Case Report

Loss of D expression associated with hematologic disease progression: a case report and review of the literature

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We report a case of a 61-year-old male patient with a complex hematologic history including pre-B acute lymphocytic leukemia, allogeneic stem cell transplant, and newly treated colon cancer followed by diagnosis of high-risk myelodysplastic syndrome (MDS), who exhibited abolishment of D antigen production. The patient's red blood cells (RBCs), which originally typed as group A, D+, again typed as group A, D+, after receiving stem cells from a female donor with the same blood type. The reactivity of the patient's RBCs was strong when tested with anti-D reagent until he was treated for colon cancer. Within 6 months of diagnosis of cancer, he developed a mixed-field D typing result followed by a complete D– phenotype over the course of a few weeks, coinciding with the new diagnosis of MDS and initiation of immunosuppressive therapy. Polymerase chain reaction (PCR) testing revealed no weak or partial D variants, and subsequent Sanger sequencing confirmed the presence of a conventional *RHD* gene. A more focused analysis by chromosomal microarray identified deletions involving the *RHD* locus, supporting the hypothesis that active MDS disrupted gene expression. The patient received another stem cell transplant, this time from a group AB, D+ male donor. In a short period of time, MDS re-emerged along with re-identification of microarray mutations encompassing *RHD* in female cells (from the first donor) even after the second stem cell transplant. However, D expression remained strong, underscoring the presence of enough male donor *RHD* expression to maintain the D+ phenotype. ***Immunohematology* 2025;41:80–83. DOI: 10.2478/immunohematology-2025-012.**

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ABO and D typing are a routine part of testing in cases of hematologic malignancy before stem cell transplantation. It is well described that hematologic—more specifically, myeloid—malignancies may cause discrepancies in ABO testing. *RHD,* on the other hand, seems more stable and less prone to such interferences.

We describe a 61-year-old male patient with a history of allogeneic stem cell transplant (from a D+ female donor) who later developed colon cancer followed by myelodysplastic syndrome (MDS). During follow-up over a relatively short period, his D expression progressively declined from 4+ in strength to 3+ and eventually became undetectable. Genetic analysis confirmed the presence of the conventional *RHD* gene in his white blood cells (WBCs); however, additional microarray testing identified a deletion in chromosome 1p affecting *RHD* expression in his red blood cells (RBCs). It was concurred that this acquired chromosomal abnormality, likely caused by MDS, gradually diminished his D expression, ultimately leading to its complete loss.

The patient had a second allogeneic stem cell transplant from a group AB, D+ male donor with re-identification of some of the MDS-related microarray mutations shortly after in female cells from the first donor. However, his D typing has been persistently 4+ since the transplant.

In contrast to ABO, which can be used to predict early emergence of myeloid disease, D expression is reasonably stable and persists in hematologic diseases with rare exceptions. The D phenotype is often the result of a hemizygous *RHD* genome, since the complete deletion of *RHD* is common among the extant population. Thus, deterioration or loss of heterogeneity of the distal end of chromosome 1p in the case of a hemizygous *RHD*-positive individual may result in the loss of D expression.

Case Report

We present a 61-year-old male patient who underwent allogeneic stem cell transplantation in 2015 from a female relative to treat pre-B acute lymphoblastic leukemia. His post-transplant course was complicated by deep vein thrombosis and chronic obstructive pulmonary disease. He had been under regular follow-up since 2015 with fluorescence in

situ hybridization (FISH) and bone marrow studies, which consistently showed normal findings. In 2023, he was diagnosed with colorectal cancer and subsequently underwent a colectomy followed by chemotherapy.

As in prior years, FISH performed in early 2024 showed a 100 percent XX (female donor) chromosomal pattern. However, bone marrow examination at that time revealed new abnormalities showing deletion of 5q and monosomy 7 in 66.5–74 percent of bone marrow cells and three or four copies of both 8q and 20q in 15.5 percent of bone marrow cells. Bone marrow biopsy revealed high-risk MDS with loss of p53. The patient started chemotherapy (azacitidine), this time for MDS, and was evaluated for a second stem cell transplant.

The patient was seen by a geneticist 1 month later for possible Lynch syndrome due to loss of MSH2 and MSH6 in his colon resection. Single gene analysis of multiple selected genes was significant for an *IKZF1* variant that is likely pathogenic and is associated with both common variable immunodeficiency and susceptibility to acute lymphocytic leukemia. The patient's testing was negative for Lynch syndrome.

Blood bank testing revealed his birth ABO/D type to be group A, D+, the same as his female donor's type. His blood sample was typing as group A, D+ with 4+ reactivity with anti-D reagent (most recent typing ~8 months before MDS diagnosis) until he typed as mixed-field 1 month after MDS diagnosis on a gel card (DG Gel 8; Grifols, S.A., Barcelona, Spain) that used two different forms: one with IgM clone P3X61 and the other with P3X61 and ESD1M. Because of the discrepancy, the testing was repeated with a new sample 10 days later at the bench using Grifols tube reagent (Anti-D [RH1] IgM/IgG; Grifols) that consisted of a blend of P3X61, P3X21223B10, P3X290, and P3X35. The result was 0 (negative). The patient was started on immunosuppressive therapy (fludarabine and melphalan) around this time in preparation for the second stem cell transplant. His sample was sent to a national immunohematology reference laboratory for evaluation of a variant *RHD* by sequence-specific polymerase chain reaction (PCR) with endpoint fluorescence detection. The testing did not reveal any evidence of a weak or partial D genotype. To screen for abnormalities related to RhCE antigens, the patient's RBCs were serologically typed at our hospital for the first time and found to be negative for C and D and positive for c and e. Per the medical director's decision, the patient's D type was kept as D+ in the blood bank's record system based on these results. *RHCE* gene testing was not performed.

The *RHD* genotyping was repeated at another national immunohematology reference laboratory using peripheral blood and buccal swab to differentiate donor- versus recipient-related causes. This lab performed extended D testing by Sanger sequencing using WBCs from peripheral blood, reported a hemizygous status of *RHD*01/RHD*deletion* (*RHD*01N.01*), and predicted a D+ phenotype, confirming the initial reference laboratory's results. The same hemizygous genotype was identified by the buccal swab, confirming that both the patient and the first donor had conventional *RHD*.

Finally, microarray testing was performed on a bone marrow specimen at our institution using a whole-genome DNA analysis method (Affymetrix OncoScan Assay; Applied Biosystems, Thermo Fisher Scientific, Santa Clara, CA), which confirmed clonal copy number abnormalities in donor cells. These abnormalities included chromothripsis involving chromosome 1p with a heterozygous deletion at 1p36.22p35.2 encompassing the *RHD* and *RHCE* gene loci (Fig. 1), but not *RHAG,* which is located on chromosome 6p.

The patient received another allogeneic stem cell transplant from a group AB, D+ male donor. Within 3 months of the transplant, the same chromothripsis in the *RHD* region along with p53 mutation in the female donor's cells was detectable by FISH. At this time, chimerism analysis performed using short tandem repeats showed 85 percent engraftment. His bone marrow biopsy revealed hypercellular marrow with some blasts. With treatment changes over his course, 10 months after his second transplant, the engraftment increased to 100 percent myeloid cells. The bone marrow was hypocellular. FISH could not be performed because of hypocellularity of the sample.

At 14 months after the second transplant, the patient is under close follow-up, and his D typing reactivity has persistently remained as 4+.

Discussion

Although losing ABOH antigens is described multiple times in the literature,[1–5] disappearance of D is relatively rare. Some studies mention mosaicism causing mixed-field reactions or showing mixed populations.[6–11] A case of acute myeloid leukemia mentions loss of heterozygosity by a mutation in the *RHD* region, displaying similar characteristics as our patient's workup.[12] A previously reported case described a patient with chronic myelomonocytic leukemia initially typed as D+C+c+E-e+, and during the active phase

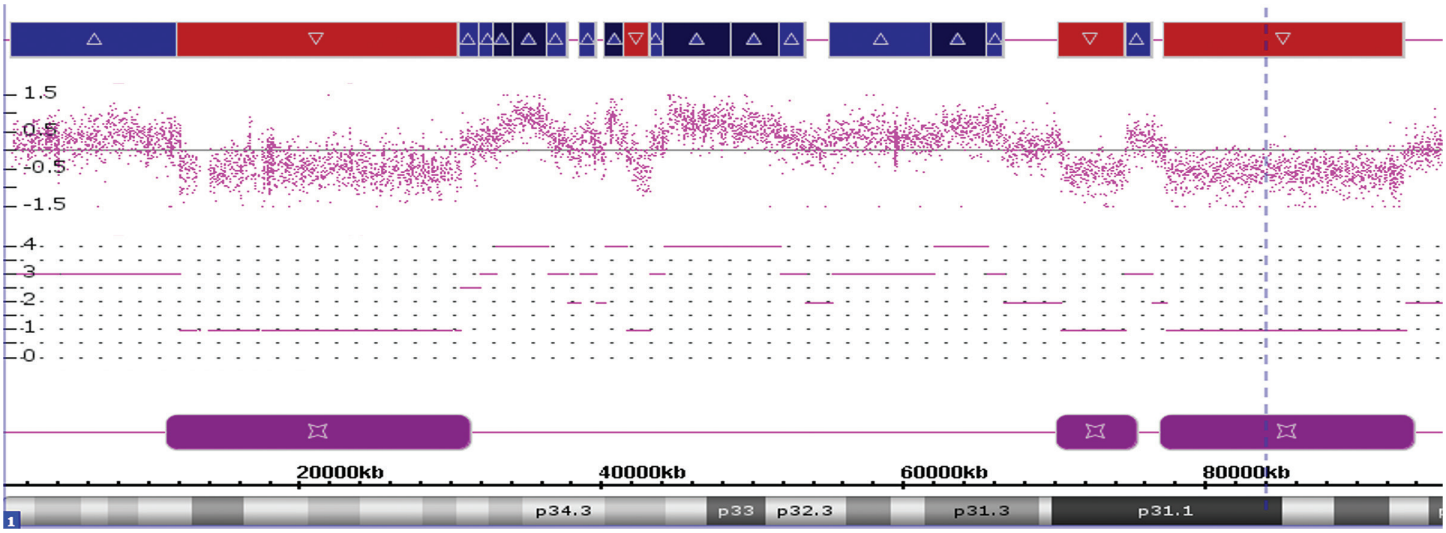

Fig. 1 Microarray testing result shows genetic variations on the p arm of chromosome 1 from the bone marrow specimen containing engrafted cells of donor 1. Upward arrowheads (light and dark blue) show copy number gains and duplications; downward arrowheads (red) represent deletions. The dashed vertical line highlights the deletion at 1p36.22p35.2 encompassing the *RHD* gene, suggesting a genetic cause for the loss of D expression.

of the disease, the expression of D and C was lost, despite the confirmed presence of *RHD* and *RHCE* genes by PCR.[13] In that case report, it was suggested the disease could be inhibiting the expression of the *RHD* gene. Another case with aplastic anemia had near total loss of D typing with a normal *RHD* genotype.[14] A more recent case report identified loss of the *RHD* gene as a result of myeloid neoplasm.[15] Finally, the most extensive work done on this topic was a study that described nine patients, four of whom had complete or progressive loss of D expression with a normal *RHD* genotype.[16] Two of these patients had bone marrow abnormalities (acute myeloid leukemia and myelofibrosis), and one had colon cancer.

Using all the information from the literature, it could be derived that D loss occurs either by inhibition and/or downregulation and rarely by deletion[15] of the available *RHD* gene. A less severe type of Rh_{null} is Rh_{mod}, which is caused by a mutation in Rh-associated glycoproteins (RhAG) or CD47 leading to decreased or diminished expression of Rh proteins.[17–19] Loss of RhAG can lead to stomatocytosis, spherocytosis, and hemolytic anemia due to cytoskeleton abnormalities and cation leak.[18,20,21] In one report, Mu et al.[22] report a case of decreased *RHD* expression caused by a single mutation in *RHAG* with spherocytes and an intact *RHCE* gene.

In our case, the initial reference laboratory evaluated for certain weak D and partial D genotypes and did not find them. The second reference laboratory performed Sanger sequencing and confirmed the presence of the *RHD* gene. However, because Sanger sequencing was done using nucleated WBCs, possible anomalies in the myeloid lineage were left unidentified. Finally, microarray identified deletions in the region including the *RHD* gene in myeloid cells. With this information, it was clear that D production was abolished over a short period of time due to MDS-related mutations in the gene itself. We believe the most interesting part of our case was the persistence of strong D typing without mixed-field reactivity despite presence of the disease in the cells remaining from the first (female) donor after the second stem cell transplant when the engraftment was not yet 100 percent. This finding is in contrast to ABO, which causes antigen typing discrepancies when the disease is in its early stages,[5] sometimes even before FISH shows a discrepancy. Presence of some of the *RHD* gene from the second (male) donor was sufficient to produce strong D expression.

Conclusion

Unlike ABO antigens that are very sensitive to aberrations in the *ABO* gene, D expression will remain undisturbed unless there are major mutations/deletions effecting the function of the *RHD* gene.

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