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Genetic mapping of the major histocompatibility complex in the zebra finch (*Taeniopygia guttata*)

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Abstract Genes of the major histocompatibility complex (MHC) have received much attention in immunology, genetics and ecology because they are highly polymorphic and play important roles in parasite resistance and mate choice. Until recently the MHC of passerine birds was not well described. However, the genome sequencing of the zebra finch (*Taeniopygia guttata*), has partially redressed this gap in our knowledge of avian MHC genes. Here we contribute further to the understanding of the zebra finch MHC organization by mapping SNPs within or close to known MHC genes in the zebra finch genome. MHC class I and IIB genes were both mapped to zebra finch chromosome 16 and there was no evidence that MHC class I genes are located on chromosome 22 (as suggested by the genome assembly). We confirm the location in the MHC region on chromosome 16 for several other genes (BRD2, FLOT1, TRIM7.2, GNB2L1 and CSNK2B). Two of these (CSNK2B and FLOT1) have not previously been mapped in any other bird species. In line with previous results we also find that orthologs to the immune related genes B-NK and CLEC2D, which are part of the MHC region in chicken, are situated on zebra finch chromosome Z and not among other MHC genes in the zebra finch.

Key words MHC, Bird, Chromosome, Linkage map, SNP

Introduction

Major histocompatibility complex (MHC) genes have attracted a considerable amount of attention in many different fields of biological research due to their important role in immunity and ecology and their exceptionally high levels of genetic variation (Edwards and Hedrick 1998; Sommer 2005). Immunoecological studies in different groups of vertebrates have demonstrated links between MHC variation and several fitness related traits such as disease susceptibility, mating success and survival (Bernatchez and Landry 2003; Piertney and Oliver 2006). In birds, however, research in this field has been severely hampered by a lack of knowledge of the basic features of MHC structure and organization.

Almost all of the detailed information available on large scale bird MHC organization comes from investigations in chicken and a few other galliform birds (Chaves et al. 2007; Miller et al. 2004; Shiina et al. 2006). In those species the classical MHC genes (class I and class IIB) form a small and closely linked cluster on chromosome 16 (one of the chicken microchromosomes), characteristics that, together with strong links between specific MHC haplotypes and disease resistance, have given rise to the concept of a "minimal essential MHC" (Kaufman et al. 1999b; Kaufman et al. 1995). While MHC sequence variation has been studied in a large number of other bird species (e.g. Alcaide et al. 2008; Burri et al. 2008; Ekblom et al. 2003; Hughes et al. 2008; Tsuda et al. 2001; Westerdahl 2007), these studies have only characterized a small part of one or a few loci, while ignoring the large scale structure and organization of the genes. Preliminary results on MHC organisation from avian taxa outside the galliformes (primarily the passerine birds) indicate that MHC organization is more complex than in chicken, and is characterised by a higher degree of gene duplication, longer introns and intergenic distances resulting in the MHC spanning a larger region (Hess and Edwards 2002; Hess et al. 2000).

Recent technological advances have opened up the field of genomics to researchers studying a wide variety of non-model organisms (Ekblom and Galindo 2011; Lister et al. 2009; Wheat 2010). Using data from the sequence assembly of the second bird genome (the zebra finch; *Taeniopygia guttata*), together with targeted sequencing of MHC-containing BACs, FISH mapping and next generation digital transcriptomics data (RNA-Seq), MHC organization in this passerine species is now beginning to be described (Balakrishnan et al. 2010; Ekblom et al. 2010; Warren et al. 2010). Initial findings indicate that the zebra finch MHC is complex and may have undergone more gene duplication events (especially among the class IIB genes) than chicken. Intriguingly, this previous work also suggests that the zebra finch homologues of MHC genes found on chicken chromosome 16 are located on two discrete chromosomes, with class I genes on a separate chromosome compared to the TAP (antigen peptide transporter) genes. Furthermore the expressed MHC class I gene is placed on chromosome 22 in the zebra finch genome assembly, while two other genes linked to the chicken MHC region (B-NK and CLEC2D) are found on zebra finch chromosome Z (Balakrishnan et al. 2010).

Here we set out to identify and confirm the location of MHC related genes in the zebra finch, using linkage mapping of specific single nucleotide polymorphism (SNP) markers. MHC related genes are here broadly defined as genes known to be situated within the MHC region in this or other species and genes involved in the function of MHC immune pathways. Specifically, we tested whether linkage mapping would confirm that zebra finch MHC genes are situated on several different chromosomes as the current genome assembly suggests, possibly resulting from a fission (or lack of fusion) of chromosome 16 in this lineage. We also investigated whether B-NK and CLEC2D genes, linked to the MHC in galliform birds, were located on chromosome Z in zebra finch, as suggested by the genome sequence assembly.

Methods

Mapping population

The zebra finch International Mapping Flock (IMF) consists of a 3-generational, 354-bird pedigree that is part of a larger captive population that has been maintained at the University of Sheffield since 1985 (Birkhead et al. 2005). Within the mapping population there are 60 G_0, 43 G_1 and 251 G_2 birds. The mean sibship size among the G_2 progeny is 12.1 (range 9-27). The IMF was previously used to create a linkage map of 876 SNPs spanning 1068 cM across 45 linkage groups (Stapley et al. 2008).

Identification of putative MHC SNPs

In order to produce a linkage map of the zebra finch MHC region, we mined several DNA sequence databases to compile a list of 96 putative SNPs located in different MHC genes or genetic regions mapping to the MHC in chicken (for details about these see Online Resource 1). It was our intention to genotype up to 48 of these SNPs, but the larger list was initially compiled to ensure that 48 SNP assays with a high probability of typing success could be developed. SNPs were detectable because 454 transcriptome sequencing data of expressed genes had previously been conducted in a pool of six individuals from the mapping population (Ekblom et al. 2010). To identify those SNPs that were located in MHC genes, alignments between contigs previously identified as representing MHC genes and all reads mapping to these contigs were manually searched for polymorphic sites (for details about this annotation see Balakrishnan et al. 2010; Ekblom et al. 2010). 454 sequencing reads were also mapped onto the zebra finch genome sequence (chromosome 16 and chromosome 16_random) and individually sequenced MHC-containing zebra finch BACs (Balakrishnan et al. 2010). This allowed us to identify SNPs in un-translated regions of MHC genes and in

genes placed on chromosome 16 of the genome assembly that had not been previously annotated. In addition to identifying SNPs from the 454 data (which was generated from birds in our study population) some putative SNPs were also identified using polymorphism data from the zebra finch genome project (which used birds from other populations). Genome project SNPs are available through the ENSEMBL genome browser (www.ensembl.org/Taeniopygia_guttata/Info/Index) or BioMart (www.ensembl.org/biomart/martview). Note that because the genome project SNPs were not identified from our mapping population it is possible that many of these SNPs will not be segregating in our study population.

SNP typing

Of the 96 putative SNPs, we designed a panel of 48 SNPs for typing (for flanking sequences see Online Resource 2). These were chosen based on Illumina assay design scores and also in order to get good coverage of the regions of interest. This 48-SNP panel, containing putative MHC genes and chromosome 16 regions, was genotyped in the IMF as a custom Veracode GoldenGate kit on the Illumina BeadXpress platform. Genotypes were called using the Illumina Genome Studio Genotyping Module v1.0, and genotypes were then combined with the data used to build the linkage map reported in Stapley et al. (2008). SNPs typed in this study were not selected based on minor allele frequency or sequence depth of the assemblies, but rather chosen because they were situated in genetic regions of particular interest. This, combined with the highly variable and extensively duplicated nature of many of the genes of the MHC in most species, and the fact that some SNPs were identified in a different population, meant that we expected a rather low genotyping success rate.

Annotation of SNPs

All SNPs used in this study (Table 1) were annotated using a blast approach (Altschul et al. 1997). The SNP flanking sequences (65 – 141 bp, and in a few cases extended genomic regions surrounding these) were blasted against zebra finch, chicken and human gene predictions (using blastn) and protein sequences (using blastx). The best blast hits were used to identify the genes for annotation of each of the SNPs. In addition the SNPs were blasted against the zebra finch genome assembly to identify the chromosome location. A stand-alone blast version (2.2.18) was used for the gene annotation while the blat search engine on ENSEMBL (http://www.ensembl.org/Taeniopygia_guttata/blastview) was used for the chromosome location survey. A SNP was classified as either being synonymous, non-synonymous, intronic, UTR or intergenic, by identifying the position of the SNP within the alignment to either the zebra finch or chicken genome sequences.

Map construction

The SNPs that were successfully typed in this study were combined with the 876 SNPs used to build the previously published linkage map of the whole zebra finch genome (Stapley et al. 2008). Map construction followed the procedure previously described in Stapley et al. (2008). A version of CriMap v2.4 (Green et al. 1990) modified by Xuelu Liu (Monsanto) was used to estimate two-point linkage between all pairs of markers, assign markers into linkage groups and build a genetic linkage map for the linkage groups containing the new MHC SNPs. Linkage groups were created between markers that were linked to at least one other marker with LOD score > 5. For each linkage group a framework map was built; framework maps contained only those markers whose relative position could be assigned with LOD > 3. Any remaining markers were then added to the map iteratively using the BUILD command in CriMap at a lower stringency, such that the final build included all markers at their most likely positions.

LD analysis

The extent of linkage disequilibrium across chromosome 16 was estimated using the program Haploview v4.2 (Barrett et al. 2005). Unphased genotype data from only the founders was used for this analysis. All markers and founder individuals passed the selection criteria for inclusion in analysis (minor allele frequencies, MAF > 0.05, markers with higher than 65% call frequency and individuals that were genotyped at > 75% of loci). Information from the LD analysis was also used to infer the order of markers that had zero recombination distance (cM) between them. In cases where markers share the same map position, changing their order relative to each other does not change the likelihood of the linkage map. However, LD analysis can sometimes be informative in this respect and suggest the most parsimonious order. As a result we used estimates of LD to order three markers at the same genetic map position (1363, 1365, flot1); marker pairs in greatest LD were placed next to each other. Ordering tightly linked markers in this way also facilitates the interpretation of the LD heatmap.

Haplotype Inference

We estimated the number of distinct haplotypes within the founders (G_0 birds) of the mapping panel. By combining marker distance information with the genotypes at all chromosome 16 SNPs in the founder birds, it is possible to infer the haplotypes (i.e. the phased chromosomes) in those founders. Haplotype inference was performed using fastPHASE (Scheet and Stephens 2006) using default parameter settings. We estimated the number of haplotypes across the length of chromosome 16 and also across the 5 markers spanning the "core MHC" including the class I and class IIB genes.

Results

Genetic map

Out of 48 genotyped SNPs from zebra finch MHC genes and chromosome 16, eleven were polymorphic in the mapping population and had sufficiently high GenTrain scores (sufficient genotype clustering) for genotyping (see Online Resource 1). The mean (SE) call rate and minor allele frequencies of the 11 SNPs were 0.98 (0.007) and 0.29 (0.029) respectively. Two of these were found to be identical to SNPs used in the genetic linkage map (Stapley et al. 2008) and were not considered further. None of the 9 remaining SNPs represented amino acid altering substitutions (Table 1).

All but one SNP was linked (LOD > 5) to other markers on the linkage map, and they were assigned to two chromosomes. Five SNPs (situated in the genes TRIM7.2, BRD2, FLOT1, MHC class I, and MHC class IIB) were mapped to chromosome 16, and three SNPs (in genes RCL1, B-NK and CLEC2D) were mapped to chromosome Z (Fig. 1). Two SNPs located in presumably functional MHC class I and IIB genes (as inferred from genetic, and transcriptomic sequences, see Balakrishnan et al. 2010; Ekblom et al. 2010), as well as several other genes, were thus found to be linked and situated within the zebra finch MHC region on chromosome 16. The SNP that could not be mapped was from a putative MHC class I pseudogene (ψC). This maker was weakly linked (LOD > 1) to 44 other markers that were spread across 10 chromosomes. The maximum LOD observed for this marker was 2.21 to a marker on chromosome 19. However, there was no linkage (LOD > 1) to markers on chromosome 16 or Z. The resulting genetic sex-average map of chromosome 16 contains 9 markers and spans 46 cM (Female map = 46 cM, Male map = 44.5 cM). The genes coding for two lectin proteins (B-NK and CLEC2D) have been shown to be integrated in the MHC of the chicken as well as other galliform birds (Hosomichi et al. 2006). Here we verify the finding

from Balakrishnan and co-workers (2010) that the zebra finch homologs of these genes are situated on the Z chromosome, close to the RCL1 (RNA terminal phosphate cyclise-like 1) gene (Fig. 1). None of the MHC SNPs typed in this study were assigned to zebra finch chromosome 22 or any other assembled chromosomes other than 16 and Z.

LD analysis

Overall LD on zebra finch chromosome 16 was relatively low; average r^2 across all markers was 0.07 and > 90% of pairwise LD was < 0.3 (Fig. 2). It is not possible to estimate recombination rate across the chromosome because the physical size is unknown. The genetic map length of this chromosome is slightly longer than other microchromosomes with a similar number of markers (4.7 - 39.9 cM) (Stapley et al. 2008), but the physical size of chromosome 16 is thought to be smaller than these chromosomes (International Chicken Genome Sequencing Consortium 2004; Warren et al. 2010). Recent estimates of the length of chicken chromosome 16 suggest that it may be around 10 Mb long (Solinhac et al. 2010). The genetic region corresponding to our chromosome 16 linkage group (from MHC class IIB to TRIM7.2) in chicken is about 100 kb long (Kaufman et al. 1999b) and the size in the turkey is around 140 kb (Chaves et al. 2007). Although it is likely that the physical size of the zebra finch MHC is considerably larger than this (Balakrishnan et al. 2010), our results would suggest unusually high levels of recombination rate in the zebra finch MHC region. The region containing markers Tgu_SNP_01365, Tgu_SNP_01363, flot1 and tgu_class1_7, showed slightly higher levels of LD between markers (mean $r^2 = 0.32$) compared to other parts of chromosome 16. However, without any knowledge of the physical distance between these markers it is not possible to determine the relevance of this LD, because it may simply reflect close physical linkage between these markers. The linkage map size of this region was 4.4 cM (Fig. 1).

Haplotype Inference

Within the 60 founder birds, we identified 53 unique haplotypes across chromosome 16, suggesting that recombination has helped to maintain diversity on this chromosome. However, the region around the "core MHC", spanning five markers (1363, 1365, flot1, tgu_class1_7, tmptgu393923) contained just three unique haplotypes, indicating that genetic diversity is relatively low in this part of the zebra finch genome. This finding also suggests that recombination is relatively rare in the part of chromosome 16 that contains the core MHC genes.

Discussion

Balakrishnan and co-authors (2010) reported that zebra finch MHC genes seem to be spread over several chromosomes. Our study reveals that one MHC class I and one MHC class IIB locus are closely linked and placed together on chromosome 16 (Fig. 1). All markers situated on chromosome 16 were assigned to that linkage group with very high confidence. These include two SNPs in expressed MHC genes corresponding to locus 1 for the class I gene and locus 2 for the class IIB gene following the nomenclature of Balakrishnan et al. (2010). Thus, there is no evidence that the homologue of chicken chromosome 16 is represented by two smaller chromosomes in the zebra finch. We found no support for the placement of the MHC class I locus on chromosome 22 as suggested by the recently released zebra finch genome assembly (Warren *et al.*, 2010). A similar conclusion was drawn by Balakrishnan et al. (2010) based on the fact that a class I probe did not co-hybridise with a chromosome 22 probe using FISH mapping. Note, however, that one SNP placed in a putative MHC class I pseudogene (locus ψC) was not linked to the rest of the MHC region or any other known linkage group. Futhermore, we were unable to detect any polymorphism within the TAP or TNXB genes,

which Balakrishnan et al. (2010) reported were on the BAC that did not co-hybridise with the rest of the MHC region. Therefore, we could not design SNP assays to map these two genes. Our study also demonstrated that five other genes (TRIM7.2, GNB2L1, CSNK2B, BRD2, and FLOT1) are situated in the zebra finch MHC region. These have all previously been reported as residing in the MHC of chicken and/or humans (The MHC Sequencing Consortium 1999), but CSNK2B and FLOT1 have not been previously mapped or annotated in the genome assembly of any bird species apart from zebra finch.

The inclusion of several new SNPs to the zebra finch linkage map has improved the original genetic mapping of chromosome 16. In the first generation linkage map only two markers mapped to this chromosome and in the second generation map an additional marker (TS1365) was included (Backström et al. 2010). In the present study two additional SNPs (Tgu_SNP_01363 and Tgu_SNP_01547), that were previously mapped by Stapley et al. (2008) to an unknown linkage group (TguUN4) are shown to be part of chromosome 16. Together with the new MHC markers described here the linkage group on chromosome 16 now contains nine markers, spanning the region from the MHC class IIB gene to TRIM7.2.

In chicken and other galliform birds an independent cluster of MHC genes (MHC-Y) is located on the same chromosome as the classical MHC (MHC-B) but these are separated by a region of very high recombination (Briles et al. 1993; Miller et al. 1996). In the chicken linkage map there are 18 markers on chromosome 16. These are situated in two linkage groups corresponding to the classical MHC (B) locus and the MHC-Y locus, localized about 60 cM apart, making them effectively unlinked. It has not been possible to provide fine scale resolution of the 12 markers mapping to the MHC-B region (Groenen et al. 2000). Therefore, there is a high rate of recombination between the B and Y regions on chicken chromosome 16

but low recombination rate within the B-region. It has been argued that the compact MHC region of the chicken has resulted in tight linkage between functionally interacting immune genes, enabling co-evolution between them; the minimal essential MHC hypothesis (Kaufman et al. 1999a). The B-region of chicken has traditionally been divided further into a "B-F/B-L region" containing the classical MHC class I and class IIB genes and a "B-G region" containing so called B-G genes (Kaufman et al. 1995). Low but significant rates of recombination have been observed between these two parts of the chicken MHC by investigation of crosses between different inbred lines (Skjødt et al. 1985). A more detailed mapping of the chicken MHC region was recently presented by Solinhac and colleagues (2010). There the genetic map of chicken chromosome 16 is 130.7 cM long and consists of 33 markers distributed over the MHC-B, MHC-Y and nucleolus organizing region (NOR). The only additional previous detailed genetic mapping study of the classical MHC region in birds has recently been performed on the turkey (*Meleagris gallopavo*). Here, 14 SNP and microsatellite markers were typed in a large number of offspring from two females (Chaves et al. 2010). The markers were spread over a physical distance of approximately 200 kb across the complete B-locus (from the TRIM7.2 gene to the C4 gene). After having controlled for three gene conversion events, the resulting genetic map was 3.6 cM, giving a recombination rate of 18 cM/Mb (an order of magnitude higher than for the average of the turkey genome).

Our mapping of the zebra finch chromosome 16 found evidence of a considerable amount of recombination between markers in the MHC region. The low level of LD on this chromosome is similar to the pattern of LD found on other microchromosomes (Stapley et al. 2010). There is a strong negative relationship between recombination rate and chromosome length in the zebra finch (Stapley et al. 2008). Given that chromosome 16 is one of the smallest chromosomes in the avian genome, it is thus not surprising that we find a high degree of

recombination here. However, our finding of a considerable genetic distance (9.1 cM) between classical MHC class I and class IIB loci is in contrast with the chicken where recombination within the classical MHC region (B-F/B-L) seems rare (see discussion above regarding the minimal essential MHC hypothesis). However, our estimate of genetic distance across chromosome 16 needs to be treated with care, because our estimated genetic map distance could be inflated by recent gene conversion events, as was observed in the turkey MHC region (Chaves et al. 2010). Also, since we don't know the physical length of the mapped zebra finch chromosome 16 region we can not obtain a reliable estimate on local recombination rate. The fact that we only identified three distinct haplotypes in our 60 founders in the "core MHC" is perhaps consistent with recombination being relatively rare in this particular region of chromosome 16.

Even though there has been two studies of genome-wide linkage disequilibrium of the zebra finch (Backström et al. 2010; Stapley et al. 2010), neither of these have been able to successfully map and analyse chromosome 16. Difficulty in mapping this chromosome in the past is most likely due to a combination of a high recombination rate and very small size. Recombination is considered an important process creating genetic and haplotype variability (e.g. Begun and Aquadro 1992; Jaramillo-Correa et al. 2010) and this may be an important factor contributing to the variability of MHC loci too. Thus, even though different MHC genes are linked on a physically small chromosome, a high recombination rate in this region would mean that novel haplotypes between these loci are constantly being generated by recombination (Schaschl et al. 2006), however our finding of a rather limited number of haplotypes in the "core MHC" region would argue against this mechanism at least for MHC class I and IIB loci. Recombination rates across different parts of the MHC region may have

profound implications on how the adaptive immune system is evolving in this lineage of birds.

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Table 1 Information, gene annotation and chromosomal positions of all SNP markers used in this study (for sequences see Online Resource 2). Markers with names beginning with "Tgu_SNP" were typed in the original genetic map of Stapley et al. (2008). Names beginning with "tmptgu" were taken from the ENSEMBL polymorphism data and other markers where designed from transcriptome and BAC sequencing data. The locus designations of MHC class I and II genes follow the nomenclature of Balakrishnan et al. (2010).

SNP name	Gene name	Gene description	Chromosome	Position	Type
Tgu_SNP_00837	RPL17	60S Ribosomal protein L17	Z	1213283	Synonymous
Tgu_SNP_01249	ACAA2	Acetyl-coenzyme A acyltransferase 2	Z	1464495	3'UTR
Tgu_SNP_01537	ACAA2	Acetyl-coenzyme A acyltransferase 2	Z	1464313	3'UTR
Tgu_SNP_01362	LOC100223824	Similar to tetraspanin-3	Z	1609192	Downstream
Tgu_SNP_01309	FBP1	Fructose-1,6-bisphosphatase 1	Z	10110645	Synonymous
Tgu_SNP_01100	CLTA	Clathrin light chain A	Z	2053857	Synonymous
Tgu_SNP_01413	CLTA	Clathrin light chain A	Z	2057898	3'UTR
Tgu_SNP_00108	BHMT	Betaine--homocysteine S-methyltransferase	Z	56027090	Synonymous
Tgu_SNP_00366	PSIP1	PC4 and SFRS1-interacting protein (Lens epithelium growth factor)	Z	59919835	3'UTR
tmptgu2430803	RCL1	RNA terminal phosphate cyclase-like 1	Z	64096600	Intronic
tmptgu2430825	B-NK	NK receptor-like; Blec2	Z	64154902	Intronic
tmptgu2430826	CLEC2D	C-type lectin domain family 2, member D; Blec1	Z	64164380	Synonymous
Tgu_SNP_00708	GHR	Growth hormone receptor	Z	44207137	Upstream
Tgu_SNP_00517	SMU1	smu-1 suppressor of mec-8 and unc-52 homolog	Z	31446385	Downstream
Tgu_SNP_00109	BHMT	Betaine--homocysteine S-methyltransferase	Z	56026996	3'UTR
Tgu_SNP_00582	LMNB1	Lamin B1	Z	68946310	Downstream
Tgu_SNP_01007	TBCA	Tubulin-specific chaperone A	Z	56676486	Synonymous
Tgu_SNP_00183	VCAN	Versican core protein Precursor	Z	70951190	Synonymous
trim7.2-2	TRIM7.2	Tripartite motif protein 7	16_random	157419	Synonymous
Tgu_SNP_00725	GNB2L1	Guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1; C12-3	16 (Chicken)	Unknown	Synonymous
Tgu_SNP_01547	CSNK2B	Casein kinase II subunit beta	16	270	Synonymous
brd2	BRD2	Bromodomain containing 2; RING3	UN	5958501	Synonymous
Tgu_SNP_01365	-	Unknown Zink finger protein	MHC BAC Contig 93	22810	Unknown
Tgu_SNP_01363	-	Unknown Zink finger protein	MHC BAC Contig 93	23065	Unknown
flot1	FLOT1	Flotillin 1	MHC BAC Contig 93	21298	Intronic
tgu_classI-7	-	MHC class I alpha chain (locus 1)	MHC BAC Contig 93	10081	3'UTR
tmptgu393923	-	MHC class II beta chain (locus 2)	UN	115905700	Synonymous
tmptgu347102	-	MHC class I alpha chain (ψ-locus C)	UN	70834777	Downstream

Figure captions

Fig. 1 Sex averaged genetic maps of zebra finch chromosomes 16 and Z. Linkage map position (cM) to the left and marker names to the right (with corresponding gene symbols within brackets). For brevity TGU_SNPs are represented by the last 4 numbers only (e.g. TGU_SNP_00725 is represented by 0725). Markers "1363" and "1365" are situated in an unknown zink finger protein gene, "tgu_classI-7" corresponds to MHC class I locus 1 and the marker "tmptgu393923" to MHC class IIB locus 2

Fig. 2 Linkage disequilibrium heat map for zebra finch chromosome 16. In each square are the pairwise LD values (r^2*100) between markers and shading corresponds to the amount of LD, ranging from black for high LD ($r^2 = 1$) to white for low LD ($r^2 = 0$). On the top is the genetic map for the linkage group. For clarity TGU_SNPs are represented by the last 4 numbers only (e.g. TGU_SNP_00725 is represented by 0725) and corresponding gene symbols are given within brackets. Markers "1363" and "1365" are situated in an unknown zink finger protein gene, "tgu_classI-7" corresponds to MHC class I locus 1 and the marker "tmptgu393923" to MHC class IIB locus 2

Electronic supplementary material

Online Resource 1: Table with information on all the MHC related SNPs identified in this study (96SNPinfo.xls)

Online Resource 2: Sequences of all SNPs included in this study (SNPs.fas)

Fig. 1

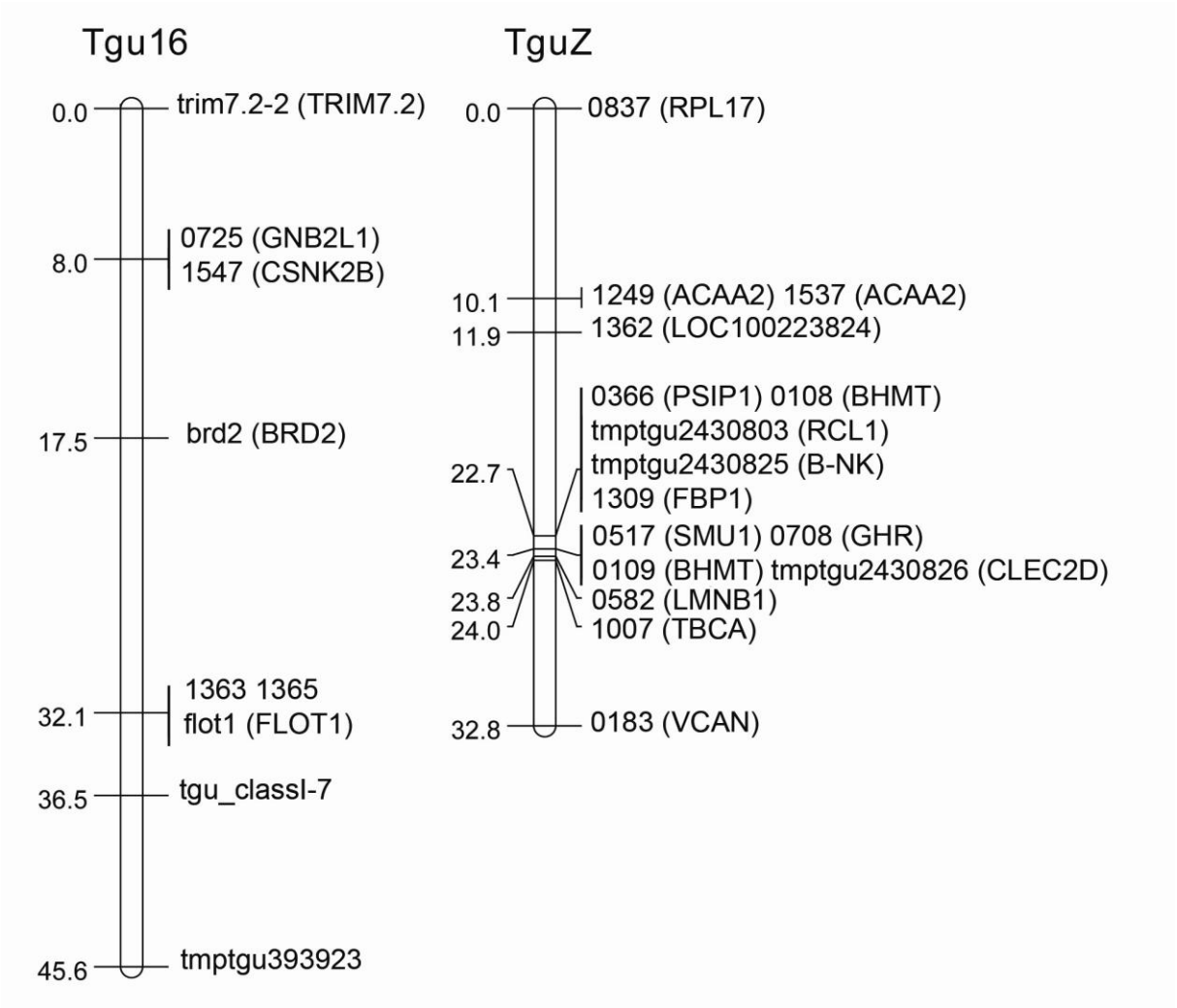
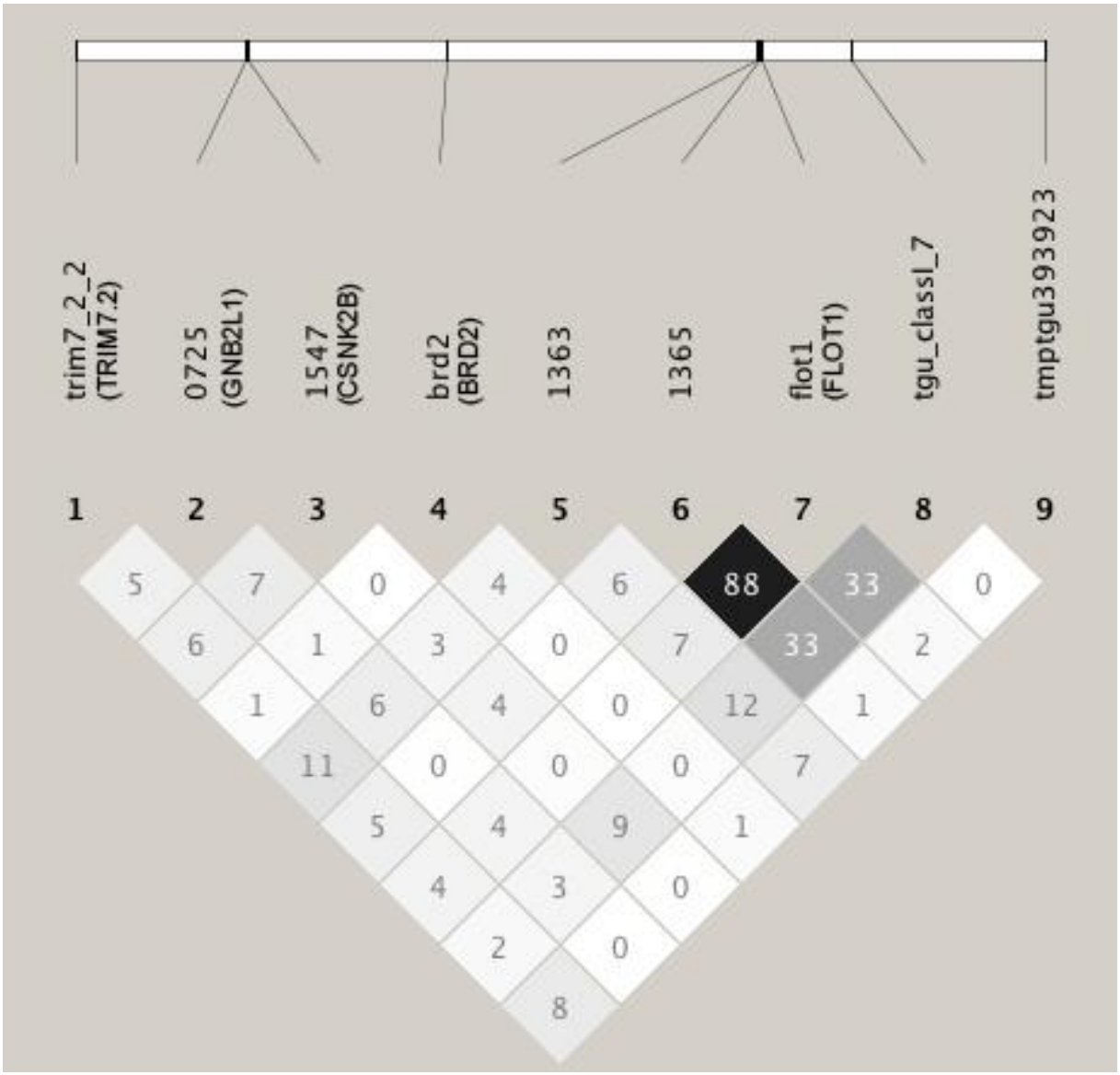

Tgu16
0.0
trim7.2-2 (TRIM7.2)
8.0
0725 (GNB2L1)
1547 (CSNK2B)
17.5
brd2 (BRD2)
32.1
1363 1365
flot1 (FLOT1)
36.5
tgu_classI-7
45.6
tmptgu393923
TguZ
0.0
0837 (RPL17)
10.1
1249 (ACAA2) 1537 (ACAA2)
11.9
1362 (LOC100223824)
22.7
0366 (PSIP1) 0108 (BHMT)
tmptgu2430803 (RCL1)
tmptgu2430825 (B-NK)
1309 (FBP1)
23.4
0517 (SMU1) 0708 (GHR)
0109 (BHMT) tmptgu2430826 (CLEC2D)
23.8
0582 (LMNB1)
24.0
1007 (TBCA)
32.8
0183 (VCAN)

Fig. 2


trim7_2_2 (TRIM7.2)
0725 (GNB2L1)
1547 (CSNK2B)
brd2 (BRD2)
1363
1365
flot1 (FLOT1)
tgu_classl_7
tmptgu393923
1
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4
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0
8

Figure 1

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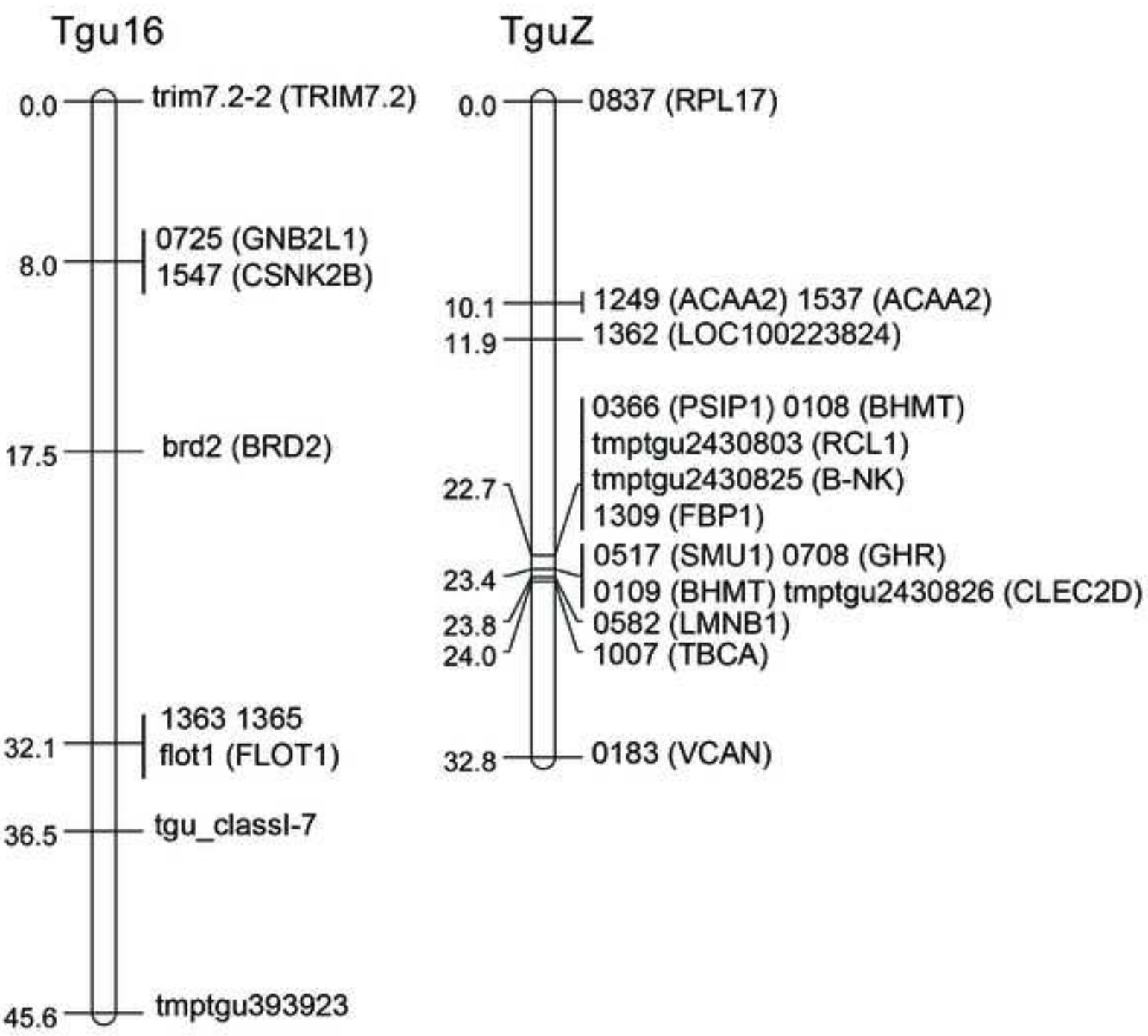

Figure 2

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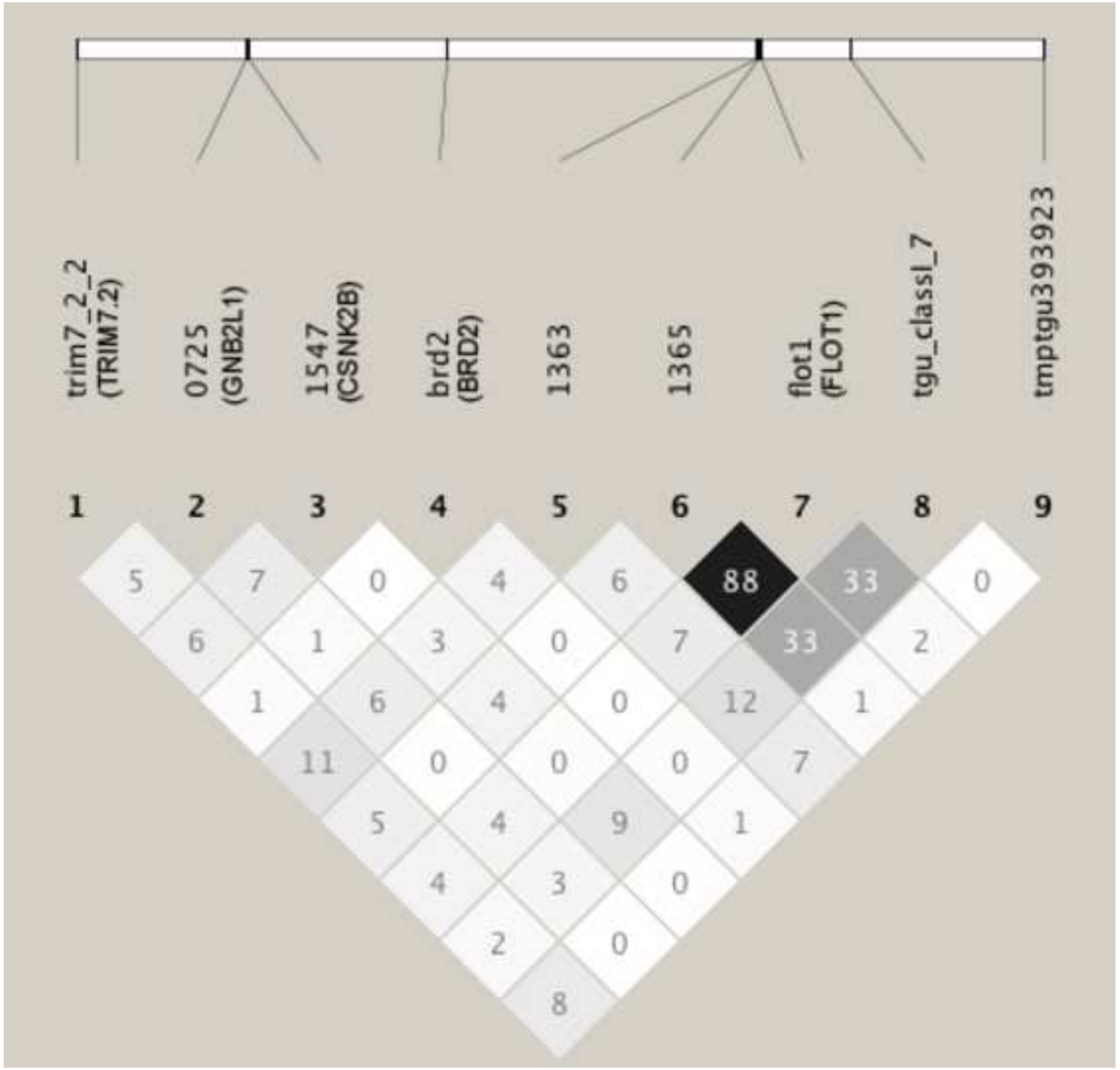

Online resource 1

Click here to download Supplementary Material: 96SNPinfo.xls

Online Resource 2

Click here to download Supplementary Material: SNPs.fas