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Analysis and Interpretation of the HLA DQα "1.1 Weak-Signal" Observed During the PCR-Based Typing Method

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ABSTRACT: The Perkin-Elmer (PE) AmpliType DQα Forensic Kit is currently available for amplification and typing of a polymorphic region in the Human Leukocyte Antigen (HLA) DQα DNA sequence. Following amplification of the DQα region with the PE kit, typing strips are processed. These strips contain immobilized DNA probes designed to distinguish six possible HLA DQα alleles. It has been observed in this laboratory and others that in a single source DNA sample, it is possible to detect a weak signal on the 1.1 specific allele dot when the samples' genotype clearly does not contain the 1.1 allele. It has been suggested that a potential source of this weak-signal is the non-specific amplification of a HLA DXα gene sequence. To demonstrate the relationship of the DXα gene to the 1.1 non-specific signal, we designed biotinylated DXα PCR primers specific for a 178 bp region in which the amplified product spans the homologous DQα region encompassing the DNA probes present on the typing strips. DXα DNA sequences from various DQα genotypes were amplified and hybridized to DQα typing strips. We have demonstrated that DXα PCR products do not always hybridize to the 1.1 probe on the typing strips. Sequence analysis of DXα PCR products show that this region is polymorphic which may explain why the occurrence of the "1.1 weak-signal" is unpredictable. We have further analyzed the effect of DNA template concentration for the DQα amplification protocol and have shown that regulation of PCR input DNA optimizes the amplification and typing protocols for HLA DQα alleles and minimizes the potential observation of the "1.1 weak-signal."

KEYWORDS: pathology and biology, DNA, PCR, HLA DQα, genetic identification

Analysis of DNA and amino acid sequence variability associated with the HLA DQα alleles has been an area of intense interest for nearly a decade [*1–3*]. As a result, DNA sequence polymorphisms within exon two of the HLA DQα region has provided a unique opportunity to use PCR technology to type DQα alleles in individuals [*4,5*]. The reagents necessary for this technique are contained in the AmpliType Forensic DNA Amplification and Typing Kit (Perkin-Elmer Corporation, Norwalk, CT). Prelabeled nylon membrane typing strips contain DNA probes specific for the identification of six different HLA

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DQα alleles, 1.1, 1.2, 1.3, 2, 3, and 4 for a total of 21 possible DQα genotypes. The HLA DQα alleles are identified by hybridizing PCR amplified DQα sequences to the typing strips followed by an enzyme/substrate reaction which produces blue dots indicative of the presence of a specific allele(s).

The AmpliType™ Users Guide cautions that on occasion, there may be a weak hybridization signal on the 1.1 dot when there is no detectable signal on the 1 dot [*6*]. This "1.1 weak-signal" has been attributed to the low-level non-specific amplification of another gene in the HLA complex known as DXα. The DQα primer sequences have been reported to have a region of high homology when compared to the DXα sequence in that there is a one base pair difference in the 5′ primer and a two base pair difference in the 3′ primer [*7*]. The HLA DXα DNA sequence is closely related to HLA DQα sequences [*8,9*]. DQα sequence information provided in the AmpliType Users Guide shows that when DXα exon 2 DNA sequences are aligned with the DQα 1.1 DNA probe sequence, there is 100% sequence homology within this genomic region. It has been suggested that if DXα genetic sequences are nonspecifically amplified during the DQα PCR procedure, the DXα PCR product could hybridize the 1.1 probe generating a weak signal. When the "1.1 weak-signal" does occur, the signal is usually weaker than or in rare cases equal to the "C" dot. Therefore, it has been suggested in the AmpliType Users Guide that the result should be noted but not considered part of the genotype.

This laboratory and others have noticed the "1.1 weak-signal" to varying degrees and in addition, a weak hybridization signal has been observed with the 1.3 dot when the single source DNA sample clearly does not have the 1.3 DQα allele. There does not appear to be a relationship between the presence of the "1.1 or 1.3 weak-signal" and any particular HLA DQα genotype. It would be beneficial for the forensic scientist to have data supporting a scientific basis for the presence of this weak 1.1 signal that may occur in the absence of the DQα 1.1 allele.

We have experimentally analyzed the hypothesis that HLA DXα genomic sequences may hybridize to the HLA DQα 1.1 probe and have determined that (1) DXα exon 2 amplified sequences do not always hybridize to the 1.1 typing probe sequence, (2) alignment of DXα exon 2 genomic sequences with the DQα 1.1 probe sequence shows that not all DXα alleles display 100% sequence homology in this region, (3) polymorphisms of the DXα exon 2 sequence located within the homologous DQα 1.1 probe sequence region explains the lack of hybridization for some DXα exon 2 amplified sequences to the DQα 1.1 typing probe, and (4) quantitation of input template DNA into the DQα amplification reaction optimizes the AmpliType protocol for the detection of HLA DQα genomic sequences.

Materials and Methods

DNA Isolation, Extraction and Gel Electrophoresis

DNA was isolated from 1 cm^2 dried whole blood stains using an organic extraction protocol previously described [*10*]. A 2 μL aliquot of the DNA was quantitated on a 0.7% TBE (Tris Borate EDTA, AMRESCO, Solon, OH) agarose gel using a Lambda Hind III (BRL, Gaithersburg, MD) digested standard. The gel was stained with 0.5 μg/mL ethidium bromide (AMRESCO, Solon, OH). Quantitation of extracted DNA was also done using reagents from the Roche Molecular Systems "QuantiBlot" kit [*11*]. The reagents were a generous gift from Sean Walsh, RMS.

Amplification

HLA DQα—DNA was amplified using the HLA DQα Forensic Amplification and Typing Kit (Perkin Elmer Corporation, Norwalk, CT). Approximately 10 to 30 ng of

DNA was added to 50 μL of amplification pre-mix and 50 μL of the 8 mM $MgCl_2$ solution for a final volume of 101 μL. Amplification was conducted in the Perkin Elmer 480 Thermocycler (Perkin Elmer, Norwalk, CT) using the following parameters for 32 cycles: 94°C for 60 s; 60°C for 30 s; 72°C for 30 s with a 7 min auto extension at 72°C on the last cycle.

HLA DXα2—Amplification of the DXα exon 2 DNA sequence (DXα2) was a modification of that described by Saiki et al. [2]. The 5′ and 3′ primer (respectively) were custom synthesized as follows: 5′ Biotin—CTC GTC AGC TGA CCA TGT T-3′ and 5′-CTC TGC GGG TCA AAA CTT AT-3′ (National Biosciences, Plymouth, MN) generating a 178 base pair amplified PCR product. The PCR reaction conditions included 200 μM dNTPs, 1 μM of each primer, approximately 30 ng template DNA, 10 μL 10X reaction buffer containing 100 mM Tris.HCl, pH 8.3, 500 mM KCl, 15 mM MgCl2, 0.1% (w/v) gelatin and stabilizers, 2.5 Units of Taq polymerase (Perkin-Elmer, Norwalk, CT) and water to a final volume of 100 μL (Stratagene, LaJolla, CA). The cycling parameters included 94°C, 60 s; 57°C 30 s; 71°C 60 s for 32 cycles on the Perkin Elmer 480 Thermocycler. Verification of DXα2 amplification was done by agarose electrophoresis of 10% of the PCR products on a 4% GeneAmp agarose gel (Perkin Elmer, Norwalk, CT) using 0.5 μg/mL ethidium bromide (AMRESCO, Solon, OH) and 1X TBE buffer (AMRESCO, Solon, OH). The molecular weight standard was a 1 Kb DNA ladder (BRL, Gaithersburg, MD).

HLA DQα Typing

PCR products (HLA DQα or DXα2) were hybridized as per protocol to the AmpliType typing strips provided in the HLA DQα AmpliType kit using 35 μL for each strip. The DNA was hybridized at 54°C or 56°C for 20 min. Posthybridization, the typing strips were washed at 54°C or 56°C for 12 min. After the washes, color development was allowed to proceed until the C dot was evident on all strips. Photography was done using an MP4+ with a #8 yellow filter and Polaroid 667 film with a 4.5 aperture at $t = {}^{1}/_{60}$.

Sequencing of DNA PCR Products

1. DXα2 amplified products from samples C106, B300, H600 were sequenced by direct PCR sequencing using the Circumvent™ sequencing protocol (N.E. Biolabs, Beverly, MA). Prior to sequencing, the DXα2 DNA sequences were amplified by the protocol already described, using the DXα2 primers except the 5′ primer was synthesized without the 5′ biotin label. The PCR products were electrophoresed on 1.0% SeaPlaque agarose gel (FMC, Rockland, MA) and the DNA excised from the gel followed by organic extraction with phenol. The purified DNA fragments were quantitated by gel electrophoresis as described. The DXα2 sequence was generated using an internal primer 5′-CAG CTG ACC ATG TTG CCT CC-3′ specific for DXα2. Sequencing was as per manufacturers protocol. Briefly, 30 ng of purified DNA template was combined with 1 μL primer (1.2 pmole), 1.5 μL 10X buffer, 1 μL Triton X, water for a final 12.0 μL volume then 2 μL S^{35} dATP and 1 μL Vent™ polymerase was added. The template/primer was added to the appropriate deoxy/dideoxynucleotide mix and placed in a Stratagene thermocycler for 20 cycles at 95°C 20 s; 55°C 20 s; 72°C 20 s.

Cloning and Transformation—DXα2 sequence information was also obtained by cloning PCR fragments from the DXα2 PCR products from samples C106, B300, H600, and the HLA DQα homozygous genotypes 1.2, and 4 using the TA Cloning™ System (Invitrogen, San Diego, CA). A single clone was sequenced from each of the samples in order to obtain sequence polymorphism information from individual DXα2 alleles. Each

ligation reaction consisted of 1 μL 10X TA-Cloning ligation buffer, 2 μL pCR™ II vector (25 ng/μL), 7 μL PCR product (50 ng) and 1 μL T4 DNA Ligase. Ligation mixtures were incubated at 14°C overnight. Transformation of the ligated DNA was performed as per manufacturers instructions. Verification of insert into the pCR™ II vector was done by restriction digest of 5 μL of isolated plasmid, 5 units of Eco RI (BRL, Gaithersburg, MD), and 1X digestion buffer at 37°C overnight in the presence of 1 μL RNAse (10 mg/mL) followed by gel electrophoresis as described.

A Sequenase kit (United States Biochemical, Cleveland, OH) was used according to manufacturers protocol. The isolated cloned DNA was purified using the ELUTIP-d protocol (Schleicher and Schull, Keene, NH). The SP6 primer was annealed to the DNA for 2 min at 65°C and slowly cooled to room temperature, 2.5 μL of DNA/primer mixture was added to the appropriate deoxy/dideoxynucleotide test tube and incubated for 5 min at 37°C.

Sequencing Electrophoresis—Sequencing reactions were electrophoresed on a 5.5%, 0.4 mm Sequagel™ (National Diagnostics, Atlanta, GA) consisting of 17.6 mL Sequagel concentrate, 54.4 mL Sequagel Diluent, 8 mL Sequagel Buffer, 640 μL 10% ammonium persulfate, and 32 μL TEMED was poured into a Biorad Sequi-Gen™ apparatus (Biorad, Richmond, CA). Following an overnight polymerization, 4.0 μL (Method 1) or 2.5 μL (Method 2) of sequencing reaction mix was loaded into the appropriate lane and electrophoresed at 150 Watts. Following electrophoresis, the gel was washed with acetic acid, dried on a gel drier and exposed on Kodak X-OMAT film for 4 days at −70°C.

Nomenclature

The WHO Nomenclature Committee reported a complete list of all Class I and Class II genes and sequences of alleles officially recognized in the scientific community [*1*]. The DQ alpha locus has been designated according to the following: DQα 1.1 is now DQA1*0101 and DQα 1.2 is now DQA1*0102; DQα 1.3 is now DQA1*0103; DQα 2 is now DQA1*0201; DQα 3 is now DQA1*0301; and DQα 4.1 is now DQA1*0501. the 4.2 is *0401 and the 4.3 is *0601. Since the AmpliType strips are marked with the designated alleles "1.1, 1.2, 1.3, 2, 3, and 4," for the sake of continuity, in the present paper, we will refer to the HLA DQα alleles as indicated on the typing strips. The DXα gene is now referred to as DQA2 according to the WHO nomenclature, but in this paper will be referred to as DXα2 since past literature refers to this region as DXα and it is the second exon of the DXα genetic sequence that we are investigating.

Results

Amplification and "AmpliTyping" of DXα2 Genetic Sequences

In order to determine if HLA DXα2 genomic sequences will contribute to the low-level signals observed on the AmpliType™ typing strips' 1.1 probe (Fig. 1), PCR oligonucleotide primers specific for DXα2 DNA sequences were synthesized as described. The DXα2 DNA primer sequences correspond to a genomic region in the second exon of DXα and were designed to amplify DXα exon 2 sequences representing the region of greatest homology with the AmpliType™ HLA DQα target sequences (Fig. 2). The 5′-biotinylated labeled primer was demonstrated to be efficacious by hybridizing the DXα2 5′ primer to slot blotted human DNA followed by a posthybridization enzyme-substrate reaction with color development reagents provided in the AmpliType™ kit (data not shown). DXα2 and DQα genomic sequences from selected DQα genotypes were amplified and the PCR fragments hybridized to typing strips (Fig. 3). Amplified DXα2

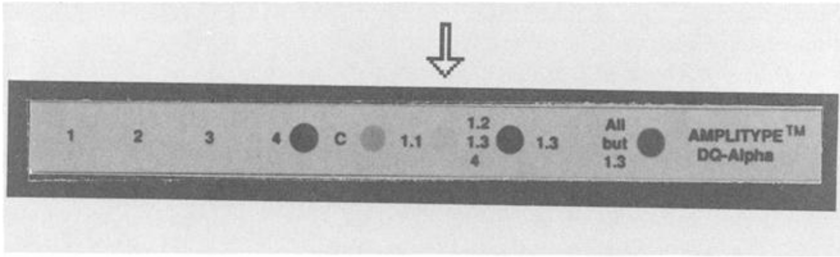

FIG. 1—*Detection of the "1.1 weak signal" during the HLA DQα AmpliType™ protocol. DNA was extracted from a dried blood stain. Amplification for DQα was done as per AmpliType protocol using 30 ng of DNA. DQα hybridization and washes were done at 56°C with 35 μL of the amplified product and color development was for 15 min. The single source DNA sample types correctly for the homozygous 4 alleles. In addition, a weak signal is observed on the 1.1 dot (*arrow*).*

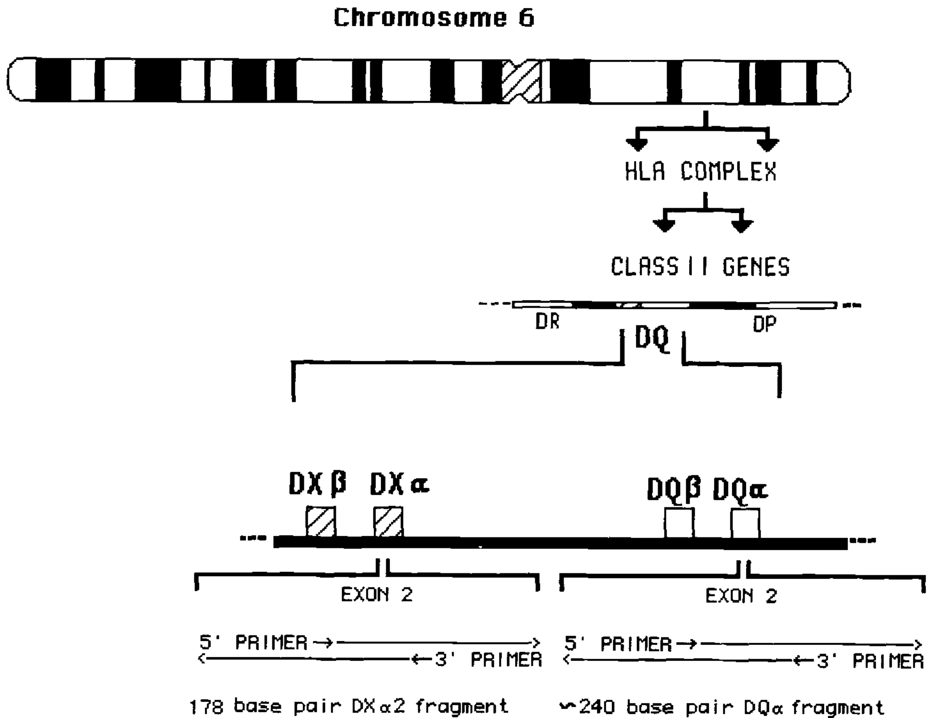

FIG. 2—*Region of HLA amplification on chromosome 6. The HLA DQα and HLA DXα genes are closely linked within the DQ region of Class II genes. The AmpliType HLA DQα kit uses PCR to exploit a region of polymorphism within exon 2 of the HLA DQα genes resulting in an approximate 240 base pair amplified fragment. The DXα genes are 5′ to the DQα genes. The DXα2 PCR primers amplify a 178 base pair fragment within exon 2, which is the region of greatest homology with the DQα amplified product and encompasses the area that includes the DQα probes immobilized on the AmpliType typing strips.*

(178 bp) and DQα (239 or 242 bp) products for each of the samples were electrophoresed to verify success of the amplification process. DXα2 and DQα amplified DNA was hybridized at 54°C or 56°C to AmpliType typing strips in order to determine if the 1.1 weak-signal was related to the temperature of hybridization. Figure 3 shows the result of hybridizing the DXα2 or DQα PCR products to the typing strips at 56°C. The results detected on the typing strip were the same regardless of hybridization temperature (54°C results not shown). The HLA DQα genotype of the six samples each typed correctly on the AmpliType typing strips (Fig. 3). The DXα2 PCR fragments only hybridized to the 1.1 DNA probe on three of the six typing strips, those with samples homozygous for the DQα 1.2, 2, and 4 alleles. The samples homozygous for the 1.1, 1.3 and 3 alleles typed correctly for DQα, however, DXα2 PCR products from these three genotypes did not elicit any hybridization signal on the 1.1 dot on the typing strip probe even though the DXα2 sequences were successfully amplified.

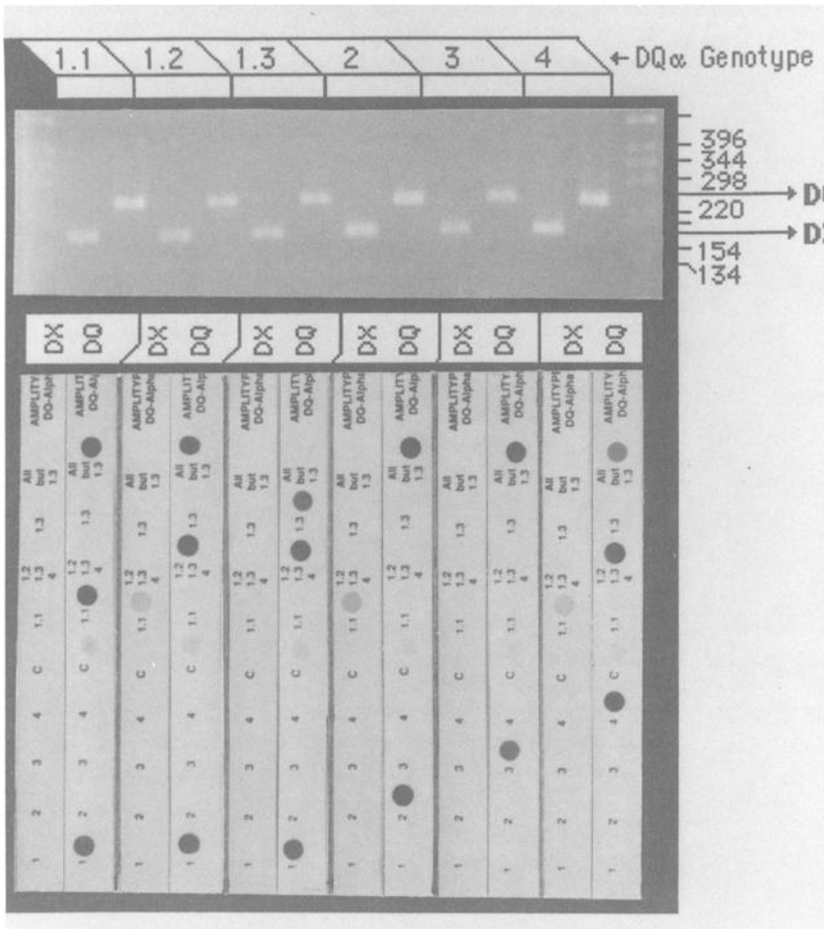

FIG. 3—Amplification and hybridization of DXα2 and DQα PCR products to AmpliType typing strips. DNA was phenol/chloroform extracted from six human blood stains with known homozygous DQα genotypes. The DNA was amplified for DQα DNA sequences or DXα2 sequences and 10% of the amplified product electrophoresed on an ethidium bromide stained 4% GeneAmp™ gel. The molecular weight standard was the BRL 1 kb DNA ladder. Typing was done at 56°C with 35 μL of DQα or DXα2 amplified product and color development was for 15 min.

Three additional donors heterozygous for DQα alleles in which all six DQα alleles are represented (C106 has 1.3,4 genotype; H600 has 1.2, 2 genotype; and B300 has 1.1, 3 genotype) were also used in these studies. Amplification of the DXα2 genomic sequence from these three samples and subsequent hybridization of the DXα2 PCR fragments to the typing strips showed that there was no hybridization to any of the AmpliType™ probes after 20 min of color development incubation from any of the samples at either 54°C or 56°C (data not shown). The 1.1 dot was weakly visible on the C106 DQα typing strip even though the amplified DXα2 sequences did not hybridize to the DQα 1.1 probe sequence.

We have experimentally eliminated contamination as the source of the "1.1 weak-signal" as a) all DNA samples were extracted at different times indicating there has been no DNA template carry over of those DNAs containing the 1.1 DQα allele, b) not all typing strips contain the "1.1 weak-signal" therefore this is not a PCR preparation problem, c) the RMS positive control DNA, which contains the 1.1 DQα allele, is never prepared at the same time the samples are prepared for amplification, and d) negative controls are consistently negative indicating this is not a quality control issue with the AmpliType kit reagents (data not shown).

Sequencing of DXα2 Genomic PCR Fragments

In order to verify that the amplified product hybridized to the discussed AmpliType typing strips were sequences synthesized from the DXα2 gene region, amplified DXα2 PCR fragments from samples C106, H600, and B300 were sequenced using the method of direct sequencing of PCR products. In addition, DXα2 PCR fragments were cloned and sequenced from these three samples. Figure 4 shows two representative examples of the DXα2 sequence information generated from cloned samples of C106 and sample H600 DXα2 PCR fragments. The sequence shows that sample C106 has a two base pair difference and H600 a one base pair difference in the analogous DQα 1.1 probe region. Direct PCR product sequencing of the C106 show that this sample is homozygous for the DXα2 allele, and the H600 and B300 samples are heterozygous for the DXα2 sequence having one allele with the two base pair difference and another DXα2 allele with one base pair difference (Fig. 5). This polymorphism explains why the amplified DXα2 PCR products do not always hybridize to the 1.1 probe on the AmpliType typing strip.

Because the DXα2 PCR products from two additional individuals homozygous for DQα alleles (B341 homozygous 1.2 genotype; and C72 homozygous 4 genotype) elicited a hybridization signal on the 1.1 dot (Figure 3), the DXα2 PCR products were cloned and an individual clone from each of these samples was sequenced. When comparing the DNA sequence for the B341 and C72 DXα2 sequence, which hybridized to the typing strip 1.1 probe, to the published 1.1 DNA sequence (5), there is 100% sequence homology between the DXα2 sequence and the 1.1 typing probe sequence (Fig. 5).

DXα2 sequence information generated from this laboratory and previously reported DXα2 sequence information is summarized in Fig. 5. The DXα2 polymorphisms in the homologous region of the DQα 1.1 probe sequence are restricted to one or two base pair changes. When aligning the DXα2 DNA sequences with the DQα 1.1 AmpliType probe sequence, there are three polymorphic DXα2 sequences including a) a T at position 6 and a C at position 18, or b) a C at position 6 and a T at position 18, or c) a C at position 6 and a C at position 18.

Controlling the Amount of Template DNA for HLA DQα Amplification

It is apparent from the studies presented here, that the low-level hybridization of the amplified DNA to the DQα 1.1 probe signal that occurs in the absence of the 1.1 allele

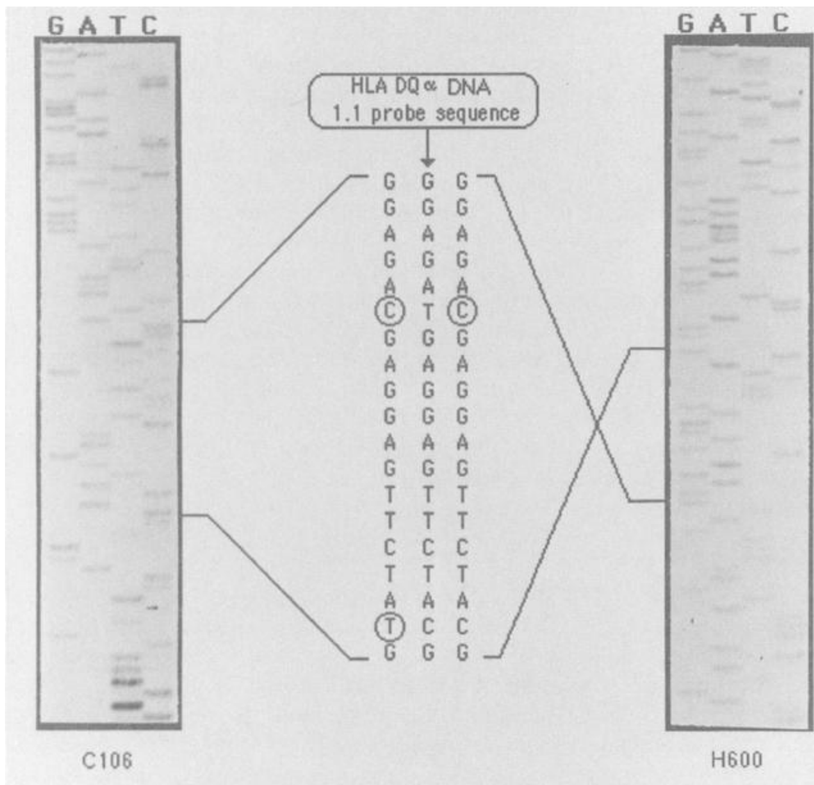

FIG. 4—*DNA sequence alignment of DXα2 genomic sequences with DQα 1.1 probe sequence. DNA was extracted from sample C600 (DQα 1.3, 4) and H600 (DQα 1.1, 2) and DXα2 primers used to amplify DXα2 DNA sequences. The PCR product was purified by gel extraction, cloned into a vector and sequenced using the Sequenase™ protocol. The DXα2 genomic sequences were aligned with the DQα 1.1 probe sequence to demonstrate DXα2 polymorphism within the DXα2 and sequence differences when compared to the DQα 1.1 probe sequence. The C106 DXα2 DNA sequence is complementary to the autoradiography sequence.*

is not always a result of non-specific amplification and subsequent hybridization of DXα2 gene sequences to the 1.1 probe since the DQα sequences from sample C106 (1.3, 4 genotype) can still elicit a signal to the 1.1 probe even though the DXα sequences do not hybridize to the 1.1 probe. Samples that were known not to have the 1.1 allele yet exhibited the "1.1 weak-signal" were amplified for DQα sequences at template DNA concentrations ranging from 50 ng to 1.5 ng. Figure 6 shows the results of amplification and hybridization at six DNA template concentrations of a sample homozygous for the DQα genotype 1.2. This sample has DXα2 gene sequences which hybridize to the 1.1 probe. Amplification of DQα DNA sequences using 50 ng of template DNA clearly shows a low-level 1.1 signal. As the PCR input template concentration is decreased, the 1.1 background signal becomes weaker and is eventually lost at DNA concentrations below 6 ng with little or no decrease on the hybridization signal from the DQα alleles. One of the samples investigated contained DXα sequences that hybridize to the 1.1 probe, yet reduced template concentrations of this sample did not totally eliminate the 1.1 weak signal even when 1.5 ng of DNA was used in the amplification mixture (data not shown). However, for all other samples tested the 1.1 weak-signal was eventually

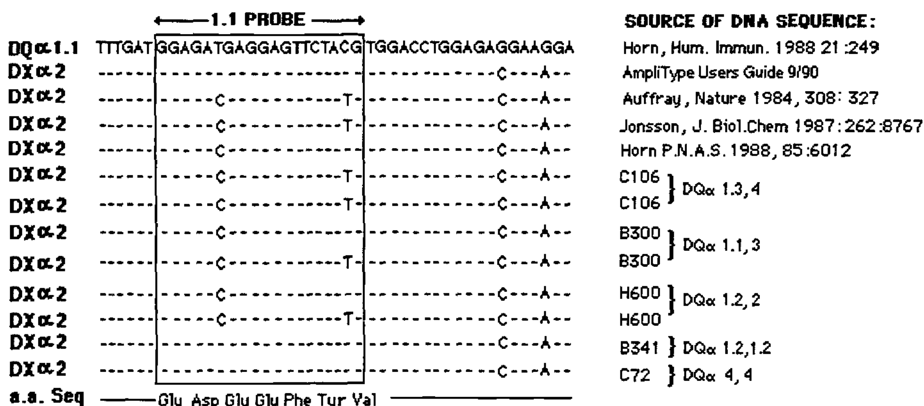

FIG. 5—*Summary of DXα2 genomic sequence data. DXα2 genomic sequence data generated from this laboratory and previously reported DXα2 DNA sequences are aligned with the DQα 1.1 probe sequence. The putative DXα2 amino acid sequence is also shown.*

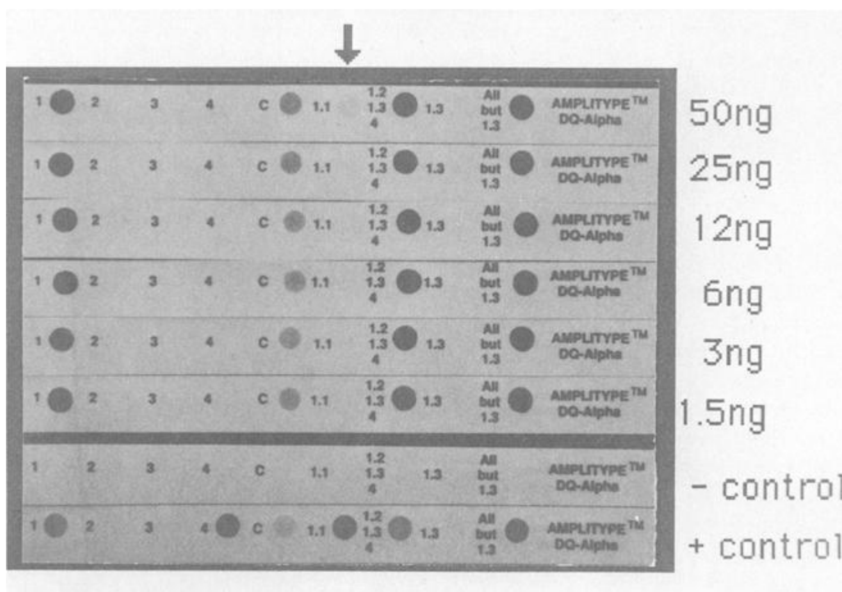

FIG. 6—*Loss of "1.1 weak signal" by controlling template DNA concentration in amplification reaction. DNA was extracted from a specimen homozygous for the 1.2 DQα allele. The DNA was quantitated by using the RMS Quantiblot kit reagents. Various DNA template concentrations were amplified for DQα sequences then hybridized to AmpliType typing strips. The arrow indicates the 1.1 dot with a weak hybridization signal and the eventual loss of this signal as the input PCR template DNA is decreased.*

lost whether the signal was the result of DXα2 gene sequence hybridization or excess template concentrations by reducing the amount of DNA template for amplification.

Discussion

We have demonstrated that DXα2 genomic sequences are polymorphic in the analogous DQα 1.1 probe region and as a result, may not necessarily contribute to the low-level 1.1 probe hybridization signal that is occasionally observed during the DQα typing protocol.

The three DNA sequence polymorphisms we have detected in the DXα2 sequence when aligned with the 1.1 probe region include: 1) a DXα2 sequence which is 100% homologous to the 1.1 DQα probe sequence, 2) a two base pair difference within the homologous 1.1 probe sequence region and 3) a one base pair difference within this DXα2 sequence. DXα DNA sequence data has previously been reported by several laboratories [*4,8,9*]. Two of these papers show that the DXα sequence codes for a two base pair difference when aligned with the DQα 1.1 sequence [*8,9*]. We found this same DXα sequence in four of the eight DXα2 alleles that we sequenced and the resulting amplified DXα PCR products do not hybridize to the DQα 1.1 probe on the typing strip. Horn et al. reported a single base pair difference when comparing the DXα sequence to Auffray's published DXα sequence [*4*]. This polymorphic sequence was detected in two of the eight DXα2 fragments we sequenced and this sequence polymorphism does not hybridize to the DQα 1.1 probe. Further, we report here that in two of the eight DXα2 fragments we sequenced there is a region of 100% sequence homology with the DQα 1.1 DNA sequence which does allow hybridization of the DXα sequences to the DQα 1.1 probe. The DXα DNA sequence is non-transcribed, however, previous reports in the literature have discussed the putative DXα amino acid sequence. The nucleic acid polymorphisms discussed in this paper do not change the reported DXα amino acid sequence.

It is clear from these studies that a hybridization signal on the DQα 1.1 probe when the 1.1 allele is not part of the samples' genotype is not necessarily due to the non-specific low-level amplification of DXα genomic sequences. It was observed that some samples exhibit the "1.1 weak-signal" yet the DXα DNA sequences from these samples do not hybridize to the 1.1 probe due to sequence differences in this homologous DQα 1.1 probe region. As a result of this observation, we investigated the reduction or loss of the "1.1 weak-signal" by changing the amount of template DNA in the DQα amplification reaction. Optimizing parameters for any PCR technique is crucial to the specificity and the sensitivity of the ultimate amplification reaction. The DQα amplification reaction conditions have been investigated extensively such that the AmpliType kit may be used by nearly anyone who maintains the proper quality control measures indicated in the users manual [*6,12*]. A technical note is presented in the AmpliType users manual that suggests that the amount of DNA template used in the amplification reaction be carefully considered [*6*]. One of the reasons for this is to prevent an unnecessary amplification of a large quantity of DQα DNA which potentially may lead to weak signals on the typing strips due to excess amplified products. The specificity and sensitivity of the DQα amplification and typing is not affected by using excessive template DNA in the PCR mixture, but there are situations when very high levels of amplified DNA may cause weak hybridization on typing strip probes. Our findings are consistent with the AmpliType Users Guide recommendations to carefully consider template concentration and currently, we are using no more than 5 ng of template DNA in the DQα amplification mixture. When using an appropriate concentration of template DNA for the DQα PCR reaction, we lose any weak hybridization signals with the DQα 1.3 probe as well. Quantitation of template DNA allows the color development reaction to proceed at approximately the same rate for all samples and as a result, the color development is stopped

at the same time for all of the typing strips being processed; that is, the "C dot" should appear for all typing strips at the same relative time.

In conclusion, we have investigated the contribution of DXα DNA sequences in the low-level hybridization signal that is sometimes detected on the 1.1 probe sequence AmpliType typing strip during the DQα typing protocol and have found that even if DXα DNA sequences are non-specifically amplified during the DQα protocol, there are polymorphisms in the DXα sequence that may not allow hybridization to the DQα 1.1 probe sequence on the typing strip. We have also shown that quantitation of template DNA used in the DQα amplification procedure is useful for optimizing the amplification and subsequent typing of HLA DQα DNA sequences.

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