Asymmetric Linkage Disequilibrium: Tools for Assessing Multiallelic LD

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Abstract

Standard measures of linkage disequilibrium (LD) provide an incomplete description of the correlation between two loci. Recently, Thomson and Single (2014) described a new *asymmetric* pair of LD measures (ALD) that give a more complete description of LD. The ALD measures are symmetric and equivalent to the correlation coefficient r when both loci are bi-allelic. When the numbers of alleles at the two loci differ, the ALD measures capture this asymmetry and provide additional detail about the LD structure. In disease association studies the ALD measures are useful for identifying additional disease genes in a genetic region, by conditioning on known effects. In evolutionary genetic studies ALD measures provide insight into selection acting on individual amino acids of specific genes, or other loci in high LD (see Thomson and Single (2014) for these examples). Here we describe new software for computing and visualizing ALD. We demonstrate the utility of this software using haplotype frequency data from the National Marrow Donor Program (NMDP). This enhances our understanding of LD patterns in the NMDP data by quantifying the degree to which LD is asymmetric and also quantifies this effect for individual alleles.

1. Introduction

Linkage disequilibrium (LD) is the non-random association of alleles at two or more loci. Understanding and incorporating the LD structure of a genetic region into analyses is necessary for detecting disease predisposing variants, as well as for understanding the evolutionary history of a genetic region.

The basic unit of LD is defined as $D_{ij} = f_{ij} - p_{Ai} p_{Bj}$, where f_{ij} is the frequency for the $A_i B_j$ haplotype and p_{Ai} and p_{Bj} are frequencies for alleles A_i and B_j , respectively. Since the maximum value that D_{ij} can assume is a function of the observed allele frequencies, there are several normalized measures of the strength of LD for *bi*- and *multi*-allelic data [1, 2]. The two most common measures of the strength of LD are: (1) the normalized measure of the individual LD values [3], $D_{ij}' = D_{ij} / D_{max}$, and (2) the correlation coefficient r for *bi-allelic* data, often reported as $r^2 = D_{ij}^2 / (p_{A1} p_{A2} p_{B1} p_{B2})$.

The ALD measures [4] can be written as normalized versions of an overall allele specific homozygosity statistic. The ordinary homozygosity (F) statistic, $F_A = \sum_i p_{Ai}^2$, extends to a conditional two locus statistic, called Haplotype or Allele Specific Homozygosity (ASF). It measures the level of genetic variation at locus A on haplotypes with the B_j allele at locus B (and vice versa), $F_{A/Bj} = \sum_i (f_{ij} / p_{Bj})^2$ [5]. When $ASF=1.0$ there is complete correlation of the genetic variation at the two loci *on the specified conditional haplotype*. Lower values of ASF indicate a relatively larger amount of variation at the other locus on the conditional haplotype. A weighted average of ASF values, over all alleles at the conditioned upon locus, gives the overall measure of ASF, $F_{A/B} = \sum_j (F_{A/Bj}) (p_{Bj})$. A similar result, swapping the roles of locus A and B in the above formula, is obtained by conditioning on the A locus.

The squared ALD statistic, conditioning on locus B, is a standardized measure of the difference between these conditional and unconditional homozygosity measures: $W_{A/B}^2 = (F_{A/B} - F_A) / (1 - F_A)$. Swapping the roles of the A and B subscripts gives the measure conditioned on variation at the A locus. When the relationship is symmetric, the squared ALD measures are equal to W_n^2 , the multi-allelic extension of the r^2 measure also known as Cramer's V statistic[6]. In the bi-allelic case they collapse to the well-known r^2 measure.

2. Methods - Asymmetric LD Web Application

Using the R shiny server [7] we developed a web application that computes the ALD measures and visualizations of the measures and their components.

The ALD app allows the user to upload data in two formats. The first format is a file of multilocus haplotypes for any number of loci followed by the haplotype frequency. The first row must be a header with the locus names and an arbitrary name for the column of frequencies. There are $k+1$ columns, where k is the number of loci (Table 1). The second file format has a list of pairwise haplotype frequencies. There are five columns that must have the names *locus1*, *locus2*, *allele1*, *allele2*, and *haplo.freq*. The order of the loci in the input file determines the order for plotting ALD statistics.

For a set of haplotypes, the sum of the frequencies should be equal to one, but there are situations where the sum may be less than one (e.g., when very low frequency haplotypes are not reported). In order to accommodate this situation, the ALD app has a “tolerance” parameter that can be set by the user (Figure 1). In order for the app to begin computations, the sum of the haplotype frequencies must be within plus or minus tolerance of 1.0. The default value for the tolerance is 0.01.

The combination of ASF and allele frequency directly indicates the extent to which specific haplotypes contribute to the overall extent of asymmetric LD. Because of this, we decided to conditionally format the entries in the ASF table to visually highlight those alleles that have large contributions to overall LD when conditioning on one of the loci. This was accomplished using the d3.js javascript library to scale colors from green to red based on a linear scale from zero to the observed maximum for haplotype frequencies and zero to one for the ASF values. Scaling is done separately for the ASF data and allele frequency data.

3. Results - Application to NMDP Haplotype Frequency data

We applied the ALD measures to data for high resolution HLA haplotypes in the US population [8] downloaded from the NMDP since this is the largest publically available dataset of HLA haplotype

frequencies used for clinical and research applications. The data consist of haplotype frequency estimates for HLA-A, -C, -B, -DRB1, and -DQB1 from 21 detailed populations and five broad U.S. Census categories of race and ethnicity. In what follows we describe results from the European American/Caucasian (abbr: CAU) and Hispanic (abbr: HIS) broad race/ethnicity populations.

Figures 1 and 2 have ALD plots for the CAU and HIS broad groups, respectively. The figures show a good deal of asymmetry in the LD relationships for the C~B and the DRB1~DQB1 locus pairs. For the CAU population, the overall variation for DQB1 is relatively low given specific DRB1 alleles ($W_{DQB1/DRB1} = 0.92$) while overall variation at DRB1 is greater given specific DQB1 alleles ($W_{DRB1/DQB1} = 0.74$). A similar, but even more extreme degree of asymmetry is seen in the HIS population.

Figures 3 and 4 have allele specific homozygosity (ASF) values for a set of DRB1~DQB1 pairs. Figure 3 also contains the allele frequency for specific alleles (focal alleles) at each locus. The combination of these values provides insight into the asymmetric pattern of LD seen for this pair of loci. For example, in the HIS population the ASF value is 1.0 for DRB1*01:02, indicating that there is no variability at the DQB1 locus for haplotypes containing DRB1*01:02. However, the contribution to overall ALD by this haplotype is moderated by the lower allele frequency for the focal allele in the HIS population and even lower frequency in the CAU population. The DRB1*03:01 allele has a relatively high frequency in both population along with a high ASF indicating that it contributes substantially to ALD in each population.

The highlighting/coloring of the ASF and allele frequency values allows one to easily see combinations that contribute to high LD. The higher $W_{DQB1/DRB1}$ values for the CAU and HIS populations are explained by the larger fraction of red-orange and orange-orange pairings (high ASF and high allele frequency) in the CAU and HIS populations. The lower $W_{DRB1/DQB1}$ value in the HIS population compared to the CAU population is highlighted by the lack of pairings in the red-orange end of the spectrum for the HIS population compared to the CAU.

From Figure 3 it is clear that the DQB1*06:02 allele makes a large contribution to $W_{DRB1/DQB1}$ in the CAU population, since it has high ASF and allele frequency values. DQB1*06:02 has an allele frequency of 0.1287 and ASF value of 0.9906. The DRB1*15:01~DQB1*06:02 haplotype accounts for 99.5% of the DRB1~DQB1 haplotype frequencies where DQB1*06:02 is the focal allele. (see supplementary Table 1). Thus, although there are a large number of DRB1 alleles (24 different ones) that occur with DQB1*06:02 in haplotypes, their frequency contribution is very small.

In the HIS population, there is no DQB1 allele with frequency greater than 0.05 and ASF value greater than 0.70. Figure 3 shows that DQB1*06:02 is relatively common with a frequency of 0.0759, but an ASF value of only 0.6825. While the DRB1*15:01~DQB1*06:02 haplotype is the most frequent DRB1~DQB1 haplotype with DQB1*06:02 as the focal allele, it only accounts for 81.2% of the DRB1~DQB1 haplotype frequency where DQB1*06:02 is the focal allele (see supplementary Table 2).

4. Discussion

Since the input to the ALD app is haplotype frequency data, it can be used with data from a variety of public data repositories. There is a large literature on the accuracy of haplotype frequency estimation (see [9] for recent references). Issues of estimation accuracy are particularly relevant for highly polymorphic data and the accuracy of LD estimates will be affected by that of the haplotype frequency data used to compute them.

ALD is a valuable tool for Hematopoietic Stem Cell (HSC) registries because it can inform optimal typing strategies. The Bone Marrow Donors Worldwide (BMDW.org) database lists HLA typing information on over 25 Million volunteer donors and cord blood units. Although the clinical standard for matching is currently to match at 10/10 alleles (5 HLA loci with 2 alleles each) the majority (54.6%) of donors in the database lack HLA-C typings and an even larger proportion lack HLA-DQB1 typings (72.6%).

Based on the ALD results (Figures 3 and 4) these are the two least informative loci since the ALD values are 0.87 & 0.85 for HLA-C conditional on HLA-B and 0.92 and 0.94 for HLA-DQB1 conditional on HLA-DRB1 in two representative populations. As clinical standards evolve to include more loci (e.g. HLA-DPB1, HLA-DQA1, MHC SNPs) the ALD measure will be a useful tool for informing decisions about how many donors to retype at these loci and how much information one can expect to glean from this additional typing beyond that which can be recovered through imputation-based matching algorithms [10].

Acknowledgments

This work was supported in part by a REACH grant from the University of Vermont and the Office of Naval Research (ONR) grant N00014-14-1-0848. The content is solely the responsibility of the authors and does not necessarily represent the official views of the ONR. The authors would like to thank Jordan Bakerman for initial feedback on the software. RS and GT developed the methodology. RS developed the software framework. RS, NS, VP, and MA developed the web application. RS and MM wrote the manuscript. NS, GT, VP, and MA reviewed the manuscript.

A	C	B	DRB1	DQB1	freq
01:01g	01:02	15:01g	01:01	05:01	0.00006
01:01g	01:02	15:01g	09:01	03:03	0.00006
01:01g	01:02	15:01g	13:01	06:03	0.00007
01:01g	01:02	27:05g	01:01	05:01	0.00016
...					

Table 1: multilocus haplotype frequency file format

locus1	locus2	allele1	allele2	haplo.freq
A	C	01:01	07:01	0.07435
A	C	02:01	03:04	0.05002
...				
DRB1	DQB1	07:01	06:02	0.00168
DRB1	DQB1	11:01	03:02	0.0025

Table 2: pairwise haplotype frequency file format

Figure 1: Screenshot of the ALD web app. Note that the tolerance value needed to be set to 0.02 since the sum of the haplotype frequencies was 0.98. The plot has ALD measures for Classical HLA Genes in the Caucasian (CAU) broad race category of the NMDP Donor population. Data Source: Six-locus high resolution HLA haplotype frequencies derived from mixed-resolution DNA typing for the entire US donor registry (Gragert et al. 2013).

Figure 2: ALD Measures for Classical HLA Genes in the Hispanic (HIS) broad race category of the NMDP Donor population. Data Source: as in Figure 1.

Figure 3: Allele specific homozygosity (ASF) and allele frequency values for certain DRB1~DQB1 pairs in the Caucasian broad race category (CAU). The focal allele is the allele conditioned upon and the ASF value is the homozygosity value computed by conditioning on this allele. High ASF values for higher frequency alleles make a large contribution to overall ALD. Data Source: as in Figure 1.

Figure 4: Allele specific homozygosity (ASF) and allele frequency values for certain DRB1~DQB1 pairs in the Hispanic broad race category (HIS). Data Source: as in Figure 1.

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Figure 1

Figure 2

Asymmetric LD
row gene conditional on
column gene

Figure 3a

ALD Table	Allele Specific Homozygosity		Raw Data	Citation
Show	25	entries	Search: <input type="text"/>	
loci	focal	allele	allele.freq	as.homz
DRB1-DQB1	DRB1	DRB1*07:01	0.130515064	0.6160090
DRB1-DQB1	DRB1	DRB1*15:01	0.130195915	0.9685337
DRB1-DQB1	DRB1	DRB1*03:01	0.114896983	0.9984061
DRB1-DQB1	DRB1	DRB1*01:01	0.085754340	0.9890753
DRB1-DQB1	DRB1	DRB1*04:01	0.080899916	0.5088757
DRB1-DQB1	DRB1	DRB1*11:01g	0.063742614	0.9964338
DRB1-DQB1	DRB1	DRB1*13:01	0.062135957	0.9971113
DRB1-DQB1	DRB1	DRB1*13:02	0.046375042	0.6925585
DRB1-DQB1	DRB1	DRB1*11:04	0.036406796	0.9498116
DRB1-DQB1	DRB1	DRB1*04:04	0.034139333	0.9274368
DRB1-DQB1	DRB1	DRB1*14:01g	0.027103872	0.9961778
DRB1-DQB1	DRB1	DRB1*08:01	0.023698311	0.9901462
DRB1-DQB1	DRB1	DRB1*16:01	0.017360643	0.9996086
DRB1-DQB1	DRB1	DRB1*12:01g	0.017355707	0.9983291
DRB1-DQB1	DRB1	DRB1*01:02	0.015078181	0.9999215
DRB1-DQB1	DRB1	DRB1*04:02	0.012664294	0.9997942
DRB1-DQB1	DRB1	DRB1*13:03	0.011921988	0.9990803
DRB1-DQB1	DRB1	DRB1*04:07g	0.009666515	0.9812778
DRB1-DQB1	DRB1	DRB1*09:01	0.009648458	0.9351685

Figure 3b

ALD Table

Allele Specific Homozygosity

Raw Data

Citation

Show 25 entries

Search:

loci	focal	allele	allele.freq	as.homz
DRB1-DQB1	DQB1	DQB1*02:01g	0.2131512198	0.4959878
DRB1-DQB1	DQB1	DQB1*03:01g	0.1954839580	0.1870543
DRB1-DQB1	DQB1	DQB1*06:02	0.1287325386	0.9905562
DRB1-DQB1	DQB1	DQB1*05:01	0.1166058473	0.5613106
DRB1-DQB1	DQB1	DQB1*03:02g	0.1034732672	0.3188934
DRB1-DQB1	DQB1	DQB1*06:03g	0.0645293692	0.9251309
DRB1-DQB1	DQB1	DQB1*03:03g	0.0431398013	0.6594968
DRB1-DQB1	DQB1	DQB1*06:04g	0.0376412925	0.9989085
DRB1-DQB1	DQB1	DQB1*05:03g	0.0281808686	0.9223514
DRB1-DQB1	DQB1	DQB1*04:02	0.0277836033	0.7266238
DRB1-DQB1	DQB1	DQB1*05:02	0.0197760260	0.7779145
DRB1-DQB1	DQB1	DQB1*06:01	0.0089113792	0.9844526
DRB1-DQB1	DQB1	DQB1*06:09	0.0086210536	0.9972235
DRB1-DQB1	DQB1	DQB1*03:04	0.0019364082	0.5119334
DRB1-DQB1	DQB1	DQB1*03:05	0.0015106747	0.9946769
DRB1-DQB1	DQB1	DQB1*05:04	0.0004507781	1.0000000
DRB1-DQB1	DQB1	DQB1*04:01g	0.0000482776	1.0000000
DRB1-DQB1	DQB1	DQB1*06:16	0.0000039864	0.7825367
DRB1-DQB1	DQB1	DQB1*06:07	0.0000028984	0.7172841

Figure 4a

ALD Table	Allele Specific Homozygosity		Raw Data	Citation
Show	25	entries	Search: <input type="text"/>	
loci	focal	allele	allele.freq	as.homz
DRB1-DQB1	DRB1	DRB1*07:01	0.10966997	0.8084626
DRB1-DQB1	DRB1	DRB1*03:01	0.07145245	1.0000000
DRB1-DQB1	DRB1	DRB1*08:02	0.07055771	0.9945510
DRB1-DQB1	DRB1	DRB1*04:07g	0.06617131	0.9601870
DRB1-DQB1	DRB1	DRB1*15:01	0.06235957	0.9762197
DRB1-DQB1	DRB1	DRB1*04:04	0.04736959	0.9953561
DRB1-DQB1	DRB1	DRB1*13:01	0.04573315	0.9818664
DRB1-DQB1	DRB1	DRB1*01:01	0.04572409	0.9987113
DRB1-DQB1	DRB1	DRB1*11:01g	0.04056052	0.8774626
DRB1-DQB1	DRB1	DRB1*13:02	0.04049713	0.5421140
DRB1-DQB1	DRB1	DRB1*01:02	0.03500178	1.0000000
DRB1-DQB1	DRB1	DRB1*11:04	0.03057164	0.9386669
DRB1-DQB1	DRB1	DRB1*14:06	0.02843409	1.0000000
DRB1-DQB1	DRB1	DRB1*04:05	0.02349153	0.7058320
DRB1-DQB1	DRB1	DRB1*16:02	0.02035360	0.8888536
DRB1-DQB1	DRB1	DRB1*04:11	0.02019289	0.7761968
DRB1-DQB1	DRB1	DRB1*14:02	0.01995422	0.8567913
DRB1-DQB1	DRB1	DRB1*04:01	0.01835482	0.5008228
DRB1-DQB1	DRB1	DRB1*14:01g	0.01807481	0.9921390

Figure 4b

ALD Table	Allele Specific Homozygosity		Raw Data	Citation
Show	25	entries	Search: <input type="text"/>	
loci	focal	allele	allele.freq	as.homz
DRB1-DQB1	DQB1	DQB1*03:01g	0.1995703480	0.1093010
DRB1-DQB1	DQB1	DQB1*03:02g	0.1908488026	0.2106270
DRB1-DQB1	DQB1	DQB1*02:01g	0.1777759519	0.4658340
DRB1-DQB1	DQB1	DQB1*05:01	0.1080202594	0.3103092
DRB1-DQB1	DQB1	DQB1*04:02	0.1020147361	0.5018572
DRB1-DQB1	DQB1	DQB1*06:02	0.0758982654	0.6825254
DRB1-DQB1	DQB1	DQB1*06:03g	0.0466191968	0.9452953
DRB1-DQB1	DQB1	DQB1*06:04g	0.0281114658	0.9962983
DRB1-DQB1	DQB1	DQB1*05:03g	0.0197771867	0.8331541
DRB1-DQB1	DQB1	DQB1*03:03g	0.0179383161	0.5336197
DRB1-DQB1	DQB1	DQB1*05:02	0.0115725451	0.6486216
DRB1-DQB1	DQB1	DQB1*06:01	0.0114406465	0.9979798
DRB1-DQB1	DQB1	DQB1*06:09	0.0097213128	0.9980222
DRB1-DQB1	DQB1	DQB1*03:04	0.0005890360	0.3731651
DRB1-DQB1	DQB1	DQB1*03:05	0.0000452299	0.7210507
DRB1-DQB1	DQB1	DQB1*05:04	0.0000373923	0.6664078
DRB1-DQB1	DQB1	DQB1*03:25	0.0000085954	0.5078882
DRB1-DQB1	DQB1	DQB1*04:03	0.0000043704	1.0000000
DRB1-DQB1	DQB1	DQB1*06:11	0.0000037579	1.0000000