Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.2.1

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (2): 141–145

ORIGINAL PAPER

Mehmet Ali Karaselek [1], Hasan Kapaklı [1], Şükrü Nail Güner [1], Ercan Kurar [2], Serkan Küççüktürk [3], Sevgi Keleş [1], İsmail Reisli [1]

A family screening of *CD19* gene mutation by PCR-RFLP

[1] Department of Pediatric Allergy and Immunology, Meram Medical Faculty, Necmettin Erbakan University, Konya, Turkey
[2] Department of Medical Biology, Meram Medical Faculty, Necmettin Erbakan University, Konya, Turkey
[3] Department of Medical Biology, Medical Faculty, Karamanoğlu Mehmetbey University, Karaman, Turkey

ABSTRACT

Introduction and aim. Mutation(s) in the gene encoding the CD19 molecule affect CD19 protein expression and primary immunodeficiency (PID) occurs. The PCR-RFLP method, which is faster and cheaper than other mutation detection methods, is rarely used in the diagnosis of PID. The study aimed to genetically identify CD19 deficiency, which is a PID, using the PCR-RFLP method.

Material and methods. A total of 8 patients and two healthy controls were included in the study and the relevant region genotypes in the CD19 gene were determined by performing PCR-RFLP analysis.

Results. The index case, newborn baby and mother were also included in the study. It was determined that the index case (P6) was homozygous mutant, the newborn baby (P7) and mother (P8) had heterozygous genotype. Based on this situation, one child (P1) was found to be homozygous mutant, mother (P2), father (P3) and other children (P4 and P5) had heterozygous genotype in the family, which was determined to be related to the first case.

Conclusion. In our study, it has been shown that PCR-RFLP is a method that can be used in the diagnosis of PID by determining genotypes using PCR-RFLP, and especially in terms of rapid genetic testing of family screenings.

Keywords. CD19, PID, RFLP

Introduction

Primary immunodeficiencies (PID) are a group of diseases that result from the development and/or dysfunction of various components of the immune system. In recent years, many genes that cause PID have been identified. Primary antibody deficiencies, mainly predisposed to bacterial infections, can be caused by mutations in genes involved in B cell differentiation.[1] Mutations in these genes inhibit immature B cell differentiation in bone marrow and cause agammaglobulinemia and low B cell in peripheral blood.[2] Although many genes (*CD20, CD21, CD81, CD225*) play a role in B cell development, one of the most important is the *CD19* gene.[3,4]

The *CD19* is a gene located in the p11.2 region of chromosome 16, and consists of 15 exons and 7.41 kb length (NC_000016.10).[5] This gene encodes the CD19 protein, a transmembrane glycoprotein of the immunoglobulin (Ig) superfamily. CD19 is also expressed in follicular dendritic cells as well as in normal and neoplastic B cells, which expression begins for the B cells in the bone marrow and continues until the plasma cell. In addition, CD19 forms a complex with CD21, CD81 and CD225 on the surfaces of mature B cells (Fig. 1).[6]

Corresponding author: Mehmet Ali Karaselek, e-mail: malikaraselek@gmail.com

Received: 15.09.2021 / Revised: 15.11.2021 / Accepted: 22.11.2021 / Published: 30.06.2022

Karaselek MA, Kapaklı H, Güner ŞN, Kurar E, et al. *A family screening of "CD19" gene mutation by PCR-RFLP*. Eur J Clin Exp Med. 2022;20(2):141–145. doi: 10.15584/ejcem.2022.2.1.

This complex co-operates with the B cell receptor (BCR) and stimulates both BCR-dependent and BCR-independent intrinsic B cell signaling pathways. Despite the mutation in the *CD19* gene, normal B cell differentiation occurs in the bone marrow, and the absolute number of B cells in the peripheral blood is normal. However, stimulation on BCR is impaired and the number of memory B cells in peripheral blood is decreased. This results in hypogammaglobulinemia and impaired antigen-specific humoral response.[7-8]

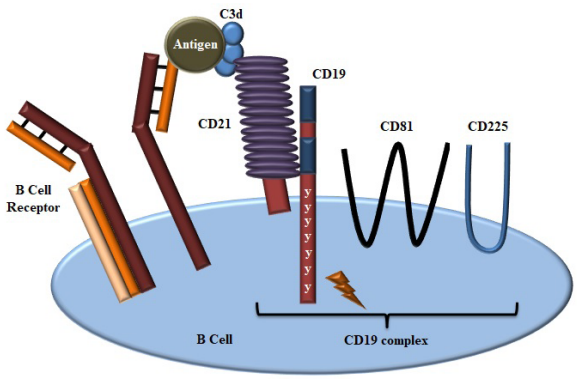

Fig. 1. B cell and CD19 complex

The first case of PID due to mutation in the *CD19* gene was described by us in a 10-year-old girl.[9] Following the identification of this case, a village screening of 208 cases was carried out and carrier was detected in 20 cases. Screening was performed by determining flow cytometric CD19 expressions.[10] She was admitted to us from the same village that was related to the previously described patient. According to the clinical and laboratory results of the patient, it was thought that *CD19* gene mutation might be present and genetic identification was aimed. In order to determine the mutation, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method which was used in *DCLRE1C* mutation detection.[11]

Aim

Therefore, in this study, it was aimed to determine *CD19* gene mutation by PCR-RFLP which is a cheap, reliable and fast method.

Material and methods

Patients

The index family has 4 children (Fig. 2). The patient was admitted to our clinic at 7 years old with complaints of frequent illness.

The history of the patient revealed that she had a fever 3-4 times a month and had a frequent fever since she started school and diagnosed at Idiopathic Thrombocytopenic Purpura (ITP) at 5 years old. Parents had a consanguineous marriage. It was learned that there was a baby who had intrauterine exitus at the age of 6 months in the family. We were learned that patient was related to the first case described by van Zelm et. al. in 2006.[11] As a result of this information and laboratory examination, it was thought that *CD19* gene mutation might be present. The region to be studied for mutation was selected as the previously defined region (c.973_973insA; p.Arg325AlafsX4; GenBank: AH005421.2). The patient (P1), mother (P2), father (P3), sister (P4), brother (P5), the patient identified in 2006 (P6), newborn baby of P6 (P7) and mother of P6 (P8) were included in the study. The studies were performed with 2 healthy controls (C1 and C2). Patient recruitment and the studies reported herein were approved by Institutional Review Board at the Necmettin Erbakan University Meram Medical Faculty. Written informed consent was obtained from participating patients' guardians and healthy controls.

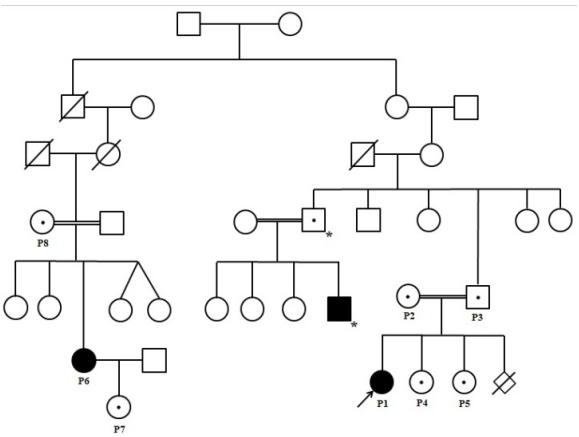

Fig. 2. Pedigree (*:Patients who had previously detected CD19 mutation and carriage)

Flow cytometry

Peripheral lymphocyte subgroup study of the patient and family members were performed. After the study, cell counts were performed with Becton Dickinson Canto II (BD Biosciences, Heidelberg, Germany) flow cytometry and their analysis were performed. In addition, CD19 expression was performed with monoclonal antibody from three different clones (SJ25C1, 4G7, HIB19).

PCR and PCR-RFLP

Polymerase chain reaction (PCR) primers covering mutation regions in *CD19* gene were designed (forward primer: 5'-CCTGAGGAGGAAAAGAAT-3' and reverse primer: 5'-GGAAACAGTAAGTGCAAGGCATA-3'). The mutation regions were amplified by PCR using these primers. A PCR protocol was used and cycling conditions were initial denaturation of 95°C for 10 minutes followed by 45 cycles of 95°C for 40 seconds, annealing beginning at 49,9°C and 72°C 15 seconds. A final extension of 72°C for 10 minutes was applied. Resulting PCR products were visualized by 1% agarose gel. DNA ampl-

icons of 118 bp were obtained after PCR amplification. PCR-RFLP method was applied to PCR products The PCR products were incubated for 1 hour at 65°C with *Bsm*I (New England Biolabs®Inc.). DNA fragments obtained after PCR-RFLP procedure were visualized on 3 % agarose gel and genotypes were determined as wild type, heterozygous and homozygous mutant.

DNA fragments of 118 bp were obtained after PCR with primers designed for *CD19* mutation site. *Bsm*I enzyme recognition region 5'…GAATGCN^…3'; 3'… CTTAC^GN…5' is selected according to the wild type allele. In the absence of mutation, enzyme digestion was performed in both alleles 94 and 24 bp DNA fragment was obtained after PCR-RFLP and these results were interpreted as normal homozygous genotype. If the patient is homozygous mutant genotype 118 bp DNA fragment was obtained. In the case of heterozygote, three DNA fragments will be obtained: 118 bp, 94 bp and 24 bp.

Results

In the peripheral lymphocyte subgroup analysis, CD3+ total T, CD4+ helper T, CD8+ cytotoxic T and CD16+CD56+ NK cell numbers were found to be normal but there was no CD19 expression on CD20+B cells. The patient had low IgG (347 mg/dl) and IgA (25 mg/dl) levels. In addition, the hepatitis B vaccine response was negative (0.74 mIU/ml; normal range 0-10 mIU/ml). Clinical and laboratory characteristics of the P1 are shown in Table 1.

Table 1. Laboratory and clinical features of the patient (P1)

Parameters	P1
Clinical features on admission	Frequent illness during school Fewer ITP
Age of diagnosis (years)	7
Lymphocytes (counts/mm^3)	3700
CD3+ (counts/mm^3)	2479
CD4+ (counts/mm^3)	1369
CD8+ (counts/mm^3)	814
CD19+ (counts/mm^3)	0*
CD20+ (counts/mm^3)	740
CD16+CD56+ (counts/mm^3)	222
IgG (mg/dl) (842-1943)	347*
IgA (mg/dl) (62-390)	25*
IgM (mg/dl) (54-392)	88

* below normal value

CD19 gene mutation analysis with PCR-RFLP was performed as a result of clinical and laboratory analysis. *Bsm*I enzyme was used for PCR-RFLP. 118 bp DNA fragment was obtained after PCR-RFLP due to lack of enzyme cleavage in P1 and P6 patients previously known to be mutations were determined to be homozygous mutant genotype. DNA fragments of 118 bp, 94 bp and 24 bp were obtained in P2, P3, P4, P5, P7 and P8 and evaluated as heterozygous. Because the enzyme cuts both alleles in healthy controls, 94 bp and 24 bp bands were obtained and genotypes were evaluated as homozygous normal. All results of PCR-RFLP are shown in Figure 3.

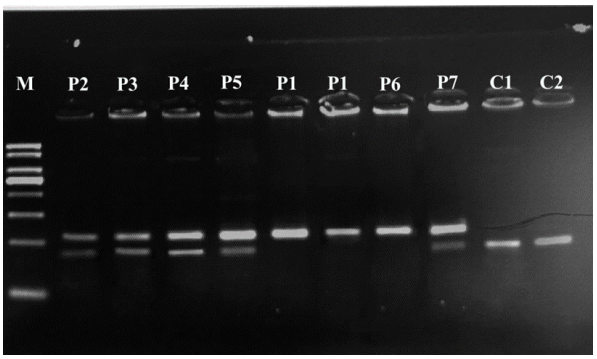

Fig. 3. PCR-RFLP results (M: Marker 50 bp; P1: Patient; P2: P1 mother; P3: P1 father; P4: P1 sister; P5; P1 brother; P6: Patient identified in 2006; P7: newborn baby of P6; C1 and C2: Control)

Discussion

Primary immunodeficiency caused by *CD19* gene mutation was first described in a 10-year-old girl by our team in 2006.[9] *CD19* gene mutation was identified in another patient who was living in the same village and was related to this patient. These two cases suggested that it might be in other cases living in this village and the village was screened for CD19 deficiency. As a result of this screening, 20 carrier individuals were identified.[10] Ten years after the village screening, a patient from the same village and related with index case applied to us. It was thought that *CD19* gene mutation was found in clinical and laboratory examinations. In this study, it was aimed to detect *CD19* gene mutation quickly, safely and inexpensively.

In our region where consanguineous marriages are high, the incidence of autosomal recessive diseases is higher. Therefore, in the light of clinical, laboratory and family history information, it is important to perform rapid mutation analyzes. Known mutations can also be detected by using the PCR-RFLP, which is less expensive, highly sensitive, requires basic laboratory instruments and is easier to implement. The PCR-RFLP is a reliable method and still used for the identification of some bacterial species in the laboratory.[12,13] Other than PCR-RFLP, polymerase chain reaction-single-stranded conformation polymorphism (PCR-SSCP) used for genotype determination after DNA post amplification are two independent methods used for post-amplification genotyping of DNA variations. Both techniques are used in a wide variety of screening applications to characterize single nucleotide polymorphisms (SNPs). Al-

though PCR-SSCP allows identification of a potentially causal unknown SNP that cannot be identified by PCR-RFLP, it has more complex steps than PCR-RFLP. However, it is used in many applications because it does not require complex steps to perform PCR-RFLP. On the other hand, PCR RFLP is easier to process in terms of time and interpretation experience.[15] Other of the reasons we prefer this method is that we have used it safely in the identification of *DCLRE1C* gene mutations. Therefore, the PCR-RFLP method was preferred in the study. That study also identified *DCLRE1C* gene mutation and carrier beers and the results were confirmed by DNA sequence analysis.[11] In our experience, the disadvantage of this method is that it can only be used to detect known mutations.

CD19 is a basic molecule that promotes the proliferation and survival of mature B cells. It forms a whole together with CD81 and CD225. The CD19 protein forms a whole with CD21, CD81 and CD225 on the surface of mature B lymphocytes. This complex works with the B cell receptor and plays an important role in the regulation of events after antigen stimulation.[15] To date, recurrent sinopulmonary infections, pyelonephritis, and gastritis have been reported in patients with CD19 gene mutations identified. [9,11,15,16] In our case, these findings were absent and there was a history of recurrent fever with bronchiolitis that had only been repeated once. Inadequate antibody responses, decreased memory B cell count and hypogammaglobunemia were seen in CD19 deficiency and in our case, it was laboratory compatible with the literature.

Conclusion

As with other diseases, the early detection of genes that cause primary immunodeficiency is important both for the course of the disease and for additional treatment options. In addition, identifying carriers and informing families about this issue is of critical importance. Although the PCR-RFLP method is a known method, it has been applied by us for the first time in the diagnosis of PID. Therefore, we believe that PCR-RFLP method can be used both in detection of known mutations and also in family screening.

Acknowledgments

We are grateful to all individuals participating in this study. We would like to thank to Necmettin Erbakan University Scientific Research Projects Coordinator for supporting this study with 182018008 research project numbers.

Declarations

Funding

The study was supported by Necmettin Erbakan University Scientific Research Projects Coordinator with project number 182018008.

Author contributions

Conceptualization, I.R., S.K. and Ş.N.G.; Methodology, E.K, MA.K. and S.K.; Software, S.K. and H.K.; Validation, M.A.K., H.K. and S.K.; Investigation, M.A.K., H.K., S.N.G., E.K., S.K., S.K. and I.R.; Resources, M.A.K. and H.K.; Data Curation, M.A.K.; Writing – Original Draft Preparation, M.A.K., H.K. and I.R.; Writing – Review & Editing, I.R., S.K., E.K. and Ş.N.G; Visualization, M.A.K., E.K. and S.K., Supervision, I.R. and S.K.; Project Administration, M.A.K., S.K., E.K., Ş.N.G. and I.R.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data used in the study and the data about the method can be accessed by contacting the corresponding author.

Ethics approval

The study was approved by Necmettin Erbakan University; protocol number (2017/818).

References

1. Picard C, Bobby Gaspar H, Al-Herz W, et. al. International Union of Immunological Societies:2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. *J Clin Immunol.* 2018;38(1):96-128.
2. Ballow M. Primary immunodeficiency disorders: antibody deficiency. *J Allergy Clin Immunol.* 2002;109:581-591.
3. Wang K, Wei G, Liu D. CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. *Exp Hematol Oncol.* 2012;29(1):36.
4. Otero DC, Rickert RC. CD19 function in early and late B cell development. II. CD19 facilitates the pro-B/pre-B transition. *J Immunol.* 2003;171(11):5921-5930.
5. CD19 – CD19 molecule. https://ncbi.nlm.nih.gov./gene/?term=CD19. Accessed November 2, 2021.
6. Rich RR. *Clinical Immunology: Principles and Practice*. 5th Edition. Elsevier. Chine. 2019.
7. Rheingold SR, Brown VI, Fang J, Kim JM, Grupp SA. Role of the BCR complex in B cell development, activation, and leukemic transformation. *Immunol Res.* 2003;27(2-3):309-330.
8. Şekerel BE. Çocukluk Çağında Allerji Astım İmmünoloji. *Bilnet Publishing*. Turkey. 2015.
9. Reisli İ. Artaç H, Pekcan S, et. al. CD19 deficiency: A village screening. *Tur Arch Ped.* 2009;44:127-130.
10. Karaselek MA, Kapaklı H, Keleş S, et. al. Intrauterine detection of DCLRE1C (Artemis) mutation by restriction fragment length polymorphism. *Pediatr Allergy Immunol.* 2019;30(6):668-671.
11. van Zelm MC, Reisli I, van der Burg M, et. al. An antibody-deficiency syndrome due to mutations in the CD19 gene. *N Engl J Med.* 2006;354(18):1901-1912.

12. Dib C, Faure S, Fizames C, et. al. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature.* 1996;380(6570):152-154.
13. Ota M, Fukushima H, Kulski JK, Inoko H. Single nucleotidepolymorphism detection by polymerase chain reaction--restriction fragment lengthpolymorphism. *Nat Protocols.* 2007;2(11):2857-2864.
14. Hashim HO, Al-Shuhaib MB. Exploring the potential and limitations of PCR-RFLP and PCR-SSCP for SNP detection: A review. *J Appl Biotechnol Rep.* 2019: 5;6(4):137-144.
15. Rudd CE. Disabled receptor signaling and new primary immunodeficiency disorders. *N Eng J Med.* 2006:354;1874-1877.
16. Kanegane H, Agematsu K, Futatani T, et. al. Novel mutations in a Japanese patient with CD19 deficiency. *Genes Immun.* 2007;8(8):663-670.