GENDX
personalising diagnostics

NGS unveiling immunogenic irregularities that were previously conceiled

Introduction

Next-generation sequencing (NGS) technology has great potential for generating reliable, unambiguous HLA typing results. The advantage of NGS is that it allows for reads originating from a single molecule, meaning that the paternal and maternal alleles can uniquely be identified. Currently applied NGS-based HLA-typing approaches are based on the assumption that an HLA genotype is represented by maximally two alleles per locus. Here we present a case where a chimeric HLA genotype was identified, showing >2 alleles per HLA locus. The sample could accurately be typed by NGS in combination with a new software tool optimized for typing of multiple HLA alleles.

Materials and Methods

Genomic DNA from blood of the patient was amplified using NGSgo-AmpX amplification primers for HLA-A, B, C, DRB1 and DQB1. The amplicons were further processed in the NGSgo® workflow (GenDx) for library preparation. The libraries were sequenced by NGS on the Illumina MiSeq using paired-end sequencing (2x 151 cycles). The NGS data was analysed using the software package NGSengine® (GenDx) for NGS-based HLA typing and NGSAlleleFitter™ (GenDx), a tool designed to explore NGS data for the presence of additional alleles or patterns in NGS data. The analysis was restricted to the coding regions and only alleles on the CWD list were included. For each locus, a low-fraction and a high-fraction genotype was determined. For all genotypes, the relative fraction of each of the four alleles was calculated based on a minimal distance statistics.

Results

A 1y5mo old patient, diagnosed with Severe Combined Immune Deficiency (SCID) and candidate for hematopoietic stem cell transplantation (HSCT), was directed to the HLA laboratory of Ankara for HLA typing. The patient has a history of having had several blood transfusions (potentially not irradiated whole blood). Typing with Luminex (Lifecodes SSO Typing Kits, Immucor) failed to determine the HLA-ABCDR typing of the patient due to the presence of more than 2 alleles in the blood DNA. The chimeric status was confirmed by study of the Short Tandem Repeats (Investigator IDPlexPlus kit), showing the presence of STR markers that have not originated from his parents (Figure 1).

Next-generation sequencing using NGSgo® and NGSengine® was applied to type the HLA alleles. The patient blood sample DNA was sequenced by NGS for HLA-A, B, C, DRB1 and DQB1 (Figure 2). Close examination of the base call frequencies revealed that at least four alleles are present for each HLA locus, of which not all alleles are present in the same amount (Figure 3). Applying NGSAlleleFitter showed that the data for all loci match with the presence of two genotypes, of which one is present as a lower fraction of about 10%. For all loci the alleles and their relative fraction could be identified (Tables 1 and Figure 4). In DRB1, only one allele seems present as a high fraction. This, however, may represent some unbalanced amplification in the PCR.

In parallel, SSO and SBT typing results (GenDx SBTexcellerator kit) were generated from the family members and also from hair root DNA of the patient to obtain somatic cell DNA. Comparison of the SSO and SBT results with the NGS-based typing results demonstrated that the genotype of the lower fraction matches the typing of the patient. The patient HLA typing was also found to be identical to two siblings (Table 2).

We suspect that the cells expressing the high fraction alleles originate from one of the blood transfusions. This suggests that stem cells derived from blood transfusions can nestle in the body, resulting in a mixed representation of HLA genotypes from different sources (e.g. patient vs. blood donor). Using NGS, we have been able to determine the high and low fraction HLA genotypes from chimeric DNA samples. Based on this method, we have accurately derived the patients low fraction HLA genotype from blood DNA.

Figure 1: Chimerism test results:

The results show a different STR marker distribution in the blood and somatic cells (hair root) of the patient. The results confirm the presence of >2 markers in the patient, which may have originated from the blood transfusion of an unknown donor. Comparison of the results with the STR markers of the parents, shows that some STR markers of the patient have originated from his parents (red arrows) and some have not.

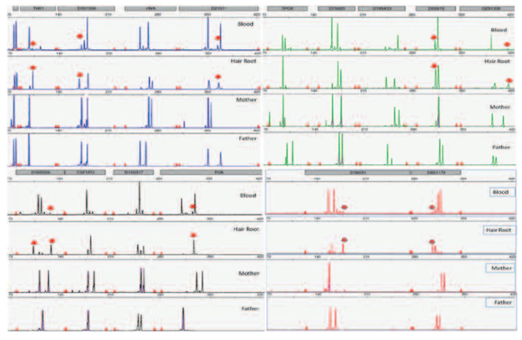
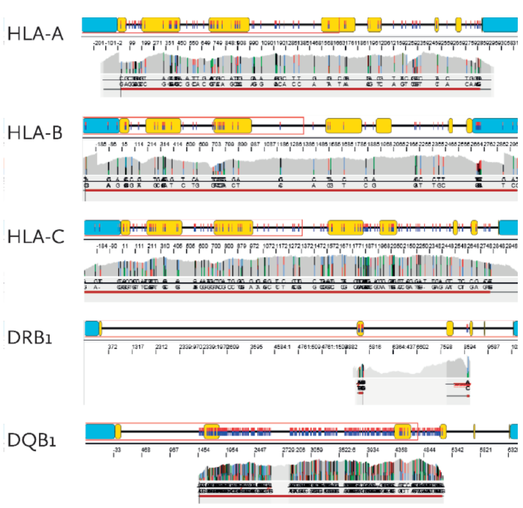


Figure 2: HLA locus coverage with NGSgo.

The images show the coverage for each of the HLA loci present in the blood DNA sample. Exons are represented in yellow, UTRs in blue. The aligned reads, indicated in grey, are covering the complete amplicon. Polymorphic positions are identified throughout the amplicons and are indicated as vertical coloured bars.



Conclusion

A chimeric HLA genotype was detected in a SCID patient due to multiple blood transfusions. Conventional NGS analysis could demonstrate the presence of multiple alleles when inspecting the heterozygosity of the sample, but failed to identify the HLA typing. Applying NGSAlleleFitter resulted in accurate typing of multiple alleles, even when alleles were present at low levels. This demonstrates that NGS data can be used for correct identification and typing of multiple HLA alleles, which will benefit the typing of chimeric patients.

Figure 3: Percentage most frequent base-calls for HLA-A, B, C, DRB1 and DQB1 loci in NGSengine®.

Each HLA locus is represented in yellow-pink colour, depicting the different exon regions of that locus. (e.g. exon 1 – yellow, exon 2 – pink, exon 3 – yellow etc.). For all exon nucleotide positions, the base variation is calculated for the major base (in blue) and the rest (in red). Patterns of polymorphic positions can be observed at a base variation level of ~50% (high fraction alleles) and ~10% (LF - low fraction alleles). A dot in the low fraction area (LF*) represents a position where the low-fraction allele has a unique base.

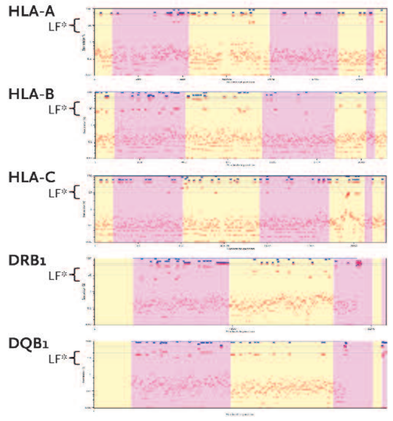


Figure 4: Patient HLA allele fractions (4 alleles) as determined by NGS

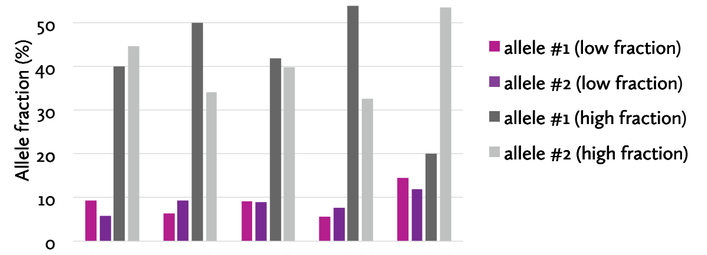


Table 1: NGS-based HLA typing result of the low and high fraction alleles.

Patient Blood DNA			
Low Fraction Alleles		High Fraction Alleles	
A*03:01:01:01	A*03:02:01	A*01:06	A*24:02:01:01
B*39:01:01:01	B*44:02:01:01	B*15:18:01	B*35:02:01
C*12:03:01:01	C*16:04:01	C*04:01:01:01	C*07:04:01
DRB1*04:02:01	DRB1*11:03	DRB1*03:01:01:01	DRB1*13:02:01
DQB1*03:01:01:01	DQB1*03:02:01	DQB1*06:09:01	DQB1*02:01:01

Table 2:

HLA typing results overview							
Relationship	**DNA source**	**HLA typing method**	**HLA-A***	**HLA-B***	**HLA-C***	**DRB1***	**DQB1***
Patient	Blood	SSOP / SBT	Not reportable[1]				n.d.
		NGS	**03:01:01:01, 03:02:01** [2]	**39:01:01:01, 44:02:01:01** [2]	**12:03:01:01, 16:04:01** [2]	**04:02:01, 11:03** [2]	03:01:01:01, 03:01:02 [2]
	Hair root	SSOP / SBT	**03:XX, 03:XX**	**39:XX, 44:XX**	**12:03, 16:04**	**04:02, 11:03**	n.d.
Mother	Blood	SSOP / SBT	**03:XX,** 26:XX	**39:XX,** 55:XX	**12:03,** 03:03	11:01, **11:03**	n.d.
Father	Blood	SSOP / SBT	02:XX, **03:XX**	13:XX, **44:XX**	06:02, **16:04**	**04:02,** 07:01	n.d.
Sibling 1	Blood	SSOP / SBT	**03:XX,** 02:XX	**39:XX,** 13:XX	**12:03,** 06:02	07:01, **11:03**	n.d.
Sibling 2 [3]	Blood	SSOP / SBT	**03:XX, 03:XX**	**39:XX, 44:XX**	**12:03, 16:04**	**04:02, 11:03**	n.d.
Sibling 3 [3]	Blood	SSOP / SBT	**03:XX, 03:XX**	**39:XX, 44:XX**	**12:03, 16:04**	**04:02, 11:03**	n.d.

1) More than 2 alleles detected
2) Low fraction allele typing
3) Sibling 2 and 3 have the same HLA-ABCDR genotype as the patient

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