**Brief Communication**

**HLA-B*44:138Q: Evidence for a confined deletion and recombination events in an otherwise unaffected HLA-haplotype**

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We discovered a new HLA-B allele, **HLA-B*44:138Q**, and confirmed its segregation. For characterisation, we used serology, sequence specific oligonucleotide (SSO), sequence specific primer (SSP), and full length sequencing by Sanger and next-generation sequencing. From an evolutionary point the 5' part of the new allele is identical with alleles from the **HLA-B*44:02** group, while its 3' part is identical to the **HLA-B*15:18:01:02** allele, the breakpoint being located somewhere between intron 3 and exon 4. The salient feature of the new allele is a deletion of codon 94 in exon 3, which is unique for HLA-alleles reported so far. Gene conversion can be hypothesised in the generation of this HLA sequence; however, the deletion seems to have occurred additionally. Other HLA-alleles of the new allele's haplotype were common alleles.

Low resolution HLA-typing of a patient indicated the existence of a novel allele. We proceeded by performing high-resolution typing by sequencing. Because the characterisation of the new allele required full length sequence analysis, we performed Sanger sequencing on cloned long-range polymerase chain reaction (PCR) products or amplicons generated by allele specific primers. The advent of next-generation sequencing (NGS) allowed us to assess the comparability of both methods in respect to effort and reliability of data on this new sequence. Biochemical tests for expression of the new allele were impossible because of lack of material. However, serological analyses were applied to check the expression of HLA epitopes on the cell surface.

Both techniques, SSO and SSP, led to inconclusive results in the HLA-B typing of an human immunodeficiency virus-positive patient; the most probable genotype seemed **HLA-B*07,*44**. Subsequent Sanger sequencing of exons 1, 2, and 3 revealed a heterozygous genotype consisting of an **HLA-B*07:02**, and a novel **HLA-B*44** related allele; thus, refining the initial findings. The sequence of the new allele could be perfectly aligned with **HLA-B*44:02:01:01**; there was a deletion of three bases at codon 94, although.

To exclude phasing artefacts, we extended the analyses by cloning and sequenced 20 clones spanning exons 1 to 3. By this strategy, the results from the heterozygous sequencing were confirmed.

To characterise the novel allele more comprehensively, we amplified the whole novel allele by long-range PCR using allele specific primers located in the 5' and 3' end of the gene. For Sanger sequence analyses, we used 11 sequencing primers (all oligonucleotides of this study are listed in Table 1) that created overlapping sequences of forward and reverse strands.

To further confirm these results, we performed full-length sequencing of HLA-B alleles from the patient and all available family members by NGS. This approach covered even longer regions of the 5'UTR and 3'UTR than we had obtained in the Sanger approach.

*HLA-B*44:138Q characterised by recombination and unique deletion events. The name B*44:138Q has been officially assigned by the WHO Nomenclature Committee in October 2011. This follows the agreed policy that, subject to the conditions in the most recent Nomenclature Report, names will be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO Nomenclature Report.

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| Designation | Sequence (5' - 3') | Coverage | Reference | Sequencing technology |
|-------------|------------------|----------|-----------|-----------------------|
| **A. Primers used for polymerase chain reaction (PCR)-amplification** |
| 5BIN1CG* | CGG GGG CGC AGG ACC CGG | HLA-B, Int1 - Ex3 | 1 | Sanger |
| 3BIN3-37* | AGG CCA TCC CCG SCG ACC TAT | | 1 | |
| CL1-14AMp-B1* | CCA GGA TGG TGG TGG TCA CGG C | HLA-B, Ex1-Ex2 | In house | Sanger |
| CL1-320G rev* | CCT CGC TCT GTG TGT AGT AGC | | In house | |
| **B.** | | | | |
| B*44--18fwd* | GCA CCC ACC CGG ACT CAG AA | HLA-B, full length | In house | Sanger |
| B*44-4347rev* | GGG GTC ACG GTG GAC ACG G | | In house | |
| A-F1fwd | AAC TCA GAG CTA AGG AAT GAT GGC AAT T | HLA-A, full length | 2 | NGS |
| A-F2fwd | AAC TCA GAG CTA TGG AAT GAT GGT AAA T | | 2 | |
| A-R1 rev | ATA TAA CCA TCA TCG TGT CTC ACC C | | 2 | |
| B-5'UTR fwd | GGC AGA CAG TGT GAC AAA GAG GC | HLA-B, full length | 3 | NGS |
| B-3'UTR-3769 | CTG CCC CAG CAC ACT GCA GC | | In house | |
| C-5'UTR fwd | TCA GGC ACA CAC ACT GTC AAC GAT | HLA-C, full length | 3 | NGS |
| C-3'UTR-3779 | CTG CAG CAC ACR ATC AGG AGC C | | In house | |
| DQB1-435 fwd | TGA CAG CAA TTT TCT TTC TCT ACC CCA | HLA-DQB1, full length | 2 | NGS |
| DQB1*04Ex1 fwd | ATG TCT TGG AAG AGA GCT TCT TG | | In house | |
| DQB1-6495 rev | TGG GGA TGA AGA GAG ATG ACC TG | | 2 | |
| DRB1-PE2-F1 | CTG CTG CTC CTT GAG GCA TCC ACA | HLA-DRB1, 5'UTR-Exon2 | 2 | NGS |
| DRB1-PE2-F2 | CTG CTA CTC CTT GAG GCA TCC ACA | | 2 | |
| DRB1-PE2-F3 | CTG CTG CTC CTT GAG GCA TCC ACA | | 2 | |
| DRB1-PE2-R1 | CTT CTG GCT GTC CTA GTC GGC AT | | 2 | |
| DRB1-PE2-R2 | CTT CTG GCT GTC CTA GTC GGC GA | | 2 | |
| DRB1-PE2-R3 | CTT CTG GCT GTC CTA GTC GGC GT | | 2 | |
| DRB1-PE2-R4 | CTT CTG GCT GTC CTA GTC GGC AG | | 2 | |
| DRB1-PE2-R5 | CTT CTG GCT GTC CTA GTC GGC GC | | 2 | |
| DRB1-PE2-R6 | CTT CTG GCT GTC CTA GTC GGC GC | | 2 | |
| DRB1-E2-1.1-F | GCA CGT TTC TTG TGG CAG CTT AAG TT | HLA-DRB1, Exon2-3'UTR | 2 | NGS |
| DRB1-E2-1.2-F | GCA CGT TTC TTG TGG CAG CTT AAG TT | | 2 | |
| DRB1-E2-2-F | TTT CCT GTG GCA GCC TAA GAG G | | 2 | |
| DRB1-E2-3568-F | CAC AGC AGC TTT CTT GGA GTA CTC | | 2 | |
| Designation   | Sequence (5’ - > 3’)                  | Coverage                        | Reference | Sequencing technology |
|--------------|--------------------------------------|----------------------------------|-----------|------------------------|
| DRB1-E2-4-F  | AGC ACG TTT CCT GGA GCA GGT TAA ACA  |                                  |           |                        |
| DRB1-E2-7-F4 | CAC AGC ACG TTT CCT GTG GCA GGG      |                                  |           |                        |
| DRB1-E2-9-F  | CAC AGC ACG TTT CCT GAA GCA GGA     |                                  |           |                        |
| DRB1-E2-10-F | ACA GCA GTG TTC TTG GAG GAG GT      |                                  |           |                        |
| DRB1-E2-12-R | ATG CAC GGG AGG CCA TAC GGT         |                                  |           |                        |
| DRB1-E2-3568-R | ATG CAC AGG AGG CCA TAG GGT |                                  |           |                        |
| DRB1-E2-4-R  | ATG CAT GGG AGG CAG GAA GCA         |                                  |           |                        |
| DRB1-E2-7-R2 | CAG ATG CAT GGG AGG CAG GAA GCG     |                                  |           |                        |
| DRB1-E2-9-R  | ATG CAT GGG AGG CAG GAA GCG         |                                  |           |                        |
| DRB1-E2-10-R | TGG AAT GTC TAA AGC AAG CTA TTT AAG AT A TGT | |           |                        |
| DRB1-5’UTR   | TCT GGC CCC TGG TCC TGT CCT GTT CTC CAG GG | HLA-DRB1, full length | In house | NGS                    |
| DRB1-3’UTR   | TGC TGA ACC AGT AGC AAC CAG GTC C   |                                  |           |                        |
| DQA1fw-310   | AGA CAT GCA ACC AGA GAA GA          |                                  |           |                        |
| DQA1rev-5441 | TGC CAC TTC CCA ATT CCC CTA C       |                                  |           |                        |
| DPB1-PRO-F2  | CCT CCT GAC CCT GAT GAC AGT CCT     |                                  |           |                        |
| DPB1-PRO-R2  | CCA TCT GCC CCT CCA GCA CCT CAA     |                                  |           |                        |
| DPB1-F2      | CTC AGT GCT CTC CCC TCC CTA GTG AT  |                                  |           |                        |
| DPB1-R2      | GCA CAG TAG CTT TCG GGA ATT GAC CA  |                                  |           |                        |
| DRB3-In1fwd*01 | GTG TGA CCG CAG CTT TCG TGT A | HLA-DRB3, Intron1-3’UTR | In house | NGS                    |
| DRB3-In1fwd*02 | GTG TGA CCG GAG CAT TCG TGT C |                                  |           |                        |
| DRB3-E2-R1   | ATG CAC AGG AGG CCA TAG GTG         |                                  |           |                        |
| DRB5-In1fwd  | ATG CCG GCG TCT CTG TCA GTA         |                                  |           |                        |
| DRB5-E2-R2   | ATG CAT GGG AGG CCG TAG GTG         |                                  |           |                        |
| DRB4-In1fwd  | CCG GAT CGT TCG TGT CCC CA          |                                  |           |                        |
| DPA1-F1      | CTC TCT TGA CCA CGC TGG TAC CTA     |                                  |           |                        |
| DPA1-R1      | TGT GCC TCT TGG CTA TAC CTC TTT T   |                                  |           |                        |
| E-fw (E00072) | CAG GTG CTC CAC GAC TCC CGA C       |                                  |           |                        |
| E-rev (E10034) | AGA CAC AGA GGT GGA CTT TCT CTC T |                                  |           |                        |
A. Lists all primers used for the generation of sequencing templates by PCR. B. Lists all sequencing primers that have been used for full length Sanger sequencing of the HLA-B*44:138Q allele. In column “Designation” the names of the primers are listed; in column “Sequence (5’->3’)” the sequence of the nucleotides is provided; in column “Coverage” the targeted gene and the location of the primers on this gene are indicated; in column “Reference” the origin of the primer sequence is listed: primers have been created by us (“in house”) or have been taken from publications; in column “Sequence technology” the technique used is stated. Oligonucleotides marked with * are also used as sequencing primers. The primers have been positioned to allow a full coverage of the allele.

| Designation       | Sequence (5’->3’) | Coverage          | Reference       | Sequencing technology |
|-------------------|-------------------|-------------------|-----------------|-----------------------|
| G-5’UTR260-fwd    | GAA GTC CCA GGG CCT CAA GC | HLA-G, full length | In house       | NGS                   |
| G-rev 3228        | CCC ATC AAT CTC TCT TGG AAA | In house         |                 |                       |
| MICA-fwd Ex1      | ACG CGT TGT TCG TCC TGG AA | MICA-exon 1-Exon2 |                 |                       |
| MICA-rev RG       | CTA CGA CGG GGG TAA GGG AAG GGT T |                       |                 |                       |
| MICA-fwd FG       | CGT TCT TGT CCC TTT GCC GTG C | MICA-Exon2-3’UTR  |                 |                       |
| MICA-rev 3’UTR    | CGT GCC TGG CCT GAG ACT |                       |                 |                       |
| B. Primers used for sequencing |                      |                   |                 |                       |
| B*44-559 rev      | TCG TCC ACG TAG CCC ACG GT | HLA-B*44 559      | In house       | Sanger                |
| B*44-1034fwd      | GGG TCT CAC ATC ATC CAG AGG | HLA-B*44 1034     | In house       | Sanger                |
| B*44-1830fwd      | GTC CTA GGG TGT CCC ATG AG | HLA-B*44 1830     | In house       | Sanger                |
| B*44-2155rev      | GAA GAG ATA TGA CCC CTC ATC C | HLA-B*44 2155     | In house       | Sanger                |
| B*44-2182fwd      | CTG GAG CCC TTC AGC AGG | HLA-B*44 2182     | In house       | Sanger                |
| B*44-2346fwd      | TGT GAT GTG TAG GAG GAA G AG C | HLA-B*44 2346     | In house       | Sanger                |
| B*44-2797fwd      | TCC CAG TCC CCT CAC AGG G | HLA-B*44 2797     | In house       | Sanger                |
| B*44-3041rev      | CCC ACC CAC CCC CAG ACC T | HLA-B*44 3041     | In house       | Sanger                |
The family study confirmed the unequivocal segregation of the new allele (Figure 1).

To further characterise the haplotypes, we typed other class I genes, HLA-A, C, E, and G and class II genes, HLA-DRB1, DRB3/4/5, DQA1, DQB1, DPA1, and DPB1; additionally the class I-related MICA genes were typed. All of those genes were typed at full length. The results are provided in Figure 1. All alleles observed have been listed in the database; it thus seems that the events, leading to the generation of the new HLA-B allele did not affect other functional genes of the haplotype.

Restricting the view on the sequence to exons 1 to 3, the relationship of the novel allele with the HLA-B*44 group seemed apparent: sequences were identical except the deletion of three nucleotides. After we had obtained the full length sequence, it became evident, that the region from the S′ end to Intron 3 matched perfectly with HLA-B*44:02:01:01 and other HLA-B*44 alleles. From position 1620 in exon 4, however, several mismatches appeared; this part of the sequence matched perfectly with HLA-B*15:18:01:02 (Figure 2), although. From position 1221 in intron 3 to position 1619 in exon 4 both putative founder alleles share the sequence, a recombination between the two alleles might therefore have occurred there. By contrast, the deletion of three nucleotides in exon 3 is a unique feature of HLA-B*44:138Q, indicating an independent event, that led to the creation of this allele.

In this respect, the new allele differs from the majority of other HLA alleles, where a simple “fixation of a single recombination event was responsible for the origin”\(^9\); whether these events have occurred simultaneously or in independent meioses remains impossible to decide.

Because of the fact that this deletion comprised three nucleotides and occurred within two identical neighbouring codons (ATC-ATC) the resulting protein was shortened by just one isoleucin. This deletion concerns the second domain at the edge between the α-helix and the β-sheet. It is not likely that this position is part of an epitope.\(^9\)\(^10\)\(^11\) However, the conformation of the molecules might change which could involve modifications of serological epitopes. This assumption would be in concordance with results from lymphocytotoxic tests: sera specific for HLA-B44 (n = 2), HLA-B12 (n = 2), or Bw4 (n = 3) did not show any reactivities with cells of the HLA-B*44:138Q-positive individual.
FIGURE 2 Sequence alignments of HLA-B*44:138Q, HLA-B*44:42-01:01, and HLA-B*15:18:01:02. Alignments have been generated using the tool of the IMGT/HLA database (https://www.ebi.ac.uk/cgi-bin/ipd/imgt/hla), version 3.33.0. Bases identical with HLA-B*44:138Q are indicated by dashes, different nucleotides are marked, and deletions are signposted with dots; "T" denotes exon/intron borders. The numbering of base positions in the genomic DNA (gDNA) is according to the IMGT/database. The HLA-B*44:138Q sequence was obtained by nucleotide Sanger sequencing (positions 10 to 3034) or NGS (positions 284 to 3804). For the preparation of an amplification template for Sanger sequencing, primers for whole gene amplification were designed that covered the gene from its 5'UTR to the 3'UTR and separated alignments have been generated using the tool of the IMGT/HLA database. The HLA-B*44:138Q allele. All primers are listed in Table 1. Cycle sequencing was performed using a Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA). The sequencing products were analysed on an ABI 3100 capillary sequencer. For confirmation, heterozygous PCR products spanning long-range amplification of the whole exons 1 to exons 3 were cloned with TA Cloning kit pCR 2.1 vector (Invitrogen, Carlsbad, USA) and also subjected to Sanger sequencing. For NGS analyses, reads was performed using two different NGS analysis software packets (TypeStream NGS Analysis Software, One Lambda, Inc. Canoga Park CA; NGSengine, GenDX, Utrecht, The Netherlands)
From a technical point, the analysis of the full-length gene by NGS was much simpler than the Sanger approach, because of the clonal nature of the NGS sequencing, there was no necessity to separate alleles beforehand and no additional sequencing primers had to be designed. The sequence of the HLA-B*44:138Q allele was concordant with the Sanger result except a homopolymer at position 3061 of the 3'UTR: With the Sanger technique, 3 cytosins were detected while the consensus of the NGS analysis indicated only 2 cytosins. Given that Sanger sequencing has represented
the golden standard for HLA typing for decades\textsuperscript{12} this discrepancy argues for a good concordance of the new technology, albeit we have compared only one of the several platforms.

In summary, the characterisation of HLA-B\textsuperscript{*}44:138Q on the IonTorrent platform allowed a fast analysis of whole genes with much less effort compared with Sanger sequencing. However, the homopolymer issue\textsuperscript{13} of NGS remains a diagnostic challenge. The generation of the new allele within a haplotype that otherwise consists of common HLA-alleles (as assessed by full length sequencing) indicates that the mechanisms leading to this new allele are restricted to a single gene.

CONFLICTS OF INTEREST

The authors have declared no conflicting interests.

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