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Extensive Haplotype Diversity in African American Mothers and Their Cord Blood Units

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Abstract

HLA-A, -B, -C, -DRB1, -DQB1 assignments were obtained for 374 pairs of African American mothers and their umbilical cord blood units (CBU) by DNA sequencing. An algorithm developed by the National Marrow Donor Program was used to assign 1122 haplotypes by segregation. 70% of the haplotypes carried assignments at all five loci. In the remainder, alleles at various loci, most often DQB1 in 48% of the haplotypes with a missing assignment, could not be assigned due to sharing of both alleles by mother and CBU. There were 652 haplotypes carrying a unique combination of alleles at the five loci; the majority (74%) were singletons. Novel B~C and DRB1~DQB1 associations were observed. The results demonstrate the genetic diversity in this population and provide validation for a publically available tool for pedigree analysis. Our observations underscore the need for procurement of increased numbers of units in the national cord blood inventory in order to identify matching donors for all patients requiring hematopoietic stem cell transplantation.

Keywords

HLA; haplotypes; umbilical cord blood; informatics

Introduction

The classical human leukocyte antigen (HLA) loci are clustered in a span of 3.5 megabases on the short arm of chromosome 6 within the major histocompatibility complex (1). The loci are highly polymorphic with hundreds to thousands of alleles described (<http://www.ebi.ac.uk/imgt/hla/>)(2). Haplotypes carrying specific alleles at each locus have been identified by segregation within families (3,4), by DNA sequencing of long stretches of

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Conflicts of Interest

There are no conflicts of interest.

cloned DNA (1,5) and, in populations of unrelated individuals, by a predictive expectation-maximization (EM) algorithm (6–8).

The frequencies of individual haplotypes within world populations (9) significantly impact the ability of individuals to find HLA matched hematopoietic stem cell donors within registries of unrelated volunteers. Enhanced search algorithms using HLA frequency data facilitate the identification of potentially matched donors even though these individuals may be typed for HLA at low to intermediate resolution (10,11). Searches for those patients unable to find a suitably matched adult donor extend to include umbilical cord blood as a source of stem cells (12). In contrast to the stringent matching requirements for bone marrow (13) or peripheral blood (14), a cord blood unit is selected based, in part, on a minimum of antigen level matching for HLA-A,-B and an allele match for HLA-DRB1 (15). However, accumulating data suggest that better HLA matching will lead to improved outcome (16). Unfortunately, limitations in the number of individuals/cord blood units typed at high resolution within specific population groups hinders the development of registry size and diversity models used to drive recruitment strategies (17).

In hematopoietic stem cell transplantation, tolerance to mismatched HLA allelic products has been noted to occur in individuals receiving a haploidentical graft from their mother or a sibling (18). Further studies of umbilical cord blood transplantation have shown an impact of non-inherited maternal antigens (NIMA) on relapse and overall survival (19,20). Thus selection of a graft sharing its non-inherited maternal antigen with the recipient may enhance outcome. Availability of maternal typing limits this selection strategy to umbilical cord blood units where the frequency with which a NIMA match occurs may be less than 10% (21).

The purpose of this study was to explore the haplotypic diversity of an African American population and to develop and validate a tool to assign haplotypes by segregation.

Methods

Samples

The maternal (n=374) and umbilical cord blood unit samples were provided by the Carolinas Cord Blood Bank. For the samples characterized by sequencing, both mother and father self-identified as Black, predominantly African American.

Typing method

DNA sequencing of HLA-A, -B, -C, -DRB1, and -DQB1 focused on the exons encoding the antigen recognition site (ARS). Alternative genotypes differing in the ARS were resolved. Alleles differing outside of the commonly typed exons (i.e., alleles in a G group) were generally not resolved in the determination of haplotypes. Methods have been previously described (22–24). Data were interpreted using the IMGT/HLA database version 2.28.0 (2). An additional 3009 pairs of various ethnicities recruited for a cord blood bank were typed for HLA-A, -B, -DRB1 by oligonucleotide hybridization using One Lambda Labtype SSO kits (RSSOH1A, RSSOH1B, RSSOH2B1)(Canoga Park, CA).

Identification of haplotypes

A pedigree analysis algorithm was developed to identify HLA haplotypes by segregation. The typings at each locus were compared among an arbitrary number of related individuals to identify the HLA types that were identical by descent (IBD) and part of a shared haplotype. With the mother-cord blood pairs, it was possible to identify three haplotypes: the inherited maternal (shared), inherited paternal, and uninherited maternal haplotypes. As

typing methods do not guarantee high resolution HLA typing, the algorithm considered overlap between sets of possible alleles to ascertain whether typings at a locus were likely to be IBD. The typings in common between mother and cord blood unit comprised the inherited haplotype.

The pedigree algorithm was designed to report results with maximum certainty, which resulted in some partial haplotypes, where the algorithm could not make a decision for a locus. In the event that a call could not be made for a locus, the algorithm returned “no_call.” In the event of a “no_call,” the algorithm returns the reason for the failure. Four situations resulted in no call being made: first, a *double match*, where mother and cord blood unit share both alleles; second, *ambiguity*, where both possible sets of alleles for one individual (either mother or unit) match a set of alleles for the other, but the individual is not homozygous; third, *ambiguous homozygosity*, similar to ambiguity except it is possible, though not likely, that an individual is homozygous; fourth, *no match is available* as a result of typing error, data reporting error, or some other factor.

The algorithm was validated by comparison with manual assignment of haplotypes. Discrepancies were addressed during the development of the algorithm until concordance was complete between the two assignment methods. Further validation was conducted on simulated families where the haplotypes were known. The simulated families were constructed from 10,000 simulated individuals with allele level typing. Following ten generations of random pairing yielding three children at each pairing, the HLA assignments of the population were converted to a probe based testing resolution level without information on the haplotype assignments. The pedigree analysis algorithm was then used to assign haplotypes based on segregation and the results compared to the known assignments.

Results

Mothers (n=374) and their cord blood units (CBU) were typed for HLA-A,-B,-C,-DRB1, and -DQB1 with resolution of alternative genotypes. An algorithm was developed to identify HLA haplotypes by segregation. Alleles at all five loci were assigned in the majority of haplotypes (69.9%, 784 of the 1122 potential). In 338 haplotypes, one or more loci could not be assigned because the mother and unit shared both alleles. For example, if mother and unit shared both HLA-A locus alleles (e.g., A*23:01:01G, *74:01:01G with maternal B*42:01, *45:01:01G and CBU B*42:01, *50:01), it was not possible to assign a specific HLA-A allele to each HLA-B allele in the three unique haplotypes found in the pair. Figure 1 shows the distribution of the failure to assign alleles by locus. Almost half of the failures (48.2%) to assign alleles to a haplotype were found to be due to identity at the DQB1 locus likely due to the more limited number of DQB1 alleles in this population (n=17) compared to the number of alleles at other loci (HLA-A with 38 alleles, HLA-B 71, HLA-C 34, HLA-DRB1 37).

To compare the impact of typing resolution on the ability to assign alleles to a haplotype, the pedigree algorithm was used to assign haplotypes in 3,009 pairs typed for HLA-A, -B, and -DRB1 by oligonucleotide hybridization. The percent of pairs without assignments doubled: HLA-A, 5.1% SBT-typed pairs vs. 9.1% probe-typed pairs; HLA-B, 3.7% vs. 5.5%; DRB1, 8.0% vs. 15.4%. Additional ambiguity was observed in probe-typed pairs when both possible sets of alleles for one individual matched a set of alleles for the other (e.g., mother A*30:GYJW, *30:GYJV vs. cord A*30:AUX, *23:DUDN) (letter codes described at www.bioinformatics.nmdp.org). In these cases, the pedigree algorithm could not assign a typing to one of the three haplotypes of the pair and could not report a confirmation of the assignment for the shared haplotype. This type of ambiguity was not observed for the samples typed by sequencing.

Of the haplotypes with alleles at all five loci identified, there were 652 distinct combinations of HLA alleles. Most, 577 (73.6%), were observed only once. Seventy five haplotypes were observed from two to 12 times (Table 1, Figure 2).

A*30:01:01G~B*42:01~C*17:01:01G~DRB1*03:02~DQB1*04:02, the most frequent haplotype, is ranked first in African Americans (7). Eight of the ten most common haplotypes in this study are found within the 20 most frequent predicted haplotypes in African Americans (7). Supplemental Table 1 lists all of the haplotypes observed.

Haplotypes were clustered based on the presence of specific HLA-A alleles. The number of distinct haplotypes observed carrying a specific HLA-A allele is shown in Figure 3.

A*02:01:01G was carried by 82 distinct haplotypes forming the largest group. Most of these haplotypes were observed once but 11 were observed from two to six times. Most frequent was haplotype

A*02:01:01G~B*44:02:01G~C*05:01:01G~DRB1*04:01~DQB1*03:01:01G. This haplotype has been ranked 11th in frequency in African Americans (7). In contrast, only a single haplotype carried A*43:01 and no haplotype was observed carrying A*69:01.

The associations between HLA-B and HLA-C and between DRB1 and DQB1 alleles were generally those previously observed in other studies based on data provided in <http://bioinformatics.nmdp.org> and <http://www.hlaexplorer.net>. Table 2 lists associations not previously noted.

To get a sense of the novelty of the haplotypes observed, the completely defined haplotypes carrying A*30 were compared to predicted haplotypes found in the study by Maiers et al. (7) and listed at <http://bioinformatics.nmdp.org>. A*30 was chosen because all of the observed alleles are most frequent in African populations. Of the 82 unique haplotypes observed carrying either A*30:01:01G (41 haplotypes), A*30:02:01G (39), A*30:04 (1) or A*30:10 (1), the majority (65.4%) were not listed in the A~B~C~DRB1~DQB1 haplotypes reported within the National Marrow Donor Program bioinformatics database. This is likely due to the small number of haplotypes with assignments for all five loci (n=894) predicted for African Americans in the earlier study. Of the 28 haplotypes found in the database, 23 (82.1%) had been previously observed in African Americans.

Discussion

To facilitate the interpretation of family typing data when beginning a search for a patient requiring a transplant and to assist with the search for a tolerant mismatched unit, the National Marrow Donor Program has developed a pedigree algorithm to identify haplotypes within a family. The pedigree analysis tool has been made available on a publically accessible web site, <http://bioinformatics.nmdp.org/>. This tool complements allele and haplotype frequency data on the site as well as a look-up tool, HaploStats, to provide estimated allele and haplotype frequencies for any submitted HLA genotype. As demonstrated in this study, the greater the level of resolution of the typing, the greater the likelihood that all loci within the haplotype will be assigned.

One impact of the observation that a hematopoietic stem cell donor may be tolerant to non-inherited maternal antigens is the increased typing of maternal donors of umbilical cord blood units. While this typing will allow the search for a “tolerant” cord blood unit for a patient whose best stem cell source is a mismatched donor, the HLA data gleaned will also allow the identification of haplotypes by segregation analysis. Since paternal typing is not available, it should be noted that a small proportion of haplotypes identified may have arisen from recombination within the family. Regardless, this information will continue to build

our knowledge of haplotype diversity and will extend and substantiate the lists of haplotypes predicted from populations of unrelated individuals.

The study focused on haplotypes in African Americans because this population brings together alleles and haplotypes found in Europeans and Native Americans (26–28) with the extensive diversity of alleles and haplotypes found in Africa (29). This diversity is reflected in the large number of singleton haplotypes observed in this study. This population group has a more difficult time finding an HLA matched hematopoietic stem cell donor compared to other U.S. population groups (30) and, for this group, access to umbilical cord blood units, with less restrictive HLA matching requirements is critical. Because outcome can be improved with closer HLA matching and because of the extensive diversity of this population, efforts should continue to increase the number of available units in the national cord blood inventory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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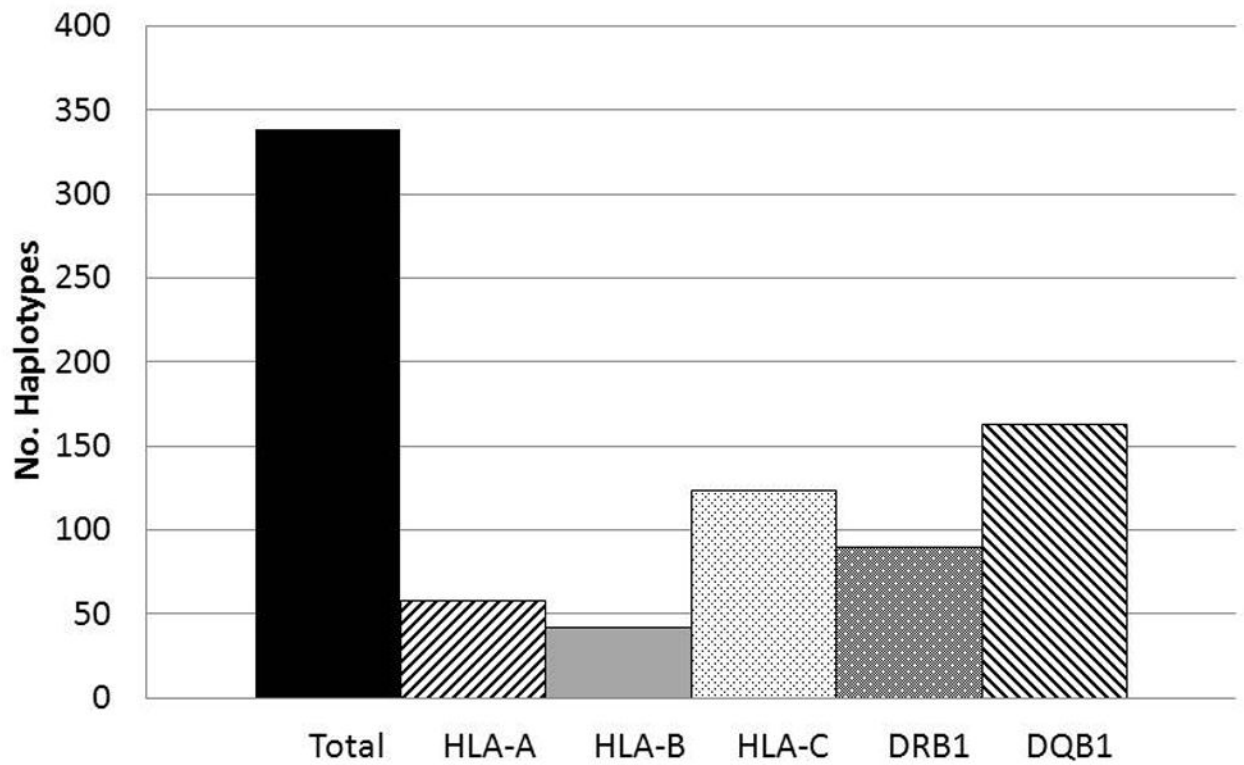


Figure 1. Distribution of failure to assign HLA alleles to haplotypes by locus. Failure is due to both mother and cord blood unit sharing both assignments at a locus.

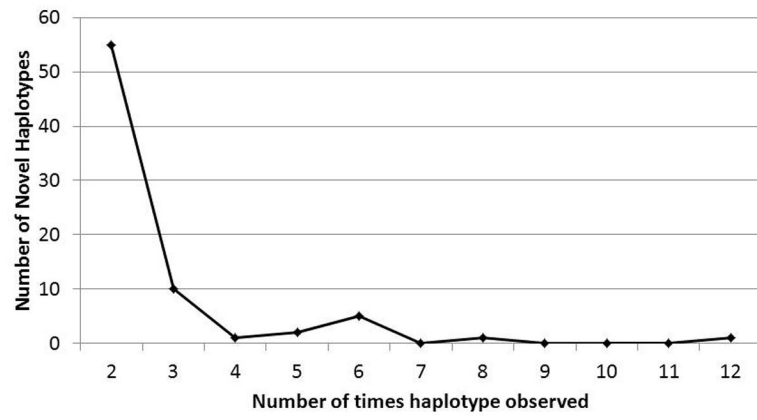


Figure 2.
Number of haplotypes observed two or more times in the study.

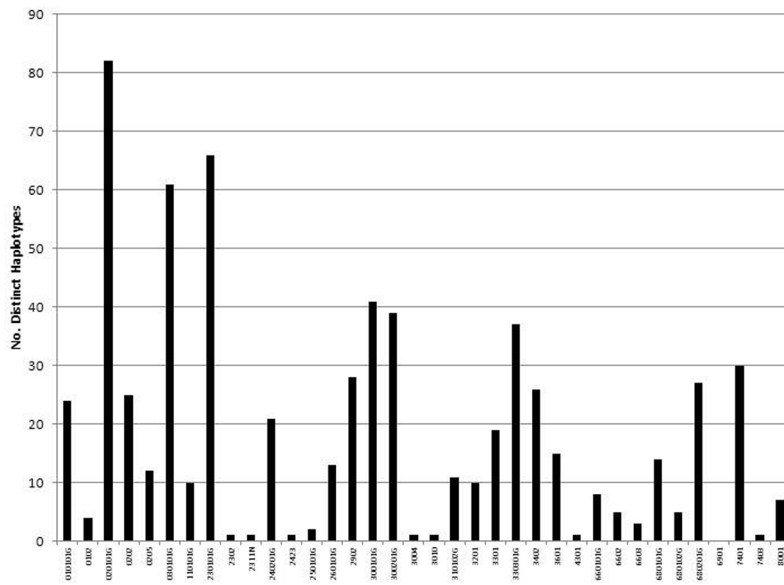


Figure 3. Distribution of distinct haplotypes carrying specific HLA-A alleles.

Table 1

Haplotypes Appearing Multiple Times in the Population

Haplotype	A_I	B_I	C_I ^a	DR_I	DQ_I	No. Times Observed
1	01:01:01G	57:01:01G	06:02:01G	07:01	03:03:02G	2
2	01:01:01G	57:03	07:01:01G	09:01	02:01:01G	2
3	02:01:01G	52:01:02	16:01	13:03	03:01:01G	2
4	02:01:01G	15:10	03:04:02	03:01	02:01:01G	2
5	02:01:01G	40:01:01G	03:04:01G	13:02	06:04:01G	2
6	02:01:01G	44:02:01G	05:01:01G	13:01	06:03	2
7	02:01:01G	45:01:01G	16:01	13:01	06:03	2
8	02:01:01G	45:01:01G	16:01	13:02	05:01	2
9	02:01:01G	50:01	06:02:01G	08:04	02:03	2
10	02:01:01G	51:01:01G	16:01	07:01	02:01:01G	2
11	02:02	18:01:01G	05:01:01G	14:01:01G	05:03	2
12	03:01:01G	07:02:01G	07:02:01G	13:02	06:04:01G	2
13	03:01:01G	15:10	08:04	11:04	05:02	2
14	03:01:01G	53:01	04:01:01G	08:04	03:01:01G	2
15	03:01:01G	57:03	07:01:01G	15:03	06:02	2
16	23:01:01G	07:05:01G	04:01:01G	14:01:01G	05:03	2
17	23:01:01G	08:01:01G	07:01:01G	11:01	06:02	2
18	23:01:01G	14:03	08:02:01G	03:02	04:02	2
19	23:01:01G	14:03	08:02:01G	10:01	05:01	2
20	23:01:01G	15:03:01G	02:10	07:01	02:01:01G	2
21	23:01:01G	15:03:01G	02:10	11:01	03:01:01G	2
22	23:01:01G	42:01	17:01:01G	03:02	04:02	2
23	23:01:01G	44:03	04:01:01G	15:03	06:02	2
24	23:01:01G	45:01:01G	06:02:01G	07:01	02:01:01G	2
25	23:01:01G	50:01	06:02:01G	07:01	02:01:01G	2
26	23:01:01G	53:01	04:01:01G	03:01	02:01:01G	2
27	23:01:01G	58:01:01G	03:02	13:01	06:03	2
28	23:01:01G	58:02	06:02:01G	13:01	03:03:02G	2

Haplotype	A_1	B_1	C_1 ^a	DR_1	DQ_1	No. Times Observed
29	29:02:01G	15:10	03:04:02	03:01	02:01:01G	2
30	29:02:01G	42:01	17:01:01G	13:02	06:09	2
31	29:02:01G	49:01	07:01:01G	15:03	06:02	2
32	29:02:01G	78:01	16:01	13:01	06:04:01G	2
33	30:01:01G	13:02	06:02:01G	07:01	02:01:01G	2
34	30:01:01G	53:01	04:01:01G	11:01	06:02	2
35	30:02:01G	15:10	03:04:02	03:01	02:01:01G	2
36	30:02:01G	39:10	12:03:01G	13:02	06:09	2
37	30:02:01G	57:03	07:01:01G	15:03	06:02	2
38	31:01:02G	40:01:01G	03:04:01G	04:04	03:02	2
39	32:01	14:02	08:02:01G	03:01	02:01:01G	2
40	33:03:01G	15:03:01G	02:10	07:01	02:01:01G	2
41	33:03:01G	15:16	14:02:01G	01:02	05:01	2
42	33:03:01G	35:01:01G	04:01:01G	07:01	02:01:01G	2
43	33:03:01G	53:01	04:01:01G	15:03	06:02	2
44	33:03:01G	53:01	04:13	03:02	02:03	2
45	34:02	44:03	04:01:01G	15:03	06:02	2
46	36:01	53:01	04:01:01G	08:04	03:01:01G	2
47	36:01	58:01:01G	06:02:01G	12:01:01G	05:01	2
48	66:01:01G	58:02	06:02:01G	13:01	03:03:02	2
49	68:02	15:10	03:04:02	03:01	02:01:01G	2
50	68:02	15:10	03:04:02	03:02	04:02	2
51	68:02	53:01	04:01:01G	07:01	02:01:01G	2
52	68:02	53:01	04:01:01G	15:03	06:02	2
53	74:01:01G	07:05:01G	15:05:01G	15:03	06:02	2
54	74:01:01G	57:03	07:01:01G	15:03	06:02	2
55	80:01	15:03:01G	02:10	11:01	03:01:01G	2
56	01:01:01G	07:02:01G	07:02:01G	15:01	06:02	3
57	02:01:01G	42:01	17:01:01G	03:02	04:02	3
58	02:01:01G	57:01:01G	06:02:01G	07:01	03:03:02G	3
59	03:01:01G	35:01:01G	04:01:01G	01:1	05:01	3

Haplotype	A_1	B_1	C_1 ^a	DR_1	DQ_1	No. Times Observed
60	03:01:01G	53:01	04:01:01G	15:03	06:02	3
61	23:01:01G	07:02:01G	07:02:01G	11:02	03:01:01G	3
62	23:01:01G	53:01	04:01:01G	08:04	03:01:01G	3
63	30:01:01G	42:01	17:01:01G	08:04	03:01:01G	3
64	30:02:01G	14:02	08:02:01G	15:03	06:02	3
65	74:01:01G	15:03:01G	02:10	13:02	06:09	3
66	24:02:01G	07:02:01G	07:02:01G	15:01	06:02	4
67	33:03:01G	53:01	04:01:01G	08:04	03:01:01G	5
68	68:02	42:01	17:01:01G	03:02	04:02	5
69	02:01:01G	44:02:01G	05:01:01G	04:01	03:01:01G	6
70	03:01:01G	07:02:01G	07:02:01G	15:01	06:02	6
71	36:01	53:01	04:01:01G	11:01	06:02	6
72	66:02	58:01:01G	07:01:01G	15:03	06:02	6
73	68:01:01G	58:02	06:02:01G	12:01:01G	05:01	6
74	01:01:01G	08:01:01G	07:01:01G	03:01	02:01:01G	8
75	30:01:01G	42:01	17:01:01G	03:02	04:02	12

^aThe presence of C*04:09N was evaluated in 56 samples typed as C*04:01:01G including 35 carrying B*44:03, four of these with A*23 (31) and one with a haplotype identical to that observed carrying C*04:09N, A*02:01~B*4403~DRB1*07~DQB1*02 (32). The nonexpressed allele was not observed.

Table 2

Unique HLA B~C and DRB1~DQB1 haplotypes

HLA-B	HLA-C	Number Observations
07:12	15:05	Shared haplotype ^a
07:14	07:02:01G	Shared haplotype
13:02	03:03:01G	Shared haplotype
14:02	03:04	1
15:01:01G	14:02	1
15:03:01G	07:02:01G	1
15:10	12:03:01G	1
41:02	08:04	1
44:03	02:14	1
44:05	03:03:01G	1
51:09	16:01	1
53:01	03:02	1
53:01	08:04	Shared haplotype
82:02	08:04	Shared haplotype
DRB1	DQB1	Number Observations
08:06	05:01	Shared haplotype, 1 other
09:01	02:02	1
13:04	05:01	1

^aObserved in both mother and her cord blood unit.