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Tri-allelic expression of HLA gene in 46,XX/46,XY chimerism

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ABSTRACT

Introduction: Chimerism is defined as coexistence of different cell lines in an individual. 46,XX/46,XY chimerism is very rare and exhibits broad range of clinical phenotypes. Most cases are detected at infancy or younger age due to disorders of sex development, while phenotypically normal cases are incidentally discovered through abnormal blood grouping results or multiple genotypes in HLA.

Objective: Aim was to determine the genetic expression of numerous HLA alleles detected in phenotypically normal 46,XX/46,XY chimerism.

Materials and methods: A patient was admitted for lung transplantation due to end-stage pulmonary disease. Pre-transplantation work-up included blood group typing and HLA DNA typing analyses. Peripheral blood and hair follicle specimens were used to confirm unusual tri-allelic results by high-resolution PCR-SBT. Cytogenetic analyses of karyotyping, FISH and chromosomal microarray were done. Flowcytometry crossmatch analysis was conducted using lymphocytes and anti-HLA sera defined by Luminex panel reactive antibody test (One Lambda, Inc., Canoga Park, CA), to determine antigen expression of HLA alleles.

Results: 46,XX/46,XY chimerism was confirmed through series of cytogenetic analyses. HLA typing of the patient revealed three alleles from HLA-A, -B and -DRB1 loci. Antigen expression of all 3 HLA alleles was confirmed by flow cytometry crossmatch.

Discussion: A case of normal phenotype 46,XX/46,XY chimerism was detected for the first time in Korean patient admitted for lung transplantation. Cytogenetic results were confirmatory for chimerism and HLA typing using PCR-SBT method was able to detect the presence of 3 HLA alleles. Flowcytometry crossmatch was proven sensitive for detecting antigen expression of different cell lines of small proportions.

1. Introduction

Human chimerism is a rare phenomenon of two or more genetically distinct cell lines coexisting in an individual. Several mechanisms of occurrence and phenotypic characteristics have been described [1]. The most common mechanisms are transplantation and transfusion of hematopoietic stem cells, capable of inducing partial chimerism limited to hematopoietic lineage. From prenatal aspect, fertilization errors such as postzygotic fusion, parthenogenesis and postzygotic diploidization are known mechanisms of true chimerism with varying degree of distribution throughout the whole body [2]. 46,XX/46,XY chimera are very rare and most cases are known to be infertile [3]. Most cases are usually detected at young age with developmental problems or ambiguous genitalia [1], while occasional phenotypically normal cases are clued by the presence of ambiguous blood grouping results or discrepant sex chromosome results. Distribution of distinct cell lines varies according to tissue types (i.e. hematopoietic and somatic) and chimerism in gonad could be normal to maintain fertility. Such cases remain undetected and those rarely reported are limited in number from previous literatures [4,5]. Detection of both male and female cells is an apparent evidence of chimerism, although chimerism with same gonosomal constitution is also possible but difficult to detect [6]. Here we present a case of phenotypically normal 46,XX/46,XY chimera, exhibiting tri-allelic expression of the human leukocyte antigen (HLA) gene.

Abbreviations: HLA, Human leukocyte antigen; PB, peripheral blood; SSP, sequence specific primer; SBT, sequence based typing; PHA, phytohemagglutinin; ISCN, International System for Human Cytogenetic Nomenclature; FISH, fluorescence in situ hybridization; CMA, chromosomal microarray; FCM-XM, flowcytometry crossmatch; PRA, panel reactive antibody; MFI, median fluorescence intensity; STR, short tandem repeat; DSD, disorders of sex development; UNOS, United Network for Organ Sharing

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2. Objective

Chimerism often display multiple alleles when genotyped. To assess the clinical influence on solid organ transplantation, expression of tri-allelic HLA genes was evaluated. We hypothesized that phenotypic analysis of tri-allelic HLA gene could affect the outcome of solid organ transplantation, hence determining the phenotypic expression was necessary.

3. Materials and methods

3.1. Blood group typing

Peripheral blood (PB) samples obtained in ethylenediaminetetraacetate containing tube was used for routine ABO blood group typing, using standard blood bank procedures.

3.2. HLA typing

PB and hair follicle samples were used for HLA typing using PCR-sequence specific primer (SSP) and PCR-sequence based typing (SBT) methods. Two HLA PCR-SBT kits (Biowithus and Biosewoom, Seoul, Korea) and PCR-SSP kit (Biosewoom, Seoul, Korea) were used for typing of HLA-A, -B and -DRB1 loci.

For PCR-SSP, the following protocol was used. Genomic DNA at 30–40 ng/μL was mixed with PCR mixture (HLA-A 150 μL and HLA-B 200 μL) and amplified using 10 μL of aliquots by DNA Engine Dyad Thermal Cycler (MJ Research Inc., Waltham, MA). PCR amplification was conducted as 2 min at 95 °C followed by 30 cycles of '20 seconds at 94°C, 50 seconds at 61°C and 40 seconds at 72°C' and finally 2 min at 72 °C. PCR products were aliquoted 5 μL on 2% agarose gel. Electrophoresis was conducted at 200 V for 25 min and agarose gel was dyed with 0.375 μg/mL ethidium bromide solution before identifying with UV transilluminator. Positive bands were then analyzed using BioSewoom SSP INT software v 1.1.1.10 (BioSewoom, Seoul, Korea) for identification of genotypes. PCR-SBT analysis was performed using methods also described previously [7]. In brief, HLA-A, -B and -DRB1 genes were sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and ABI 3730 Genetic Analyzer (Applied Biosystems, Hitachi, Japan). Laboratory-developed software, SBT analyzer v.2.7.4 (Biowithus, Seoul, Korea) was used to analyze the obtained sequences. High-resolution HLA results at 4-digit were obtained by PCR-SBT method.

3.3. Cytogenetic analysis

PB lymphocytes cultured with phytohemagglutinin (PHA) stimulation were harvested for cytogenetic analysis. Chromosome analysis was performed by standard GTL-banding techniques, and aberrations were described according to the International System for Human Cytogenetic Nomenclature (ISCN 2016) guideline [8]. A fluorescence in situ hybridization (FISH) study was also performed using PB and buccal swab specimens for comparison of chimerism distribution among different tissues. Metaphase chromosomes prepared from PHA-stimulated lymphocytes were analyzed using Vysis CEP X SpectrumOrange/Y SpectrumGreen direct labeled fluorescent DNA probe kit (Abbott Molecular, IL, USA). Chromosomal microarray analysis (CMA) was conducted on PB specimen using Affymetrix CytoScan 750 K array kit (Thermo Fisher Scientific, MA, USA) according to manufacturer's instructions. SRY PCR was conducted for resolving discrepancy between CMA result and clinical information (sex mismatch).

3.4. Flowcytometry crossmatch for confirmation of antigen expression of HLA alleles

Flowcytometry crossmatch (FCM-XM) was conducted to determine the antigenic expression of HLA alleles. Anti-HLA sera defined by Luminex panel reactive antibody (PRA) analysis (One Lambda, Inc., Canoga Park, CA) were selected for those with antibody median fluorescence intensity (MFI) value higher than 5000. FCM-XM was conducted using BD FACSCanto-II system (BD Biosciences, San Jose, CA). Patient lymphocytes were incubated with sera containing HLA Abs, adding fluorochrome-conjugated anti-human globulin before analysis. For T-cell specific detection, fluorochrome-conjugated CD3 monoclonal antibody was used. MFI ratio - sample MFI divided by negative control MFI - > 2.0 was interpreted as positive crossmatch.

3.5. Ethics

All experiments were conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). This study was approved by the institutional review board (AMC 2018–1325).

4. Results

A 65-year-old man was admitted for idiopathic pulmonary fibrosis (IPF) with concomitant usual interstitial pneumonia. Other than previous history of tuberculosis lymphadenitis 5-years before admission, previous medical history was non-specific. As IPF is an end-stage pulmonary disease, the patient proceeded with transplantation workup. The result of blood typing result was Aw, showing mixed field agglutination. Initial HLA typing of HLA-A, -B and -DRB1 loci by PCR-SBT method, revealed three alleles from HLA-DRB1 locus. The finding was initially ruled out for possibilities of sample contamination, DNA contamination and prior history of transfusion or transplantation. Presence of three HLA-A, -B and -DRB1 alleles was confirmed with additional PCR-SBT and -SSP analyses (Fig. 1). Subsequent analysis with PCR-SSP and high-resolution PCR-SBT typing using both PB and hair follicle specimens showed matching results. HLA typing of the family was also conducted, and the results are summarized in a pedigree (Fig. 2). HLA typing results are explained by haplotypes based on frequencies reported from Korean population [9].

After confirmation of additional HLA allele by HLA typing, the presence of additional HLA allele was evaluated for cytogenetic origin. Further analyses included karyotyping, FISH and chromosomal microarray. Karyotype of the patient was 45,X [2]/46,XX[45]/46,XY [3] (Fig. 3). 45,X [2] was regarded as an age-related loss of sex chromosome. FISH result was consistent with the chromosome result (Fig. 4), but showed different distribution in PB and buccal swab specimens (PB, nuc ish(DXZ1x1) [21]/(DXZ1x2)[431]/(DXZ1,DYZ1)x1[48]; buccal, nuc ish(DXZ1,DYZ1)x1[88]/(DXZ1x2) [12]). CMA result was also concordant with other cytogenetic results but showed only arr(1–22,X)x2. Although additional SRY PCR result was positive, resolution of CMA was limited for detecting presence of 46,XY cell lines. The patient was confirmed as whole body 46,XX/46,XY chimera.

FCM-XM results conducted to determine the antigen expression of the HLA alleles is summarized in Table 1. Tested anti-sera were those identified positive with HLA antibodies against A24, A30, A33 and DR15 from previous Luminex PRA analyses. Sera were selected to have only one corresponding antibody (MFI > 5000) to HLA antigens of the patient and controls. FCM-XM results using patient lymphocytes were all positive, indicating expression of all three HLA alleles. Control lymphocytes displayed anticipated positive and negative results.

In summary, a rare case of normal phenotype 46,XX/46,XY chimerism was found in a Korean patient, cytogenetically confirmed using different specimens of PB, buccal swab and hair follicle samples. While the distribution of chimerism varied between different tissues, the patient was a phenotypically normal male, showing normal body proportions and normal fertility. Most clinical features were non-specific other than end-stage pulmonary disease, IPF. All three HLA alleles were confirmed expressing HLA antigens by FCM-XM.

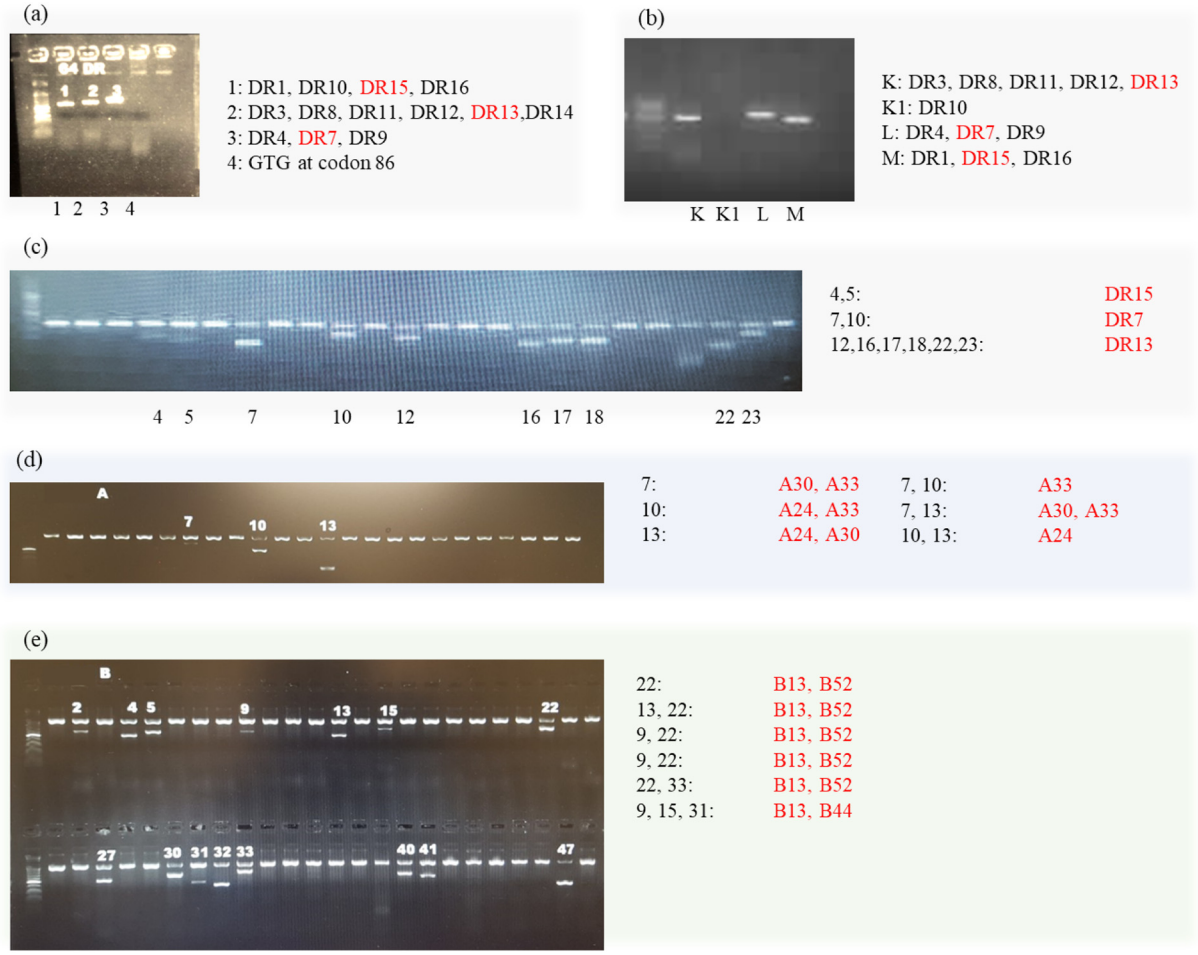

Fig. 1. Initial PCR-SBT and SSP analyses of HLA-DR showing results concordant with three HLA-DR alleles.
(a) PCR-SBT result from AVITA cross kit (Biowithus, Seoul, Korea) showing three bands. (b) Retest with different PCR-SBT kit (BioSewoom, Seoul, Korea) also showing three bands. (c)–(e) PCR-SSP result was concordant with presence of three HLA-A, -B and -DR alleles.

5. Discussion

Occurrence of chimerism can be explained by several mechanisms such as fusion of two different zygotes, dispermic fertilization of an oocyte and dispermic fertilization of a parthenogenetically activated oocyte [2]. Short tandem repeat (STR) marker analysis of the family is often useful for assessing the underlying mechanism. Unfortunately, family study to determine the origin of additional HLA allele was not possible but results indicated that chimerism and tri-allelic expression was limited to the patient. As true chimera varies in its degree of distribution among different tissues, FISH results using PB and buccal specimens showed different ratios in this patient.

Chimerism is suspected when abnormal blood group types or karyotypes are detected. Chimerism is detected early when displaying disorders of sex development (DSD) such as ambiguous genitalia but otherwise will remain undetected in phenotypically normal individuals. Such phenotypically normal 46,XX/46,XY chimera were reported before, although in limited numbers [10–18]. Most are incidentally discovered during blood group typing presenting with ambiguous results or occasionally during transplantation donor work-up. While previous cases were reported from various populations such as Taiwanese, Chinese and also from North America [10,17,19], this is the first Korean case of normal phenotype 46,XX/46,XY chimera showing tri-allelic expression of HLA alleles.

FCM-XM was used to demonstrate antigen expression of detected HLA alleles. FCM-XM was proven of highly sensitive for detecting the presence of cell lines in small proportions, compared to CMA which was unable to detect cell lineages of 46,XY, even though SRY PCR was positive. Therefore, the capability of FCM-XM as a method for demonstrating chimerism was confirmed. A previous report also utilizes FCM-XM for identifying blood group chimera using red blood cells, however HLA genotyping results by PCR-SBT were normal in HLA-A, -B and -DRB1 loci [20].

Previously suggested from trisomy 6p cases – 6p where *MHC* is localized within the chromosome - additional presence of the HLA allele does not seem to impair or augment immune function [21]. Also immunological tolerance can be observed in chimeras due to presence of two or more cell lines present at early stage of development [1]. No signs of immune deficiency or autoimmune features were exhibited, although the impact of tri-allelic expression of HLA on the prognosis of transplantation is to be determined. The patient received lung transplantation from a deceased donor (HLA type; A26/33, B35/58 and DR13/14). While development of chimerism after allogenic bone marrow transplantation has prognostic implications, its presence prior to solid organ transplantation is an ongoing matter of interest.

Presence of an additional HLA allele was problematic during reporting HLA typing result to organ allocation system, Korean Network for Organ Sharing (KONOS), which is the equivalent of United Network for Organ Sharing (UNOS) of United States. Both KONOS and UNOS systems allow two HLA alleles per loci to be reported, therefore listing an additional allele is not supported by the current system. Had the organ of interest in this case been a kidney, much serious consideration would have been necessary before transplantation. In this case, two alleles per loci were reported based on their higher haplotype

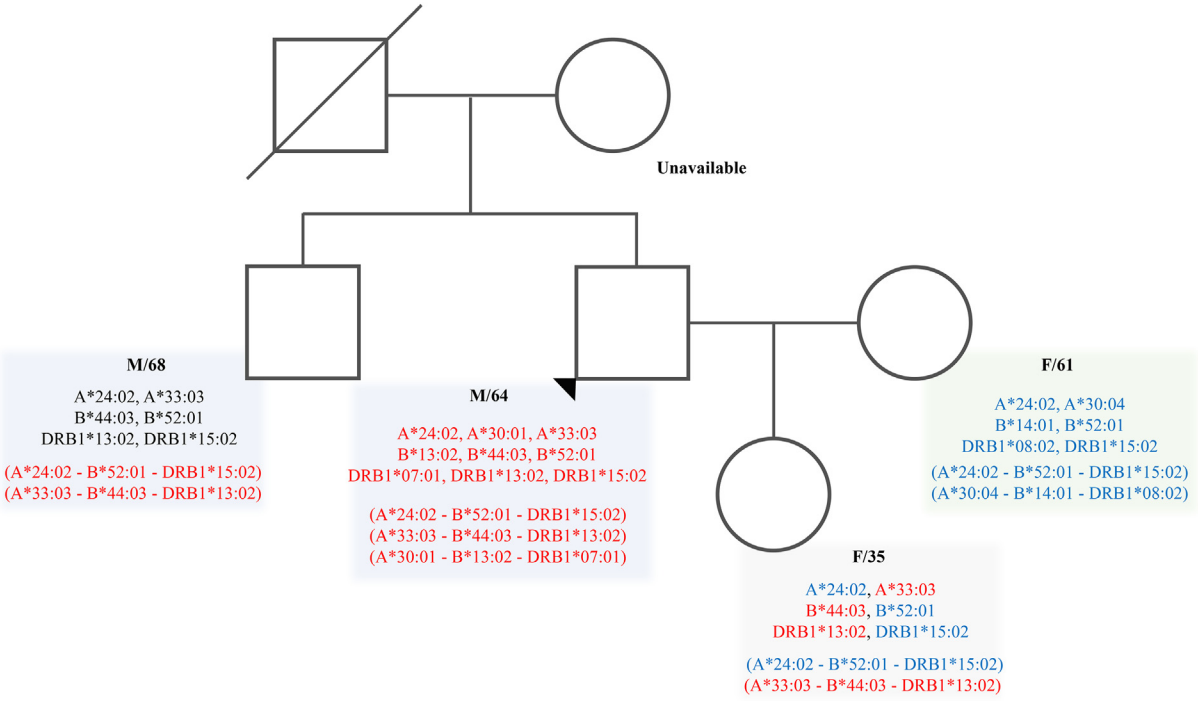

Fig. 2. HLA typing results and pedigree of the family including the proband (indicated by arrow). HLA-A, -B and –DR haplotypes are described below in each individual. Haplotype frequencies of A*24:02-B*52:01-DRB1*15:02, A*33:03-B*44:03-DRB1*13:02 and A*30:01-B*13:02-DRB1*07:01 were 2.10%, 4.60% and 2.19% respectively [7].

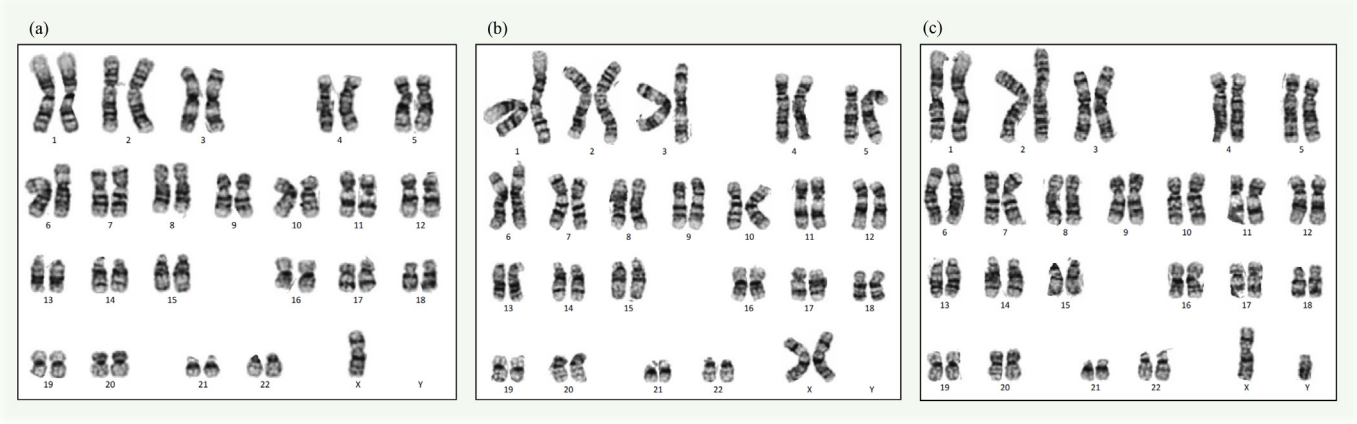

Fig. 3. Karyotype of the proband using peripheral blood specimen; 45,X[2]/46,XX[45]/46,XY [3].
(a) 45,X (b) 46,XX (c) 46,XY.

frequencies, as previously reported in the Korean population [9,22].

First Korean case of phenotypically normal 46,XX/46,XY chimera was successfully identified by cytogenetic analyses and HLA typing. FCM-XM was useful for determining antigen expression of HLA alleles, demonstrating its high sensitivity for detecting a small population of different cell lineage. Analysis of rare normal phenotype 46,XX/46,XY chimerism case using methods of different aspect provided a comprehensive understanding of how chimerism in clinical practice can be approached.

Conflict of interest

Authors declare no conflict of interest.

Author contributions

Conception and design of study: JY, HBO.

Data acquisition, analysis and interpretation: JY, SHH, DHK, EJS, HBO.

Final approval of manuscript: JY, SHH, DHK, EJS, HBO.

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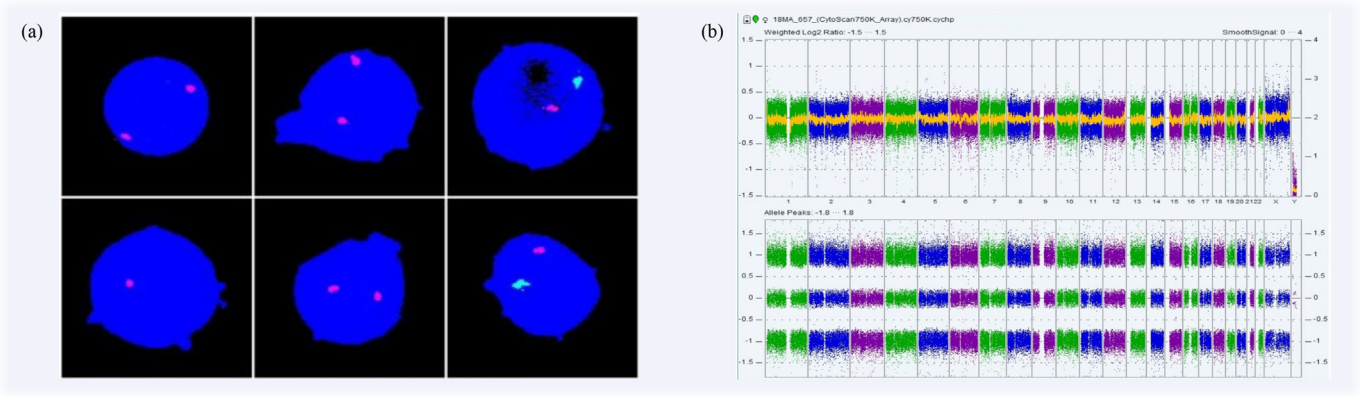

Fig. 4. Additional cytogenetic analyses of FISH and CMA.
(a) FISH results: PB: nuc ish(DXZ1x1)[21]/(DXZ1x2)[431]/(DXZ1, DYZ1)x1[48]. Buccal swab: nuc ish(DXZ1, DYZ1)x1[88]/(DXZ1x2)[12].
(b) CMA result arr(1-22, X)x2. SRY PCR result was positive (data not shown) using same specimen.

Table 1
Flowcytometry crossmatch analysis.

		HLA antibodies				Allele expression
		A24	A30	A33	DR15	
Patient	***A*24:02***–B*52:01–***DRB1*15:02***	P			P	Y
	A*30:01–B*13:02–DRB1*07:01*		P			Y
	A*33:03–B*44:03–DRB1*13:02*			P		Y
Controls	***A*30:01***–B*13:02–DRB1*07:01*	N	P	N	NT	Y
	*A*02:01–B*27:05–DRB1*01:01*					
	A*33:03–B*58:01–DRB1*13:02*	P	NT	P	N	Y
	A*24:02–B*52:01–DRB1*03:01*					
	*A*26:03–B*15:01–***DRB1*15:02***	NT	N	NT	P	Y
	*A*26:02–B*40:06–DRB1*09:01*					

Results are confirmative of the antigenic expressions of patient and three different control lymphocytes used in analyses. Alleles are displayed based haplotype frequencies reported in Korean population.
*P, positive; N, negative; NT, not tested;

HLA-related experiments.

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