used with 5ul of Quantifast Multiplex PCR mastermix, 0.25ul primer mix, 0.045ul probe mix and 4.705ul of water. qPCR conditions were 95°Celsius for 5 minutes, followed by 40 cycles of 95°Celsius for 15 seconds and 66°Celsius for 50 seconds. Data was collected at 66°Celsius. The samples were tagged with three different dyes, Fam for *KIR3DS1*, Cy5 for *KIR3DL1* and DFO for *STAT6*, and amplified on nineteen 384-well plates. On all plates, samples were replicated across four wells. So that each plate contained a maximum of 96 samples. Four calibrator samples of known *KIR3DL1/S1* copy number and one water sample were included on all but one plate. Cases and controls were distributed evenly across all plates. Four plates were analysed in duplicate.

The experiment files exported from the LightCycler gave us the crossingpoint (Ct) value for each dye-DNA conjugate. The Ct value is the number of amplification cycles it takes for the fluorescence of a dye in a given well to reach a certain detection threshold. By subtracting from the Ct value of the reference dye-DNA conjugate, DFO-STAT6, we obtained the baseline relative ΔCt value for Fam-KIR3DL1 and Cy5-KIR3DS1. Since *STAT6* is known to have two copies, negative values of ΔCt indicate two or more copies, and positive values, two copies or less.

As part of our quality control (QC), we dropped 64 samples which did not yield a DFO-STAT6 Ct reading in all four well replicates or for which the maximum difference in DFO-STAT Ct between wells was larger than 2. All remaining samples were summarised by the ΔCt median of the four well replicates.

We normalised ΔCt values for *KIR3DL1* and *KIR3DS1* across plates by a linear transformation so that the mean ΔCt of the two most distinguishable copy number groups, one and two copies, were aligned across all nineteen plates. The group means were identified using the k-means algorithm. Visual inspection of the data distributions by plate led us to drop plate 22 because it appeared excessively noisy (Figure S**??** and Figure S**??**).

After normalisation, samples repeated in different plates showed good reproducibility of the *KIR3DL1* and *KIR3DS1* ΔCt values (Figure S**??**). Samples repeated across plates were summarised by the median of their repeated value. Following QC, we were left with 1,474 individuals, 747 cases and 727 controls, for analysis (Figure S**??**).

HLA typing

In order to test for interaction with HLA-Bw4, genotyping of HLA alleles was done using the DYNAL technologies from Invitrogen in a subset of 5,603 individuals, 2,922 cases and 2,681 controls (Table S**??**). The HLA-A and HLA-B genes were typed at four-digit allele resolution using Dynal RELI SSO assays (Invitrogen, Paisley, U.K.). The epitope classification of HLA-A and HLA-B alleles is given in Table S**??**.

SNP data

All 12,106 samples were genotyped using ImmunoChip, a custom Illumina 200K Infinium high-density SNP array (Cortes and Brown, 2011), according to the manufacturer's protocol, and processed at the University of Virginia in Charlottesville, USA. At each SNP, two readings were obtained, the Log R Ratio, indicative of copy number, and theta, indicative of allelic frequency. On ImmunoChip there are 30 SNPs which fall in the *KIR3DL1* region according to the human genome reference build36/hg18. The *KIR3DS1* gene is missing from build36/hg18.

KIR3DL1/S1 Copy Number Calling

In the qPCR dataset, samples which yielded one or less Ct reading for Fam-KIR3DL1 or Cy5-KIR3DS1, but all four Ct readings for the reference DFO-STAT6, were assumed to contain zero copies of *KIR3DL1* or *KIR3DS1*. For the remainder of the samples, we called genotypes using a mixture of bivariate Gaussian distributions allowing for eight *KIR3DL1/S1* genotype groups: three common groups of two copy numbers (major homozygote, heterozygote, minor homozygote) and five rarer groups of lower or higher copy numbers (Figure 1). The initial mixing and covariance parameters for the mixture of bivariate Gaussian distributions were calculated from the clusters returned by k-means with centers set to the eight copy number groups. The parameters were then further refined using an EM algorithm (Young et al, 2009). After fitting the mixture model each sample is assigned a posterior probability of belonging to each of the eight genotype groups which allows for uncertainty in genotype calling.

We increased our sample size with the SNP data, by using the subset of samples, 747 cases and 727 controls, common between the qPCR and SNP datasets to train a k-nearest neighbour classifier to predict *KIR3DL1/S1* copy number from SNP signals on ImmunoChip. For the k-nearest neighbour classifier we chose $k = 3$ as it minimised the leave one out cross validation error (Table S**??**). The classification was achieved by using the 3 nearest neighbours on the Log R Ratio and Theta signals from 30 SNPs lying within the *KIR3DL1* region (Table S**??**) which were associated with *KIR3LD1-KIR3DS1* copy number in individual linear regression. Applying the nearest neigbour classifier independently on 10 multiply imputed data set generated from the qPCR posterior probabilities, we estimated the mean misclassification error at 1.5% (Table S**??**).

Testing for Association of *KIR3DL1/S1* Copy Number with T1D

We tested for association of T1D with the predicted copy numbers from the qPCR and SNP datasets using logistic regression. We allowed for copy number prediction uncertainty in the association test for qPCR data by doing multiple imputation (N=10) with the `R mitools package` (Lumley, 2012) on the genotype group membership probabilities (Cordell, 2006). We allowed for statistical interaction with HLA-Bw4 by repeating the association test in the subsets of individuals carriers of the respective HLA ligands, HLA-Bw4 ligand for *KIR3DL1* and the HLA-Bw4-80I putative ligand for *KIR3DS1*. We further tested for interaction with a more powerful case-only χ^2 test (Yang et al, 1999; Cordell, 2009).

Results

No Evidence of Association of *KIR3DL1/S1* with T1D

In the qPCR data association test in all individuals, 747 cases and 727 controls, no significant effect was detected in the joint *KIR3DS1-KIR3DL1* (Table 1a) common copy number groups, 0-2, 1-1, 2-0, 2-1 and 0-1, nor in the marginal *KIR3DL1* (Table 1b) and *KIR3DS1* (Table 1c) At this sample size we are underpowered to detect any effect in the rarer groups, 1-0 and 3-0, which only contain 7 and 5 individuals respectively. Repeating our analysis in the extended SNP data, 6,744 cases and 5,362 controls, our strongest association comes from the 2-0 group ($P = 0.0439$) (Table 1a), but is only nominally significant. No association is detected in the rarer groups, 1-0 and 3-0, this time at sample sizes of 44 and 24 respectively. In the marginal genotypes, we also detect weak association of two copies of *KIR3DS1* at a nominal significance ($P = 0.0224$)

(Table 1c). However none of our nominal associations would survive correction for the number of tests conducted. The second most common group, the 1-1 copy number group, allows us to exclude odds ratio outside of the range [.92; 1.08] with 95% certainty.

From the SNP data, if we consider all *KIR3DL1/S1* copy number groups then we can exclude odd ratios outside of the [0.67; 1.54] for the genotypes with frequency greater than 1%. For the marginal effect then we can exclude *KIR3DL1* odd ratios outside the [0.71; 1.07] range and *KIR3DS1* odd ratios in the [0.21; 1.08] range.

No Evidence of KIR/HLA-Bw4 Interaction in T1D

Repeating the association tests in the HLA-Bw4 subset of individuals for the joint *KIR3DS1-KIR3DL1* (Table 2a) and marginal *KIR3DL1* (Table 2b) genotypes, and in the HLA-Bw4-80I subset, for the marginal *KIR3DS1* (Table 2c), we detected no significant association.

A disadvantage of subsetting by HLA-Bw4 is that we lose power by greatly reducing the sample size. For example in the SNP dataset after further subsetting by HLA-Bw4, we only find one individual with three copies of *KIR3DS1* (Table 2c).

A more powerful interaction test, is a case-only test. If there were an interaction between *KIR3DL1/S1* and HLA-Bw4 then this should be detectable as a difference in *KIR3DL1/S1* genotype frequencies across HLA-Bw4 strata in the cases. However we found no significant evidence for association in either the qPCR or SNP data sets, before or after grouping the KIR genotypes by presence/absence to increase power by reducing the degrees of freedom (Table 3).

Discussion

Previous studies of KIR genes in type 1 diabetes

So far there have only been two studies which have reported KIR genes to be associated with T1D independently of HLA: *KIR2DS2/L2* in the Latvian population (Nikitina-Zake et al, 2004) and *KIR2DS2* and *KIR2DL5* in the South Korean population (Park et al, 2006). However, one issue with these results, as raised by Middleton et al (2006), is that the difference in frequency between *KIR2DS2* and *KIR2DL2* is suspiciously large in both these studies, 53% vs 81% in the Latvian and 20% vs 46% in the South Korean, when these genes are usually in very high linkage disequilibrium (Single et al, 2007) suggesting perhaps errors in genotyping.

Moreover, HLA independent KIR association has not been replicated in other populations

including Dutch (van der Slik et al, 2003), Finnish (Middleton et al, 2006), Basque (Santin et al, 2006), Japanese (Mogami et al, 2007), South Brazilian (Jobim et al, 2010) and Chinese Han (Zhi et al, 2011).

Nonetheless, these studies often detect association when they conduct subset analysis by HLA genotype, by age band or by grouping into activating or inhibiting composite KIR-HLA genotypes (Carrington et al, 2005; van der Slik et al, 2007). For example, van der Slik et al (2003) report association with *KIR2DS2* in the HLA-C1, *HLA-DQ2/DQ8* (high risk) subset of the Dutch cohort. Jobim et al (2010) find association with *KIR2DL1* in the HLA-C2 subset of the South Brazilian cohort. In the Chinese Han cohort, Zhi et al (2011) report association with *KIR2DL3* in the HLA-C1 subset. In the Japanese cohort, Mogami et al (2007) find association in the adult-onset diabetes subset (age of onset older than 35 years) after assignment into three KIR-HLA activation groups as defined by Carrington et al (2005). Mehers et al (2011) find association with *KIR2DS2/2DL2* and *KIR2DL3* in the early-onset (less than 5 years old), HLA-C1 subset of the UK cohort. All previous studies which have reported association with KIR have found HLA-C binding centromeric KIR genes to be associated with T1D.

Of concern in these analyses is that as the starting samples are small (no more than three-hundred individuals), further subsetting and testing for multiple hypotheses (presence/absence of up to fourteen KIR genes) can lead to false positive results. Also since the HLA region is known to be associated with T1D it difficult to tell whether the signal comes from HLA or truly from the interaction. Furthermore it is unclear whether the established biological interaction between KIR and HLA necessarily translates into the statistical KIRHLA interaction claimed in those studies. As HLA-C is significantly associated with T1D before controlling for HLA Class II and HLA-B (Nejentsev et al, 2007; Howson et al, 2009), careful interaction analysis such as case-only tests (Yang et al, 1999; Cordell, 2009) are required to assess whether there is a significant epistatic KIR-HLA effect or if the reported associations to T1D are only driven by HLA-C.

The benefits of our approach

So far, KIR association studies for T1D have involved small samples sizes and have probed for presence/absence of multiple KIR genes whilst ignoring the individual copy numbers of these genes. However, copy number variation in KIR could be important as it is a mechanism which gives rise to a large diversity of activating haplotypes (Jiang et al, 2012). In our T1D candidate

KIR gene study, we gathered the largest cohort yet (765 cases and 761 controls post-QC) and limited the analysis to the copy number variation of the *KIR3DL1/S1* genes. We chose *KIR3DL1* because it is the only known KIR gene to bind to HLA-B ligands and HLA-B is known to be significantly associated with T1D whereas HLA-C is not after controlling for HLA-B and HLA Class II (Nejentsev et al, 2007; Howson et al, 2009). We then proceeded to complement our analysis with ImmunoChip SNP data leading to the largest case-control study yet by a factor of twenty.

In calling copy number states from qPCR data, rare amplifications of three or more copies are harder to classify with certainty than more common copy number states such as zero, one, or two copies. This is mainly because copy numbers higher than two are rare but also because the Δct values in qPCR follow a positively skewed distribution. However dropping samples which can not be classified with certainty may lead to bias. A less biased approach is to allow for uncertainty by using posterior probabilities in the association tests (Plagnol et al, 2007). We implemented this approach by using model based clustering to obtain posterior probabilities, followed by logistic regression on multiply imputed data in order to estimate the effect size and variance of the regression coefficients. Jointly clustering on *KIR3DL1* and *KIR3DS1*, has the advantage of exploiting the correlation between the ΔCt values. For example, this can be seen in plate 10, where noisy cases (Figure S**??**) are hard to assign as one or two copies based solely on their *KIR3DL1* ΔCt, but are much more clearly distinguishable when we also consider their *KIR3DS1* ΔCt value (Figure **??**b).

The implications

In the largest study of *KIR3DL1/S1* copy number in T1D to date, we found no association of *KIR3DL1/S1* copy number with T1D alone or conditional on HLA-Bw4 genotype. Furthermore, we did not find significant interaction between *KIR3DL1/S1* and HLA-Bw4 genotype in a case-only analysis. Our results suggest that, despite the association of HLA-B alleles with T1D and the established biological interaction between HLA-Bw4 and *KIR3DL1*, copy number variation in *KIR3DL1/S1* is unlikely to have a significant effect on the risk of developing T1D. Other KIR genes which are in high linkage disequilibrium with *KIR3DL1* and *KIR3DS1* are also unlikely to be associated. According to the Allele Frequency Net database (Gonzalez-Galarza et al, 2011), these include *KIR2DS4* (97%) and *KIR2DL3* (86%), for *KIR3DL1* and, *KIR2DL5* (81%), *KIR2DS5* (84%) and *KIR2DS1* (92%), for *KIR3DS1* (Figure S**??**).

Copy number variation in *KIR3DL1/S1* is unlikely to be an important risk factor in T1D.

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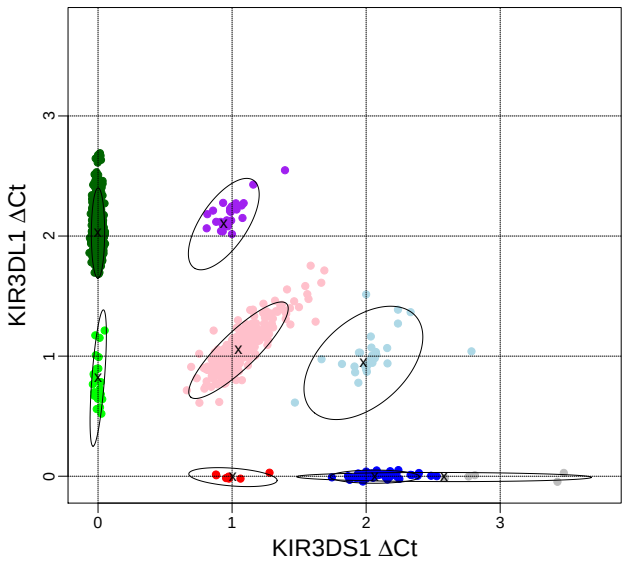
Figures



KIR3DS1-KIR3DL1	count (percentage)		
Copy Number	cases	controls	total
0-2	444 (59.44)	446 (61.35)	890 (60.38)
1-1	229 (30.66)	207 (28.47)	436 (29.58)
2-0	26 (3.48)	28 (3.85)	54 (3.66)
2-1	15 (2.01)	16 (2.2)	31 (2.1)
1-2	13 (1.74)	14 (1.93)	27 (1.83)
0-1	13 (1.74)	11 (1.51)	24 (1.63)
1-0	4 (0.54)	3 (0.41)	7 (0.47)
3-0	3 (0.4)	2 (0.28)	5 (0.34)
.-2	457 (61.18)	460 (63.27)	917 (62.21)
.-1	257 (34.4)	234 (32.19)	491 (33.31)
.-0	33 (4.42)	33 (4.54)	66 (4.48)
0-.	457 (61.18)	457 (62.86)	914 (62.01)
1-.	246 (32.93)	224 (30.81)	470 (31.89)
2-.	41 (5.49)	44 (6.05)	85 (5.77)
3-.	3 (0.4)	2 (0.28)	5 (0.34)
Total	747 (100)	727 (100)	1474 (100)

Figure 1. On the left, the median normalised Δct values for KIR3DS1 and KIR3DL1 are shown with the results of clustering into the eight genotype groups indicated by colouring points according to the group with the greatest posterior probability. The three most common genotype groups are the ones with normal copy numbers: KIR3DL1 homozygous (dark green), KIR3DL1/S1 heterozygous (pink) and KIR3DS1 homozygous (dark blue). The ellipses delimit the 95^{th} percentile. On the right, the counts of the most probable bivariate genotypes are shown for cases and controls.

a)

	qPCR					SNP				
KIR3DS1/L1	case:control	total	OR	95%CI	p-value	case:control	total	OR	95%CI	p-value
0-2	444:446	890	1.00			4091:3220	7311	1.00		
1-1	229:207	436	1.11	0.88-1.40	0.3673	2056:1628	3684	0.99	0.92-1.08	0.8828
2-0	26:28	54	0.92	0.52-1.61	0.7713	231:223	454	0.82	0.67-0.99	0.0349
2-1	15:16	31	0.94	0.46-1.93	0.8695	116:104	220	0.88	0.67-1.15	0.3422
1-2	13:14	27	0.93	0.43-2.01	0.8587	101:73	174	1.09	0.80-1.48	0.5833
0-1	13:11	24	1.19	0.53-2.68	0.6794	116:79	195	1.16	0.87-1.54	0.3273
1-0	4:3	7	1.34	0.30-6.02	0.7031	24:20	44	0.94	0.52-1.71	0.8509
3-0	3:2	5	1.52	0.27-8.62	0.6369	9:15	24	0.47	0.21-1.08	0.0756
	747:727	1474				6744:5362	12,106			

b)

	qPCR					SNP				
KIR3DL1	case:control	total	OR	95%CI	p-value	case:control	total	OR	95%CI	p-value
.-2	457:460	917	1.00			4192:3293	7485	1.00		
.-1	257:234	491	1.11	0.89-1.38	0.3702	2288:1811	4099	0.99	0.92-1.07	0.8464
.-0	33:33	66	1.01	0.61-1.66	0.9795	264:258	522	0.80	0.67-0.96	0.0159
	747:727	1474				6744:5362	12,106			

c)

	qPCR					SNP				
KIR3DS1	case:control	total	OR	95%CI	p-value	case:control	total	OR	95%CI	p-value
0-.	457:457	914	1.00			4207:3299	7506	1.00		
1-.	246:224	470	1.10	0.88-1.37	0.4096	2181:1721	3902	0.99	0.92-1.07	0.8750
2-.	41:44	85	0.94	0.60-1.47	0.7787	347:327	674	0.83	0.71-0.97	0.0224
3-.	3:2	5	1.24	0.21-7.28	0.8084	9:15	24	0.47	0.21-1.08	0.0741
	747:727	1474				6744:5362	12,106			

Table 1. No evidence of a significant joint or marginal effect of *KIR3DS1/L1* copy number with T1D in qPCR dataset, 747 cases and 727 controls, and in SNP dataset, 6,744 cases and 5,362 controls.

HLA-Bw4-80 subset

a)			qPCR			SNP				
KIR3DS1/L1	case:control	total	OR	95%CI	p-value	case:control	total	OR	95%CI	p-value
0-2	259:286	545	1.00			1025:1156	2181	1.00		
1-1	123:128	251	1.06	0.79-1.43	0.6976	556:583	1139	1.08	0.93-1.24	0.3194
2-0	16:15	31	1.22	0.58-2.57	0.5985	61:87	148	0.79	0.56-1.11	0.1733
2-1	7:13	20	0.59	0.23-1.51	0.2754	32:40	72	0.90	0.56-1.45	0.6695
1-2	8:8	16	1.10	0.41-2.98	0.8450	27:32	59	0.95	0.57-1.60	0.8513
0-1	10:7	17	1.58	0.59-4.20	0.3621	36:26	62	1.56	0.94-2.60	0.0876
1-0	2:1	3	2.21	0.20-24.50	0.5187	7:3	10	2.63	0.68-10.19	0.1614
3-0	3:0	3				3:1	4	3.38	0.35-32.51	0.2910
	428:458	886				1747:1928	3,675			

HLA-Bw4-80 subset

b)			qPCR			SNP				
KIR3DL1	case:control	total	OR	95%CI	p-value	case:control	total	OR	95%CI	p-value
.-2	267:294	561	1.00			1052:1188	2240	1.00		
.-1	140:148	288	1.04	0.78-1.38	0.7787	624:649	1273	1.09	0.95-1.25	0.2414
.-0	21:16	37	1.45	0.74-2.83	0.2822	71:91	162	0.88	0.64-1.21	0.4399
	428:458	886				1747:1928	3,675			

HLA-Bw4-80I subset

c)			qPCR			SNP				
KIR3DS1	case:control	total	OR	95%CI	p-value	case:control	total	OR	95%CI	p-value
0-.	159:187	346	1.00			650:734	1384	1.00		
1-.	93:83	176	1.32	0.92-1.90	0.1370	384:365	749	1.19	0.99-1.42	0.0578
2-.	12:14	26	1.01	0.45-2.24	0.9842	61:75	136	0.92	0.64-1.31	0.6376
3-.	2:0	2				1:0	1			
	266:284	550				1096:1174	2,270			

Table 2. KIR3DS1/L1 association with T1D in the subset of individuals carriers of an HLA-Bw4 allele. KIR3DS1 association with T1D in the subset of individuals carriers of HLA-Bw4-80I alleles.

a)		qPCR		SNP	
		HLA-Bw4-	HLA-Bw4+	HLA-Bw4-	HLA-Bw4+
KIR3DS1-	KIR3DL1+	188	269	3146	1061
KIR3DS1+	KIR3DL1-	12	21	193	71
KIR3DS1+	KIR3DL1+	119	138	1658	615
		$\chi^2_2 = 2.3612$	$p = 0.3071$	$\chi^2_2 = 2.7343$	$p = 0.2548$

b)	qPCR		SNP	
	HLA-Bw4-	HLA-Bw4+	HLA-Bw4-	HLA-Bw4+
KIR3DL1-	12	21	193	71
KIR3DL1+	307	407	4804	1676
	$\chi^2_1 = 0.3286$	$p = 0.5665$	$\chi^2_1 = 0.0916$	$p = 0.7621$

c)	qPCR		SNP	
	HLA-Bw4-80I-	HLA-Bw4-80I+	HLA-Bw4-80I-	HLA-Bw4-80I+
KIR3DS1-	298	159	3557	650
KIR3DS1+	183	107	2091	446
	$\chi^2_1 = 0.257$	$p = 0.6122$	$\chi^2_1 = 5.1171$	$p = 0.02369$

Table 3. Case-only χ^2 test in qPCR and SNP data. Distribution of KIR3DL1/S1 by relevant HLA-Bw4 genotype within cases. The test statistic and p value of chi-squared test is given for each contigency table. To reduce the degrees of freedom and improve power, we summarise copy numbers higher or equal than one by presence (+) and zero by absence (-). We find no significant association between KIR, for both compound and marginal genotypes, and HLA-Bw4 within cases.