The Usefulness of X-linked Polymorphic Loci as Gene Markers to Track X Allele and Chimerism in a Post-Allogeneic Peripheral Blood Stem Cell Transplant Patient with Wiskott-Aldrich Syndrome

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Abstract

Wiskott-Aldrich syndrome (WAS), an X-linked recessive disorder, is characterized by progressive T-cell immunodeficiency. Laboratory findings generally demonstrate reduced response to T-cell mitogens, markedly decreased serum concentration of IgM, and thrombocytopenia with small platelet volume. Allogeneic HLA-matched sibling bone marrow transplantation (BMT) can correct this disorder. We report the usefulness of X-linked polymorphic loci to detect X-allele gene tracking among WAS siblings and chimerism between a pre- and post-allogeneic matched sibling peripheral blood stem cell transplantation (PBSCT). A 3 $^1/_2$ year old boy with clinical and laboratory findings consistent with WAS underwent allogeneic matched sibling PBSCT. We used *BclI* restriction fragment length polymorphism (RFLP) of intron 18 of factor VII gene and *MseI* RFLP of the 5' flanking region of factor IX gene to detect X-allele gene tracking among siblings and family members and chimerism in patients between pre-and post-allogeneic matched sibling PBSCT. We were able to demonstrate that determination of *BclI* and *MseI* RFLP can be employed to recognize the difference in X-allele genes between the recipient and donor for allogeneic matched sibling PBSCT. The authors also were able to demonstrate that these polymorphic loci can detect full chimerism of donor hematopoietic cells in recipient blood after allogeneic PBSCT. This finding was correlated with improvement of post-PBSCT clinical and laboratory findings. *BclI* and *MseI* RFLP associated with X-chromosome can effectively track X-allele, detect carrier state, and demonstrate the different X-allele among male siblings, and chimerism of hematopoietic cells between donors and recipients in a setting of allogeneic matched sibling BMT or PBSCT for X-linked hereditary diseases such as Wiskott-Aldrich syndrome.

Key word : X-linked Polymorphic Loci, Chimerism, Allogeneic Transplant, Wiskott-Aldrich Syndrome

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Wiskott-Aldrich syndrome (WAS), an X-linked recessive disorder, is characterized by progressive T-cell immunodeficiency, impaired anti-polysaccharide antibody production, eczema, and thrombocytopenia[1,2]. Laboratory studies demonstrated variable but generally reduced response to T-cell mitogens and allogeneic cells, markedly decreased serum concentration of IgM and isoagglutinins, normal serum concentration of IgG_2, and small platelet volume associated with impaired platelet aggregation. The primary defect in X-linked WAS maps to the proximal short arm of X-chromosome (X p11.23)[3].

Full correction of both lymphoid and platelet abnormalities associated with WAS were induced by administering an HLA-matched marrow graft after treating the patient with myelosuppressive and immunosuppressive regimen[4]. Although direct methods of detection for tracking the specific mutation are likely to become the method of choice in developed countries, the technically simpler polymorphic based process of carrier analysis is likely to be favored in other parts of the world. The authors demonstrated the usefulness of X-linked polymorphic loci (*BclI* restriction fragment length polymorphism (RFLP) in intron 18 of factor VIII gene and *MseI* RFLP in the 5' flanking region of factor IX gene)[5,6] as markers to tag a defective allele, and detect chimerism in post-allogeneic matched sibling peripheral blood stem cell transplantation (PBSCT) in a patient.

Case presentation

The diagnosis of WAS was made in January 1998 in a 3 year, 6 month old boy presenting with several bouts of bloody diarrhea, respiratory tract infection, otitis media, persistent eczematous rash at face, trunk, and extremities, and persistent thrombocytopenia since birth. Physical examination revealed generalized lymphadenopathy. The patient and his HLA-identical sibling brother were evaluated. We evaluated CD4 and CD8 lymphocyte number and ratio in this patient which demonstrated CD4: 3,900 cells/mm^3 (47%), CD8 680 cells/mm^3 (8%) and CD4/CD8 ratio: 5.9. This finding is consistent with WAS which usually shows low level of CD8 count.

Laboratory profiles are shown in Table 1.

The authors tested T-cell function in this patient with panel antigens (Merieux®) consisting of proteus 8, trichophyton, candida, tetanus toxoid, diptheria, streptococcus, tuberculin, and glycerin control. The result was non-reactive to all antigens.

He underwent an allogeneic PBSCT in February, 1998. The conditioning regimen consisted of busulfan 4 mg/kg/day for 4 days and cyclophosphamide 50 mg/kg/day for 4 days which was continued after busulfan administration. Peripheral blood stem cells were collected from his 6-year-old brother and infused to the patient after conditioning regimen. Peripheral blood stem cells were mobilized with granulocyte colony stimulating factor (G-CSF) at doses of 7.5 μg/kg/day for 4 days before mobilization. The number of infused mononuclear cells and CD 34 cells was 5.1 x 10^8 cells/kg and 6.27 x 10^6 cells/kg respectively. Graft *versus* host disease (GVHD) prophylaxis was carried out with methotrexate and cyclosporin A. Hematologic recovery was prompt with polymorphonuclear count greater than 0.5 x 10^9/ L on day +11 and stable platelet engraftment on day +30. Mean platelet volume was persistently greater than 6.0 fL after platelet engraftment. At present (day + 180 post PBSCT), platelet count was greater than 150 x 10^9/L. The patient did not develop acute or chronic GVHD. Serial hematologic findings and immunoglobulin levels were followed after PBSCT procedure as shown in Table 1.

Table 1. Laboratory findings pre- and post-PBSCT.

Laboratory data	Pre-PBSCT		Day +44 post-PBSCT	Day +174 post-PBSCT	Day +242 post-PBSCT
	Recipient	Donor			
Platelet (x10^9/L)	12.0	462	60.0	152	169
Mean platelet volume (fl)	4.5	6.3	7.0	6.0	6.1
IgG (mg/ml)	13.2	12.7	14.3	13.2	22.0
IgA (mg/ml)	1.9	1.47	1.29	1.40	1.09
IgM (mg/ml)	0.32	2.67	0.51	2.01	1.75

MATERIAL AND METHOD

In order to evaluate X-allele tracking in the patient's family and chimerism after allogeneic peripheral blood stem cell transplant, we selected two X-linked polymorphic loci of intron 18 of factor VIII gene with *BclI* restriction enzyme and 5' flanking region of factor IX gene with *MseI* restriction enzyme. These two X-linked polymorphic loci were our in-house markers for hemophiliacs and were informative in this patient's family.

The buffy coat was collected from 10 ml of whole blood drawn into EDTA. The genomic DNA was extracted with Nucleon® kit. The genomic DNA was amplified by PCR using two sets of primers for intron 18 of factor VIII gene (5'TAA AAGCTTTAAATGGTCTAGGC-3', 5'-TTCGAA TTCTGAAATTATCTTGTTC-3') and 5' flanking region of factor IX gene (5'-GATAGAGAAACTG GAAGTAGACCC-3', 5'TTAGGTCTTTCACA GAGTAGAATTT-3').

The PCR reaction mixture of intron 18 of factor VIII gene was 50 µl volume consisting of 100 µM dNTPs, 1 unit Tag polymerase, 16.6 mM NH_4SO_4, 67 mM Tris HCl pH 8.8, 5 mM $MgCl_2$, 10 mM ß mercaptoethanol, 100 µg/ml BSA, and 75 ng of each primer with 250 ng of genomic DNA. The PCR reaction was done with condition initial denaturation at 92°C for 7 minutes. Thermocycling of denaturation at 92°C for 1 minute and annealing at 60°C for 4 minutes was carried out for a total of 30 cycles with a final extension at 60°C for 7 minutes. The PCR reaction mixture for 5' flanking of factor IX gene was similar to the intron 18 of factor VIII gene except for 200 µM of dNTPs, and 250 ng of each primer. The initial denaturation was at 94°C for 5 minutes. Thermocycling of denaturation at 91°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 2 minutes without final extension. After amplification, 20 µl of amplified products were digested with *BclI* restriction enzyme for intron 18 of factor VIII gene and *MseI* restriction enzyme for 5' flanking region of factor IX gene. The digested PCR products were separated by 12 per cent polyacrylamide gel electrophoresis in 1 x TBE buffer and stained with silver stain technique.

RESULTS

Gene tracking in Wiskott-Aldrich family:

The pedigree of the patient's family is summarized in Fig. 1.

Genomic DNAs from his maternal grandfather, maternal grandmother, two aunts and one uncle from his mother's side, father, mother, his brother who was his donor for marrow stem cells, and the patient were amplified and digested according to methods described above. The results of both polymorphic loci are shown in Figs. 2 and 3. The

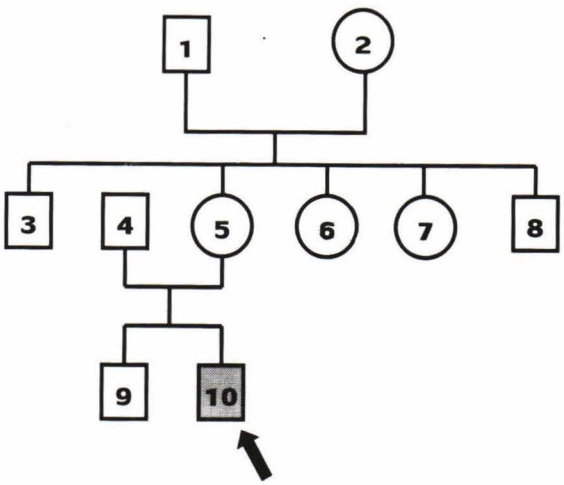

Fig. 1. The pedigree of the patient's family. No. 9 represents brother sibling and No. 10 represents WAS patient.

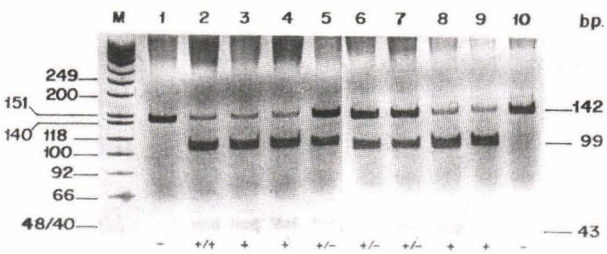

Fig. 2. *BclI* RFLP of intron 18 of factor VIII gene in the patient's family. The amplified product of intron 18 of factor VIII gene is 142 base pairs. If polymorphism is present, the amplified product will be digested by *BclI* restriction enzyme into 99 and 43 base pairs. Lane 9 represents brother sibling polymorphism and lane 10 represents WAS patient polymorphism.

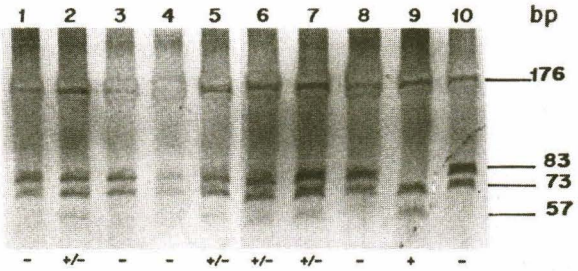

Fig. 3. ***Mse*I RFLP of the 5' flanking of factor IX gene in the patient's family. The amplified products of the 5' flanking region of factor IX gene are 176, 83, and 73 basepairs. If polymorphism is present, the amplified product of 83 basepairs will be digested by *Mse*I restriction enzyme in to 57 and 26 base pairs. Lane 9 represents brother sibling polymorphism and lane 10 represents WAS patient polymorphism.**

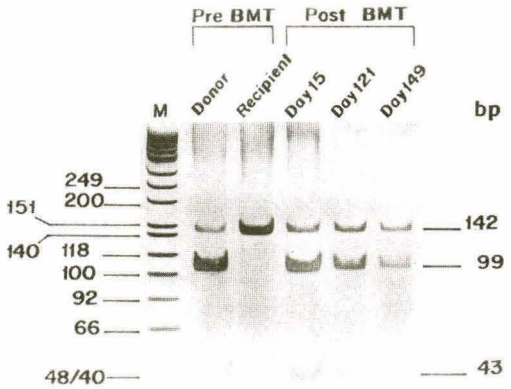

Fig. 4. ***Bcl*I RFLP to detect chimerism pre- and post-PBSCT in recipient.**

amplified product of intron 18 factor VIII gene is 142 base pairs. If polymorphism is present, the amplified product will be digested by *Bcl*I restriction enzyme into 99 and 43 basepairs. Additionally, the amplified products of the 5'flanking region of factor IX gene are 176, 83, and 73 basepairs. If polymorphism is present, the amplified product of 83 basepairs will be digested by *Mse*I restriction enzyme into 57 and 26 base pairs.

Chimerism post allogeneic PBSCT:

Genomic DNAs from the patient pre- and post-allogeneic PBSCT and his sibling were amplified and digested according to the methods described above. The results depicted in Fig. 4 showed that there was a full chimerism of donor hematopoietic cells in recipient blood post PBSCT.

DISCUSSION

WAS can be fully corrected by administering an HLA-matched marrow graft after treating patients with myelosuppressive and immunosuppressive regimen. The clinical diagnosis can be readily entertained from persistent thrombocytopenia with small platelet size, frequent infection, normal IgG, IgA levels but low IgM level and abnormal T cell function. Although the WAS gene has been cloned and well recognized, mutation detection is not widely available because there are several mutation loci which must be screened. A polymorphic dinucleotide repeat at DXS6940, adjacent to the WAS gene, has been characterized[7]. However, we have already set up several X chromosome polymorphisms associated with factor VIII gene and factor IX gene which are routinely used for gene tracking among hemophiliacs and their family members. These informative markers are helpful for carrier detection and prenatal diagnosis for hemophilia A and B[8].

In this report WAS patient and family, the *Bcl*I and *Mse*I RFLP were found to be informative. Therefore, both polymorphisms were used for gene tracking among the family members. We demonstrated that the X-allele from this male patient was different from his brother who was his donor for matched sibling PBSCT. Additionally, they were also used for chimerism detection post PBSCT. The chimerism of hematopoietic cells in the recipient was changed to donor after PBSCT. This finding was correlated with the improvement of both clinical and laboratory findings.

In general, the chimerism can be detected by the linkage analysis of restriction fragment length polymorphism, microsatellite or variable number tandem repeats of different loci on different chromosomes. The DNA analysis can be performed by Southern blot or DNA amplification. If the sex of donor and recipient are different, X and Y chro-

mosomes can be differentiated by either cytogenetic based techniques (conventional or fluorescence *in situ* hybridization technique) or DNA amplification.

However, the DNA amplification is preferable because is practical, simple, and reliable. Therefore, searching for the informative genetic marker for tracking among the family member is essential.

*Bcl*I polymorphism of the intron 18 of factor VIII gene and *Mse*I polymorphism of the 5' flanking region of factor IX gene are informative 34 per cent[5] and 33 per cent[6] in Thai populations, respectively. The combination of both *Bcl*I and *Mse*I polymorphism resulted in a heterozygosity rate of 56 per cent in Thai populations. Therefore, both polymorphisms are useful X linked polymorphic loci for tracking X allele among the X linked hereditary disease. They are also helpful in detection of chimerism in the recipient post PBSCT or BMT. However, meiotic crossing over in X allele should be considered in the laboratory interpretation. Therefore, more than one informative genetic marker should be included in the gene tracking of X allele and chimerism study.

In conclusion, *Bcl*I and *Mse*I RFLP associated with X chromosome are helpful in tracking X allele and chimerism in a post allogeneic PBSCT or BMT patient with WAS. It can be adopted for other X-linked hereditary diseases.

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REFERENCES

1. Wiskott A. Familiarer, angeborener Morbus Werlhofii?. Monatschrift Kinderheikunde 1937; 68: 212-6.
2. Aldrich RA, Steinberg AG, Campbell DC. Pedigree demonstrating a sex-linked recessive condition characterized by draining ears, eczematoid dermatitis and bloody diarrhea. Pediatrics 1954; 13: 133-9.
3. Kwan S-P, Lehner T, Hagemann T, et al. Localization of the gene for the Wiskott Aldrich syndrome between two flanking markers TIMP and DXS255, on Xp11.22-Xp11.3. Genomics 1991; 10: 29-33.
4. Parkman R, Rappaport J, Geha RS, et al. Complete correction of Wiskott Aldrich syndrome by allogeneic bone marrow transplantation. N Engl J Med 1978; 298: 921-7.
5. Goodeve AC, Chuansumrit A, Sasanakul W, et al. A comparison of the allelic frequencies of ten DNA polymorphisms associated with factor VIII and factor IX genes in Thai and Western European populations. Blood Coagulation and Fibrinolysis 1994; 5: 29-35.
6. Winship PR, Nichols CE, Chuansumrit A, et al. An MseI RFLP in the 5' flanking region of the factor IX gene: its use for hemophilia B carrier detection in Caucasian and Thai populations. Br J Haematol 1993; 84: 101-5.
7. Kwan S-P, Hagemann TL, Radtke BE, et al. Identification of mutations in the Wiskott Aldrich syndrome gene and characterization of a polymorphic dinucleotide repeat at the DXS6940, adjacent to the disease gene. Proc Natl Acad Sci USA 1995; 92: 4706-10.
8. Chuansumrit A, Goodeve AC, Sasanakul W, et al. DNA polymorphism for carrier detection of hemophilia in Thailand. Southeast Asian J Trop Med Public Health (Supplement) 1995; 26: 201-6.

การใช้ X-linked polymorphic loci เพื่อศึกษาหาการสืบทอดของ X-allele ของมารดาไปยังรุ่นลูก และใช้หา chimerism หลังการปลูกถ่ายไขกระดูกในผู้ป่วย Wiskott-Aldrich syndrome

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Wiskott-Aldrich syndrome (WAS) เป็นโรคทางพันธุกรรมที่ถ่ายทอด แบบ X-linked recessive โดยมีความบกพร่องที่ T cell เป็นเหตุทำให้มีการสร้าง immunoglobulin M ต่ำ นอกจากนี้ยังมีปัญหาเรื่องเกร็ดเลือดต่ำ โดยเกร็ดเลือดมีขนาดเล็กร่วมด้วย ทั้งนี้การปลูกถ่ายไขกระดูกสามารถรักษาโรคนี้ให้หายขาดได้

ผู้วิจัยและคณะได้ศึกษาถึงการใช้ X-linked polymorphic loci เพื่อศึกษาการสืบทอดของ X-allele ของมารดาไปยังรุ่นลูก นอกจากนี้ยังใช้เป็นตัวบอกถึง chimerism หลังการปลูกถ่ายไขกระดูก ทั้งนี้ผู้วิจัยและคณะได้ใช้ *Bcl*I restriction fragment length polymorphism (RFLP) ของ intron 18 ของ factor VIII gene และ *Mse*I RFLP ของ 5' flanking region ของ factor IX gene เพื่อหาการสืบทอด X-allele ในโรค WAS และใช้ในการตรวจหา chimerism หลังการปลูกถ่ายไขกระดูกในโรคนี้

คำสำคัญ : X-linked Polymorphic Loci, Chimerism, ปลูกถ่ายไขกระดูก, Wiskott-Aldrich Syndrome

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