

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*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,¹⁴ 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.

*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.⁸ This approach covered even longer regions of the 5'UTR and 3'UTR than we had obtained in the Sanger approach.

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TABLE 1 Oligonucleotides used for sequencing based HLA typing with Sanger or next-generation sequencing (NGS) technology

Designation	Sequence (5' ->3')	Coverage	Reference	Sequencing technology
A. Primers used for polymerase chain reaction (PCR)-amplification				
<i>5B/NTCG*</i>	CGG GGG CGC AGG ACC CGG	HLA-B, Int1 - Ex3	1	Sanger
<i>3Bn3-37*</i>	AGG CCA TCC CCG SCG ACCTAT	HLA-B, Ex1-Ex2	1	In house
<i>CLL-14AMP-B1*</i>	CGA GGA TGC GGG TCA CGGC	HLA-B, Ex1-Ex2	In house	Sanger
<i>CLL-320G rev*</i>	CCT CGC TCT GGT TGT AGT AGC	HLA-B, full length	In house	Sanger
<i>B*44-18fwd*</i>	GCA CCC ACC CGG ACT CAG AA	HLA-B, full length	In house	In house
<i>B*44-4347rev*</i>	GGG GTC ACG GTG GAC ACG G	HLA-B, full length	2	In house
<i>A-F1fwd</i>	AAC TCA GAG CTA AGG AAAT GAT GGC AAA T	HLA-A, full length	2	NGS
<i>A-F2 fwd</i>	AAC TCA GAG CTA TGG AAAT GAT GGT AAA T	HLA-B, full length	2	NGS
<i>A-R1 rev</i>	ATA TAA CCA TCA TCG TGT CCC AAG GTT C	HLA-C, full length	3	In house
<i>B-5'UTR fwd</i>	GGC AGA CAG TGT GAC AAA GAG GC	HLA-B, full length	3	NGS
<i>B-3'UTR-3769</i>	CTG CCC CAG CAC ACT GCA GC	HLA-C, full length	3	NGS
<i>C-5'UTR fwd</i>	TCA GGC ACA CAG TGT GAC AAA GAT	HLA-C, full length	In house	In house
<i>C-3'UTR-3779</i>	CTG CAG CAC ACR ATC AGG TTT C	HLA-DQB1, full length	2	NGS
<i>DQB1-453 fwd</i>	TGA CAG CAA TTT TCT CTC CCC TGA	HLA-DQB1, full length	2	NGS
<i>DQB1*04Ex1 fwd</i>	ATG TCT TGG AAG AAG GCT TTG CG	HLA-DQB1, 5' UTR-Exon2	2	In house
<i>DQB1-6495 rev</i>	TGG GGA TGA AAG GAG ATG ACC TG	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-PE2-F1</i>	CTG CTG CTC CTT GAG GCA TCC ACA	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-PE2-F2</i>	CTG CTA CTC CTT GAG GCA TCC ACA	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-PE2-F3</i>	CTG CTG CTC CCT GAG GCA TCC ACA	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-PE2-R1</i>	CTT CTG GCT GTT CCA GTA CTC GGC AT	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-PE2-R2</i>	CTT CTG GCT GTT CCA GGA CTC GGC GA	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-PE2-R3</i>	CTT CTG GCT GTT CCA GTA CTC AGC GT	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-PE2-R4</i>	CTT CTG GCT GTT CCA GTA CTC CTC AT	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-PE2-R5</i>	CTT CTG GCT GTT CCA GTG CTC CGC AG	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-PE2-R6</i>	CTT CTG GCT GTT CCA GTA CTC GGC GC	HLA-DRB1, 5' UTR-Exon2	2	NGS
<i>DRB1-E2-1-1-F</i>	GCA CGT TTC TTG TGG CAG CTA AAG TT	HLA-DRB1, Exon2-3'UTR	2	NGS
<i>DRB1-E2-1-2-F</i>	TTT CCT GTG GCA GCC TAA GAG G	HLA-DRB1, Exon2-3'UTR	2	NGS
<i>DRB1-E2-2-F</i>	CAC AGG ACG TTT CTT GGA GTA CTC	HLA-DRB1, Exon2-3'UTR	2	NGS
<i>DRB1-E2-3568-F</i>		HLA-DRB1, Exon2-3'UTR	2	NGS

TABLE 1 (Continued)

Designation	Sequence (5'->3')	Coverage	Reference	Sequencing technology
DRB1-E2-4-F	AGC ACG TTT CTT GGA GCA GGT TAA ACA	2	2	
DRB1-E2-7-F4	CAC AGC ACG TTT CCT GTG GCA GGG	2	2	
DRB1-E2-9-F	CAC AGC ACG TTT CCT GAA GCA GGA	2	2	
DRB1-E2-10-F	ACA GCA CGT TTC TTG GAG GAG GT	2	2	
DRB1-E2-12-R	ATG CAC GGG AGG CCA TAC GGT	2	2	
DRB1-E2-2568-R	ATG CAC AGG AGG CCA TAG GGT	2	2	
DRB1-E2-4-R	ATG CAT GGG AGG CAG GAA GCA	2	2	
DRB1-E2-7-R2	CAG ATG CAT GGG AGG CAG GAA GCG	2	2	
DRB1-E2-9-R	ATG CAT GGG AGG CAG GAA GCG	2	2	
DRB1-E2-10-R	TGG AAT GTC TAA AGC AAG CTA TTT AAC ATA TGT	2	2	
DRB1-5'UTR	TCT GGC CCC TGG TCC TGT CCT GTC CAG GG	HLA-DRB1, full length	In house	NGS
DRB1-3'UTR	TGC TGA ACC AGT AGC AAC CAG GTC C	HLA-DQA1, full length	In house	NGS
DQA1-fwd-310	AGA CAT GCA CAC ACC AGA GAA GA	HLA-DQA1, full length	In house	NGS
DQA1-rev-5441	TGC CAC TTC CCA ATT CCC CTA C	HLA-DRB1, 5'UTR-Exon2	In house	NGS
DPB1-PRO-F2	CCT CCT GAC CCT GAT GAC AGT CCT	HLA-DRB1, 5'UTR-Exon2	2	2
DPB1-PRO-R2	CCA TCT GCC CCT CAA GCA CCT CAA	HLA-DRB1, Exon2-3'UTR	2	2
DPB1-F2	CTC AGT GCT CGC CCC TCC CTA GTG AT	HLA-DRB1, Exon2-3'UTR	2	2
DPB1-R2	GCA CAG TAG CTT TCG GGA ATT GAC CA	HLA-DRB3, Intron1-3'UTR	In house	NGS
DRB3-In1fwd*01	GTG TGA CCG GAT CCT TCG TGT A	HLA-DRB3, Intron1-3'UTR	In house	NGS
DRB3-In1fwd*02	GTG TGA CGG GAG CAT TCG TGT C	HLA-DRB5, Intron1-3'UTR	In house	NGS
DRB3-E2-RI	ATG CAC AGG AGG CCA TAG GGT	HLA-DRB4, Intron1-3'UTR	In house	NGS
DRB5-In1fwd	ATG GCG GCG TCT CTG TCA GTA	HLA-DRB4, Intron1-3'UTR	2	2
DRB5-E2-R	ATG CAT GGG AGG CAG GAC AGT	HLA-DPA1, full length	NGS	NGS
DPA1-F1	CTC TCT TGA CCA CGC TGG TAC CTA	HLA-DPA1, full length	2	2
DPA1-R1	TGT GCC TCT TGG CTA TAC CTC TTT T	HLA-DPB1, full length	2	2
E-fwd (E08072)	CAG CGT CGC CAC GAC TCC CGA C	HLA-E, full length	4	4
E-rev (E10034)	AGA CAC AGA GGT GGA CTG TTT CTC T	HLA-E, full length	4	4

TABLE I (Continued)

Designation	Sequence (5'->3')	Coverage	Reference	Sequencing technology
G-5'UTR260-fwd	GAA GTC CCA GGG CCT CAA GC CCC ATC AAT CTG TCT TGG AAA	HLA-G, full length	In house	NGS
G-rev 3228	ACG CGT TGT CTG TCC TGG AA	MICA-exon 1-Exon2	5	NGS
MICA-fwd Ex1	CTA CGA CGG GGG TAA GGG AAG GGT T		6	
MICA-rev RG	CGT TCT TGT CCC TTT GCC CGT GTGC	MICA-Exon2-3'UTR	6	NGS
MICA-fwd FG	CGT GCC TGG CCT GAG ACT		7	
MICA-rev 3'UTR				
B. Primers used for sequencing				
<i>B*44-559</i> rev	TGG TCC ACG TAG CCC ACG GT	<i>HLA-B*44 559</i>	In house	Sanger
<i>B*44-1034</i> fwd	GGG TCT CAC ATC ATC CAG AGG	<i>HLA-B*44 1034</i>	In house	Sanger
<i>B*44-1830</i> fwd	GTC CTA GGG TGT CCC ATG AG	<i>HLA-B*44 1830</i>	In house	Sanger
<i>B*44-2155</i> rev	GAA GAG ATA TGA CCC CTIC ATCC	<i>HLA-B*44 2155</i>	In house	Sanger
<i>B*44-2182</i> fwd	CTG GAG CCC TTG AGC AGG	<i>HLA-B*44 2182</i>	In house	Sanger
<i>B*44-2346</i> fwd	TGT GAT GTG TAG GAG GAA GAG C	<i>HLA-B*44 2346</i>	In house	Sanger
<i>B*44-2797</i> fwd	TCC CAG TCC CCT CAC AGG G	<i>HLA-B*44 2797</i>	In house	Sanger
<i>B*44-3041</i> rev	CCC ACC CAC CCC CAG ACC T	<i>HLA-B*44 3041</i>	In house	Sanger

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

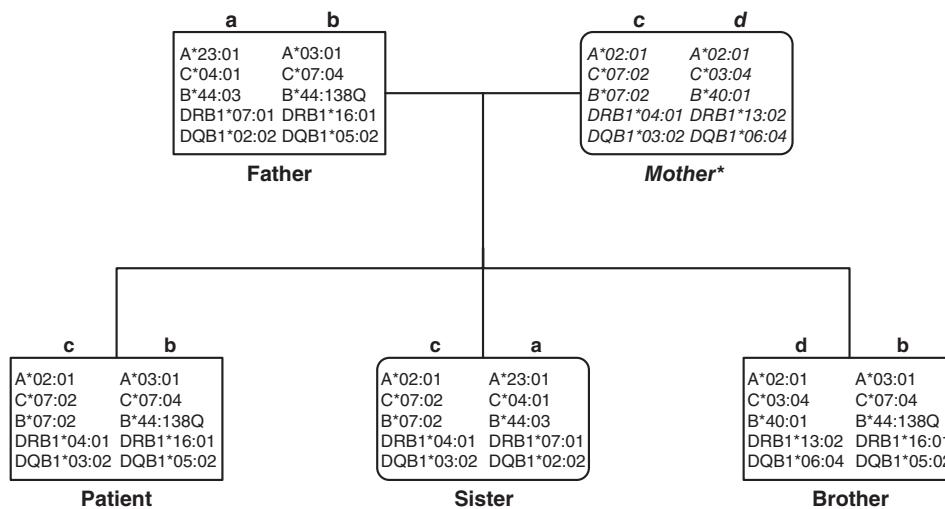


FIGURE 1 Pedigree chart of the patient's family; A-D indicate HLA-haplotypes; only HLA-A, B, C, DRB1, and DQB1 genotypes at two field resolution are depicted for easy readability. The new allele is located on haplotype b, which is observed in the father and two siblings *the mother was at the time of typing already deceased, her haplotypes have been deduced. The typing has been performed by various methods:

- Serology: we used 180 in house validated typing sera to detect HLA class I molecules on the cell surface.
- Low resolution typing: SSO typing was performed for *HLA-A*, *-B*, and *-C* genes using a commercial reverse dot blot assay (Dynal, Bloomsborough, UK). SSP typing was performed for the *HLA B* gene only using a commercial SSP assay (Genovision, Vienna, UK).
- Sequencing-based typing is described in Figure 2.
- The full characterisation of HLA-haplotypes is as follows:

Haplotype a: G*01:04:04, A*23:01:01, E*01:01:01:01, C*04:01:01:01, B*44:03:01:01, MICA*004, DRB4*01:01:01:01, DRB1*07:01:01:01, DQA1*02:01:01:01, DQB1*02:02:01:01, DPA1*01:03:01:04, DPB1*04:01:01:01. Haplotype b: G*01:01:01:05, A*03:01:01:01, E*01:03:02:01, C*07:04:01:01, B*44:138Q, MICA*008:01:02, DRB5*02:02, DRB1*16:01:01, DQA1*01:02:02, DQB1*05:02:01, DPA1*01:03:01:02, DPB1*04:01:01:01. Haplotype c: G*01:01:01:01, A*02:01:01:01, E*01:01:01:01, C*07:02:02:03, B*07:02:01, MICA*008:04, DRB4*01:03:01:01, DRB1*04:01:01:01, DQA1*03:01:01, DQB1*03:02:01, DPA1*01:03:01:02, DPB1*04:01:01:01. Haplotype d: G*01:01:01:01, A*02:01:01:01, E*01:03:02:01, C*03:04:01:01, B*40:01:02, MICA*008:04, DRB3*03:01:01:01, DRB1*13:02:01, DQA1*01:02:01:04, DQB1*06:04:01, DPA1*01:03:01:01, DPB1*02:01:02:06

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 5' 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.⁹ 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.^{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.

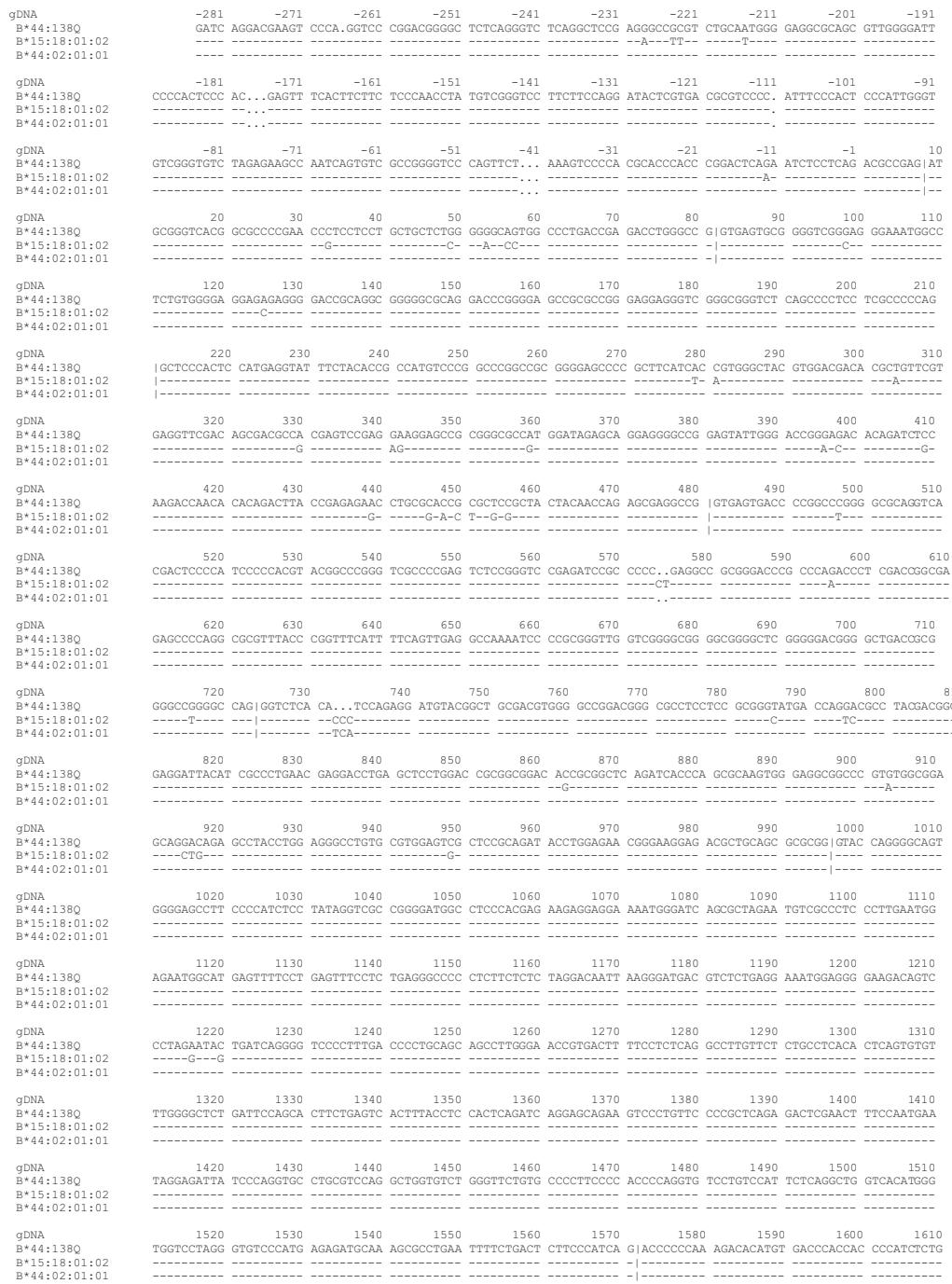


FIGURE 2 Sequence alignments of *HLA-B*44:138Q*, *HLA-B*44:02:01:01*, and *HLA-B*15:18:01:02*. Alignments have been generated using the tool of the IMGT/HLA database (<https://www.ebi.ac.uk/cgi-bin/ipd/imgt/hla>), version 3.33.0. *Bases identical with HLA-B*44:138Q* are indicated by dashes, different nucleotides are marked, and deletions are signposted with dots; “l” 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 22 to 456) and whole gene 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 *HLA-B*07* and *HLA-B*44* allele groups. For amplification the PCR Qiagen Long-Range PCR Kit (Qiagen GmbH Hilden, Germany) was used. Sequencing was performed with overlapping primers for the whole *HLA-B*44* allele. All primers are listed in Table 1. Cycle sequencing was performed using a Big Dye Terminator Cycle Sequencing kit (ABI, Foster City, CA). The sequencing products were analysed on an ABI 3100 capillary sequencer. For confirmation, heterozygous PCR products spanning 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, long-range amplification of the whole *HLA-B* gene was achieved with the primers listed in Table 1. Amplification was performed using GoTaq Long PCR Mastermix (Promega Corporation, Woods Hollow Roads Madison). Library preparation was performed according to the manufacturers' instructions. Size selection was performed on an E-Gel (Invitrogen, Kiryat Shoma, Israel); only fragments with sizes >400 bp were selected. After quantification, 26 PMol of fragments were used for emulsion PCR. Enriched Ion sphere particles were loaded onto a Ion Torrent 316 chip v2 (Life Technologies, Carlsbad, California) and subsequently sequenced on an Ion PGM device (LifeTechnologies, Carlsbad, California) with a flow number of 800, for 400 base reads. Analysis of the 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).

gDNA	1620	1630	1640	1650	1660	1670	1680	1690	1700	1710
B*44:138Q	ACCATGAGGCC	CACCGGAGG	TGCTGGCCC	TGGGCTCTA	CCCTGCAGG	ATCACACTGA	CCTGGCAGCG	GGATGGCAG	GACCAAATC	AGGACACCGA
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-T-	-	-	-	-	-	-	-	-	-
gDNA	1720	1730	1740	1750	1760	1770	1780	1790	1800	1810
B*44:138Q	GCTTGTGGAG	ACCAAGCCAG	CAGGAGATAG	AACCTTCCCAG	AAGTGGCCAG	CTGTGGTGT	GCCTCTGGG	GAAGAGCAGA	GATAACATG	CCATGTACAG
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-	-	-	-
gDNA	1820	1830	1840	1850	1860	1870	1880	1890	1900	1910
B*44:138Q	CATGAGGGGC	TGCCGAAGCC	CCTCACCTTG	AGATGGG GTA	AGGAGGGGG	TGAGGGGTC	TATCTGTTCT	CAGGAAAGC	AGGACCCCT	CTGGAGCCCT
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-	-	-	-
gDNA	1920	1930	1940	1950	1960	1970	1980	1990	2000	2010
B*44:138Q	TCAGCAGGGT	CAGGGGCCCT	CATCTTCCCC	TCCTTCTCCAG	AGCCCATCTT	CCCAGTCAC	CATCCCCATC	GTGGGCATTG	TTGCTGGCT	GGCTGTCCCTA
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-T-	-	-	-G-	-	-	-	-
gDNA	2020	2030	2040	2050	2060	2070	2080	2090	2100	2110
B*44:138Q	GCAGTTGTGG	TCATGGAGC	TGTGGTCGCT	ACTGTGATGT	GTAGGAGAAA	GAGCTCAG GT	AGGGAGGGG	TGAGGGGTGG	GGTCCTGGGT	TTCTTGTGCC
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-G-	-	-	-	-	-	-
gDNA	2120	2130	2140	2150	2160	2170	2180	2190	2200	2210
B*44:138Q	ACTGGGGTT	TCAAGCCCCA	GGTAGAACGT	TTCCCTGCCT	CATTACTGGG	AAGCAGCATC	CACACAGGGG	CTAACGCAGC	CTGGGACCT	GTGTGCCAGC
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-	-	-	-
gDNA	2220	2230	2240	2250	2260	2270	2280	2290	2300	2310
B*44:138Q	ACTTACTCTT	TTGTGCGAGC	CATGTGACAA	TGAAGGACAG	ATGTATCGG	TGATGTTGTT	TGGTGTGGG	GTCTGTGATTC	CAGCATTAT	GAGTCAGGG
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-G-	-	-A-	-G-	-	-	-
gDNA	2320	2330	2340	2350	2360	2370	2380	2390	2400	2410
B*44:138Q	AAGTCCCTG	CTAAGGGACAG	ACCTTGGAGG	GGCAGATTGTT	CCAGGACCCA	CACTGCTTT	CCTCGTTGTT	CCTGATCCCTG	CCCTGGGCT	GTAGTCATAC
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-T-	-	-	-
gDNA	2420	2430	2440	2450	2460	2470	2480	2490	2500	2510
B*44:138Q	TTCTTGAAAT	TCTTTTTGGT	TCCAAGACGA	GGAGGTTCT	CTAAGATCTC	ATGGTCTCTG	TTCTCTCCAG	CCCCCTCAC	GGACATTTC	TTCCACAG G
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-G-	-	-	-C-	-	-G-	-	-
gDNA	2520	2530	2540	2550	2560	2570	2580	2590	2600	2610
B*44:138Q	TGGAAAAGGA	GGGAGCTACT	CTCAGGCTGC	GT GTAAGTGG	TGGGGTGGG	AGTGTGGAGG	AGCTCACCA	CCCCATAATT	CCTCCGTCTC	CACGCTCCT
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-A-	-C-	-	-
gDNA	2620	2630	2640	2650	2660	2670	2680	2690	2700	2710
B*44:138Q	GCGGGCTCTG	ACCAGGCTCT	GTTTTGTCT	TACTCCAG C	ACGGCACAGTG	CCCAGGGCTC	TGATGTGCT	CTCACAGCTT	GA AAAGGTGA	GATTCTGGG
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-G-	-	-	-	-	-	-
gDNA	2720	2730	2740	2750	2760	2770	2780	2790	2800	2810
B*44:138Q	GTCTAGACTG	GGTGGGGTGG	CGGGTCTGGG	GGTGGGGTGG	CGACGTGGGA	AAGGCTCTGG	TAATGGAGAT	TCTTGTGTTG	GGATGTTTCG	CGTGTGTCGT
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-A-	-	-	-	-	-	-	-G-
gDNA	2820	2830	2840	2850	2860	2870	2880	2890	2900	2910
B*44:138Q	GGGCTGTCTCA	ACTTACCATG	ACTAACCAGA	ATTGTTCTCAT	GACTGTGTT	TCTCTGAGCC	TGAGACAGCT	GTCTGTGAG	GGACTGAGAT	-
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-T-	-	-	-	-	-	-	-
gDNA	2920	2930	2940	2950	2960	2970	2980	2990	3000	3010
B*44:138Q	GCAGGATTTC	TTCAGGCTCC	CCCTTTGTGA	CTTCAAGAGC	CTCTGCGATC	TCTTTCTGCA	AAGGACCTG	AATGTGCTG	CGTCCCCTGT	AGCATATAATG
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-	-	-	-
gDNA	3020	3030	3040	3050	3060	3070	3080	3090	3100	3110
B*44:138Q	GAGGAGGTGG	AGAGACAGCC	CACCCCTGTG	TCCACTGTGA	CCCTGTCTTC	CATGCTGATC	TGTGTTCT	CCCCAGCTAT	CTTCTGTGTT	CCAGAGAGGT
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-CC-	-	-C-	-	-CT-	-	-
gDNA	3120	3130	3140	3150	3160	3170	3180	3190	3200	3210
B*44:138Q	GGGGCTGGAT	GTCTCCATCT	CTGTCCTCAC	TTTATGTGCA	CTGAGCTGCA	ACTTCTTACT	TCCCTACTGA	AAATAAGAAT	CTGAATATAA	ATTGTTTTTC
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-	-	-	-
gDNA	3220	3230	3240	3250	3260	3270	3280	3290	3300	3310
B*44:138Q	TCAAATATT	GCTATGAGAG	GTTGATGTT	TAATTTAATA	AGTCAATTCT	TGGAATTGTA	AAGGACCAAT	AAAGACCTGA	GAACCTTCCA	GAATCTGCAT
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-	-	-	-G-
gDNA	3320	3330	3340	3350	3360	3370	3380	3390	3400	3410
B*44:138Q	GTTGGCTGTG	CTGAGCTGT	TGGAGGTTGG	GGTGGTGGAGA	GGCTGTGGGG	GGGCCAGGTGT	GGATGGGGCC	TGTGCCCATT	TGGTGTGAG	TCCATCATG
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-C-	-	-	-
gDNA	3420	3430	3440	3450	3460	3470	3480	3490	3500	3510
B*44:138Q	GCTTTATGTY	GTTAGTCGTC	AGCTGGTCA	CCTTCACCTG	TCCATTGTC	TGTGTTCTTC	AGTGGAAACT	TGTCCAGTGG	GAGCTGTGAC	CACAGAGGCT
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-T-	-	-	-	-	-	-	-
gDNA	3520	3530	3540	3550	3560	3570	3580	3590	3600	3610
B*44:138Q	CACACATCGC	CCAGGGCGCC	CCCTGCACAC	GGGGATCTCT	GTGCATCTC	AGACAAATTTC	TCAGAGCCAT	TCACCTCCCTG	CCCTGCTTCT	AGAGCTCCTT
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-T-	-	-G-	-	-	-	-
gDNA	3620	3630	3640	3650	3660	3670	3680	3690	3700	3710
B*44:138Q	TTCTGCTCTG	CTCTTCTGCG	CTCTCTCTCT	GCCCTGGTTC	TAGTGTATTT	GGTGGCTGAAT	CCAACTCCCAA	CTCATGAATC	TGTAAGGAG	AGCTTAATT
B*15:18:01:02	-	-	-	-	-	-	-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-	-	-	-
gDNA	3720	3730	3740	3750	3760	3770	3780	3790	3800	
B*44:138Q	AGACTTACAT	TTGTCGTGTA	AATTGGACCC	ATCATCAAGG	ACTGTTCTTT	CCTGAAGGGA	GAACCTGATT	GTGTGCTGCA	GTGTGCTGGG	GCAG
B*15:18:01:02	-	-	-	-	-	-	-A-	-	-	-
B*44:02:01:01	-	-	-	-	-	-	-	-	-	-

FIGURE 2 (Continued)

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 decades¹² 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*44:138Q* on the IonTorrent platform allowed a fast analysis of whole genes with much less effort compared with Sanger sequencing. However, the homopolymer issue¹³ 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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