Clinical Research

ENT Updates 2019;9(2): 159-165
DOI: 10.32448/entupdates.578602

ENT updates
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Inter-retrotransposon polymorphism polymerase chain reaction as a tool for screening HERV polymorphisms in nasal mucosal swabs

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Abstract

Objective: Inter-retrotransposon polymorphism Polymerase Chain Reaction (IRAP-PCR) technique allows for detecting insertional polymorphisms via amplification of the DNA fragment between two retrotransposons in plant genomes. However, this method has not been reported to be used for analyzing human samples to date. Recently, Human Endogenous Retrovirus (HERV) polymorphisms gained interest due to their potential effect on pathophysiology of certain diseases. Nevertheless, the association between HERV polymorphisms and the risk for developing nasal polyposis (NP) has not been studied. In this study, we aimed to investigate whether or not IRAP-PCR could be performed in nasal swab samples for comparing HERV polymorphisms in different nasal mucosal samples.

Methods: Nasal swab samples from 16 patients were used for DNA isolation. These DNA samples were used as templates for IRAP PCR of HERV-K6, HERV-K11, HERV-L1 and HERV-L2 and PCR products were analyzed by agarose gel electrophoresis.

Results: Nasal swab samples yielded enough DNA material for successfully performing IRAP-PCR. We obtained specific banding patterns the three out of four HERV sequences tested in this study. No polymorphisms was detected between samples from different patients. Similarly, polymorphic bands was not detected between the polyps or nasal mucosal swab samples obtained from the same patient.

Conclusion: We have, for the first time, shown that IRAP-PCR can be performed in nasal swabs. Our findings suggest that this technique can serve as an inexpensive and effective screening tool for investigating links between nasal mucosal diseases and HERV polymorphisms such as nasal polyposis.

Keywords: Human endogenous retrovirus, genetic polymorphism, polymerase chain reaction, nasal polyps.

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Received: 17.06.2019; **Accepted:** 24.07.2019

Online available at:
www.entupdates.org

Introduction

Integration of retrotransposon sequences into the genome creates new joints between genomic DNA and their conserved ends. Since, these changes can be detected they can also be used as genetic markers. PCR is the most commonly used technique for detecting retrotransposon insertion polymorphisms.[1] IRAP-PCR has been developed by Kalendar et al [2] in 1999 for retrotransposon-based genotyping and fingerprinting applications. IRAP method, can detect insertional polymorphisms by amplifying the portion of DNA between two retrotransposons.[2] IRAP is a simple technique which relies on resolving the PCR products obtained by using HERV-specific primers. The resulting PCR products can originate from hundreds of target sites in the genome. Basically, the PCR products form a fingerprint pattern which is specific to the source genome.[2] Typically, these IRAP fingerprints consists of bands ranging from 500 to 3000 bases.

Nasal polyposis (NP) is a multifactorial inflammatory disease of sinonasal mucosa which leads to the formation of benign polyps. It estimated to affect approximately 4% of the population worldwide.[3-6] Although its' aetiology remains elusive chronic infection, allergy, cystic fibrosis, and aspirin sensitivity seems to be associated with NP.[6] The most common symptoms of NP include nasal obstruction, postnasal drip, nasal discharge, facial pain and impairment of sense of smell.[6,7] Local and systemic use of corticosteroids is the first-line treatment of the NP, and corticosteroids can also be used for prevention of postoperative recurrence.[6]

The possibility for the involvement of hereditary factors in the development of nasal polyps has been previously proposed by Greisner et al.[8] A pedigree analysis by Delagrand A et al suggested a recessive autosomal mode of inheritance pattern for nasal polyposis.[9] Similarly, correlations between gene polymorphisms and nasal polyposis have also been investigated by a number of different groups.[5]

Findings imply that polymorphisms in several genes including MMP-9 [10], LTC4S, NOS2A, PTGDR [11], MET, COX-2 [12], OSF-2 and LF [13] may also be associated with increased risk of developing nasal polyps.[5] However, these studies show limited ability in the detection of true genetic markers due to the heterogeneity of the mixed populations and/or concentration on populations from a small geographical region.[5] Furthermore, large scale research data from genome-wide association studies (GWAS) for nasal polyposis are still not available.[5,10-14] Given the high prevalence and morbidity associated with NP, more research is required to better understand its' aetiology and develop novel therapeutic strategies.[5,6,10-15]

Previously, the association between NP and infections of DNA viruses such as Epstein-Barr virus and human herpes virus-6 infection was studied.[16,17] However, a possible link between Human Endogenous Retrovirus (HERV) and NP has not been investigated yet. HERVs are retroviral sequences which represent approximately 8% of the human genome and are distributed among ~700,000 different loci.[18] The proviral structure of HERVs consists of gag and pol genes that are flanked with 5'LTR and 3'LTR sequences. While gag and pol regions encode the proteins required for replication and integration processes, LTR sequences serve as a promoter region for transcription of HERV mRNAs.[19] Majority of the HERVs in human genome are believed to be silenced throughout the evolutionary processes with various mechanisms such as methylation of LTR sequences.

Although, HERVs are implicated in regulation of several biological processes, abnormal activation or altered expression of HERVs are also associated with a number of pathological conditions such as autoimmune, neurodegenerative, chronic inflammatory diseases, and cancer.[20] HERV integrations are shown to be able to alter gene function through induction of positional changes in the genome even if these retroviral sequences are not transcribed or translated.[21] In this context, HERV polymorphisms have gained more interest due to their potential effect on pathophysiology of certain diseases.[22-25] Especially, the determination of HERV insertions in or near disease-related genes can have implications for the identification of novel biomarker candidates.[1]

Our review of the literature revealed that IRAP-PCR technique which allows for comparison of retrotransposal polymorphisms in genomes, has not been performed for analyzing human samples to date.[26]

IRAP-PCR technique holds the potential to be used as an inexpensive and effective screening tool for investigating links between nasal mucosal diseases and HERV polymorphisms such as nasal polyposis. Therefore, we aimed to investigate whether or not IRAP-PCR could be performed in nasal swab samples for comparing HERV polymorphisms in different nasal mucosal samples.

Methods

Sample collection

This study was performed in accordance with guidelines of the Ethics Committee of Istanbul Yeni Yüzyıl University (Approval No. 16.11.2017/044). A total of 16 patients positively diagnosed with nasal polyposis (aged between 15-71 years, 7 female and 9 male) were included in the study. Nasal swab samples were collected either from polyps or from the nasal mucosal tissue by swabbing the nasal cavity of patients with a sterile cotton-tipped swab. The swabs were then placed into sterile tubes containing sterile isotonic solution (0,9% NaCl).

DNA isolation

Nasal swab samples were used for genomic DNA isolation. DNA isolation was performed according to Ghatak et al [26] with some modifications. Cotton-tipped swabs were removed and cells were harvested from isotonic solution by centrifugation at 10.000g and 4°C for 10 minutes. Then cell pellets were suspended in 500 µl extraction buffer (10 mM Tris (pH 8.0), 10 mM EDTA, 2% SDS) and 50 µl 10% SDS was added. Following incubation at 56ºC for 90 minutes, 550 µl of "phenol:chloroform:isoamyl alchol (25:24:1)" mixture was added. Tubes were mixed gently by inverting several times for 3 minutes and centrifuged at 4°C for 10 minutes at 10.000g. After centrifugation, upper phases were transferred into clean microfuge tubes and equal volume of chloroform:isoamyl alchol (24:1) was added. Then tubes were centrifuged at 4°C for 10 minutes at 10.000g and upper phases were transferred into clean tubes. 1 ml of "isopropanol:3M sodium acetate (10:1)" mixture was added onto each tube and incubated for 1 hour at -20ºC. After incubation, tubes were centrifuged at 4°C for 10 minutes at 10.000g and DNA pellets were washed with 70% ethanol. DNA pellets were dissolved in nuclease free water. Following qualitative and quantitative analysis of DNA molecules DNA concentration of each sample was adjusted to 20 ng/µl.

IRAP-PCR

IRAP-PCR analysis was performed for HERV-K6, HERV-K11, HERV-L1 and HERV-L2 which are abundant throughout the human genome. Full length sequences of target HERVs were obtained from NCBI database and their LTR sequences were determined using an online bioinformatics tool "LTR*harvester*". Forward and reverse primers targeting LTR sequence of each HERV were designed as manually (*Table 1*).

IRAP-PCR was performed in a total volume of 25 µL containing 1X PCR buffer, 4 mM of MgCl2, 1mM of dNTP, 1 nmol of each of the forward and reverse primers, 2 ng of template DNA and 0.02 U Taq DNA polymerase (GenTaq DNA polymerase, Cat. No, GM008-2). PCR conditions were set as follows: initial denaturation at 95 °C for 3 minutes followed by 30 cycles at 95°C for 30 s, 55°C (for HERV-K11, HERV-L1) or 53°C (for HERV-K6) for 30 s, 72°C for 3 minutes. The reaction was completed with a final extension step at 72°C for 7 minutes. PCR products were resolved by 2% agarose gel electrophoresis performed at 90 V for 3 h in 1X TAE buffer and gel was visualized on UV transilluminator.

Table 1: Primer sequences and annealing temperatures

Primer Name	Sequence (5'→3')	Ta (°C)	NCBI Accession Number
HERVK-6F	CCTACAGGTTTCACCATCTTG	53	AF074086.2
HERVK-6R	CTTCTTTCTACACAGACACAG		
HERVK-11F	CCACAGGTGTGGAGGGACAACC	55	DQ112099.1
HERVK-11R	CACCGAGACATTCCATTGCCC		
HERVK-L1F	GCACGTTCTGCACATGTATCCCAG	55	Z72519.1
HERVK-L1R	CCAGTGTGTGATGTTCCCCTTCCT		
HERVK-L2F	CAAAGTGCTGGGATTACAGGCGTG	*X	Z72519.1
HERVK-L2R	CTGAGGCAGGAGAATGGTGTGAACC		

* Optimization of annealing temperature was performed between 52-67°C.

Results

Nasal swab samples obtained either from polyps or nasal mucosal tissue of 16 patients yielded enough DNA material for performing IRAP-PCR. In an attempt to examine the possible association between insertional HERV polymorphisms and the risk for developing NP, we selected four HERV sequences (HERV-K6, HERV-K11, HERV-L1 and HERV-L2) which are known to occur frequently throughout the human genome. IRAP-PCR analysis was performed to amplify the HERV-K6, HERV-K11, HERV-L1 and HERV-L2 inter-genomic sequences as explained under "Material and Methods" section. PCR products were then analyzed with agarose gel electrophoresis.

IRAP-PCR of HERV-K6 yielded 8 sharp bands ranging from 200 bp to 2000 bp (**Figure 1**). Similarly, we obtained 3 bands ranging from 400 bp to 3000 bp for IRAP analysis of HERV-K11 (**Figure 2**).

IRAP-PCR of HERV-L1, produced 12 sharp bands ranging from 200 bp to 1000 bp (**Figure 3**). As shown in *Figures 1-3*, IRAP-PCR banding patterns were highly specific and monomorphic for each of the three HERVs.

In addition, each time IRAP-PCR was repeated we obtained the same band profiles. These banding patterns were the same between different samples of the same person or between different individuals. This finding clearly proved the reproducibility of the reaction. In addition to these three HERVs, we also tested HERV-L2 at the same conditions. However every optimization reaction of IRAP-PCR for HERV-L2 resulted with smear profile and any sharp bands could not be observed (data not shown).

Discussion

IRAP-PCR is a suitable technique for detecting insertional retrotransposon polymorphisms.[2] This simple and effective tool is frequently used for studying retrotransposon polymorphisms in plant genomes.[1] However, this method has not been performed for analyzing human samples to date.

Insertional polymorphisms of HERVs and distinctive expression patterns can increase the risk for developing some genetic disorders. Molecular genetic findings suggest that determination of HERV insertions in or near disease-related genes can have implications for identification of novel biomarker candidates.[25] Previously García-Montojo et al [27] pointed out the correlation between polymorphisms of HERV-W in chromosome X and the risk of multiple sclerosis (MS). Similarly, the association between HERV-K18 polymorphism and type1 diabetes was also demonstrated.[28] In addition to these examples, altered expressions and polymorphisms of HERVs were proved to be the cause of certain diseases including cancer [29,30], rheumatoid arthritis [31] and even schizophrenia [32]. All these studies proved that HERV polymorphisms can serve as a useful genetic marker to identify several genetic disorders.

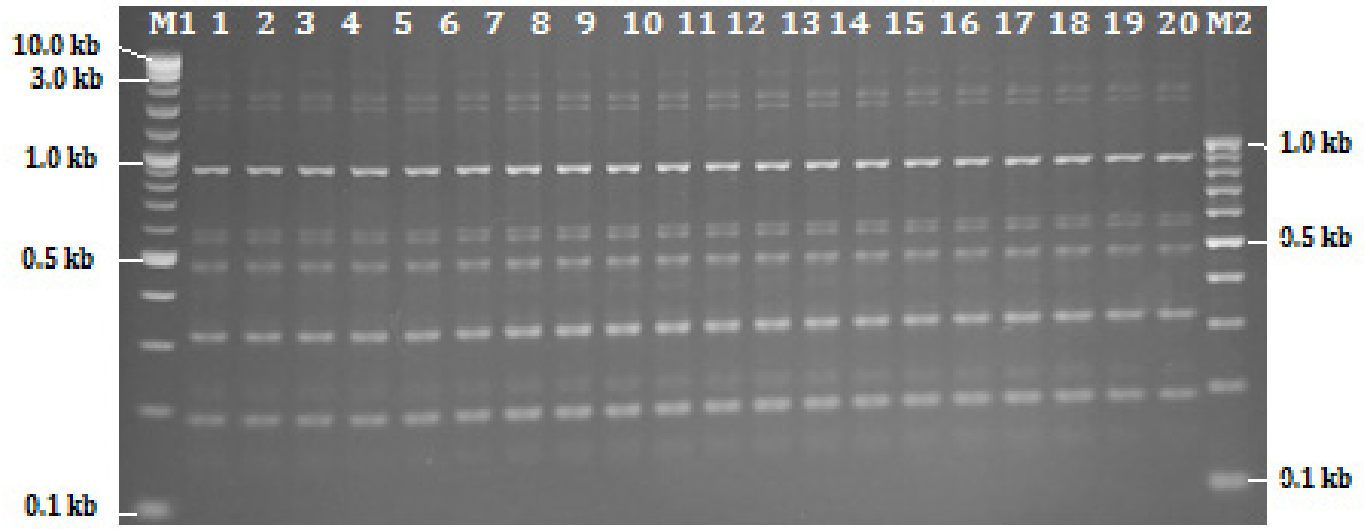

Figure 1: HERV-K6 IRAP-PCR results. In this figure only 10 patients' results are shown. Odd numbers are polyposis tissues, even numbers are nasal mucosal tissues. M1, 1kb DNA ladder (Neb, N3200S); M2 100 bp DNA ladder (Vivantis, NL1401).

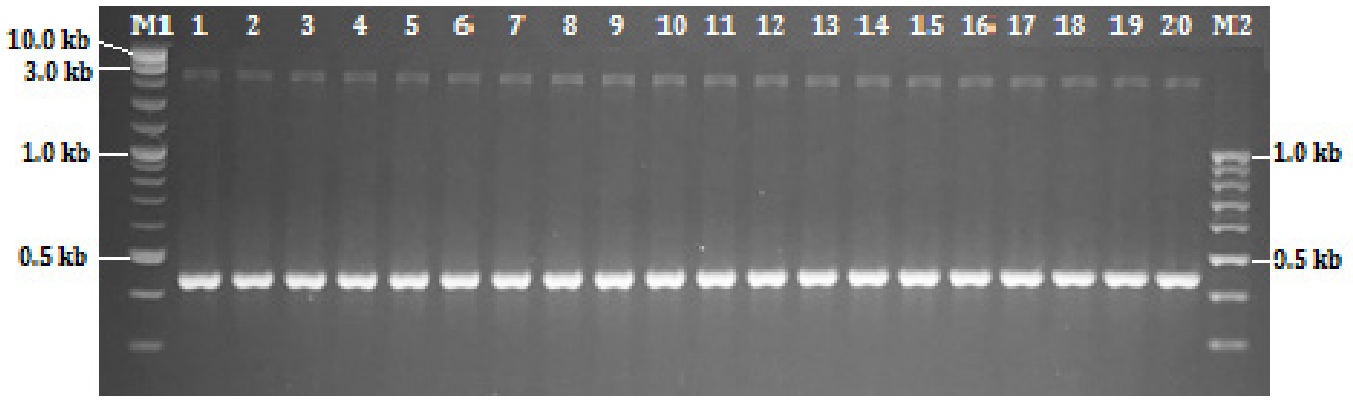

Figure 2: HERV-K11 IRAP-PCR results. In this figure only 10 patients' results are shown. Odd numbers are polyposis tissues, even numbers are nasal mucosal tissues. M1, 1kb DNA ladder (Neb, N3200S); M2 100 bp DNA ladder (Vivantis, NL1401).

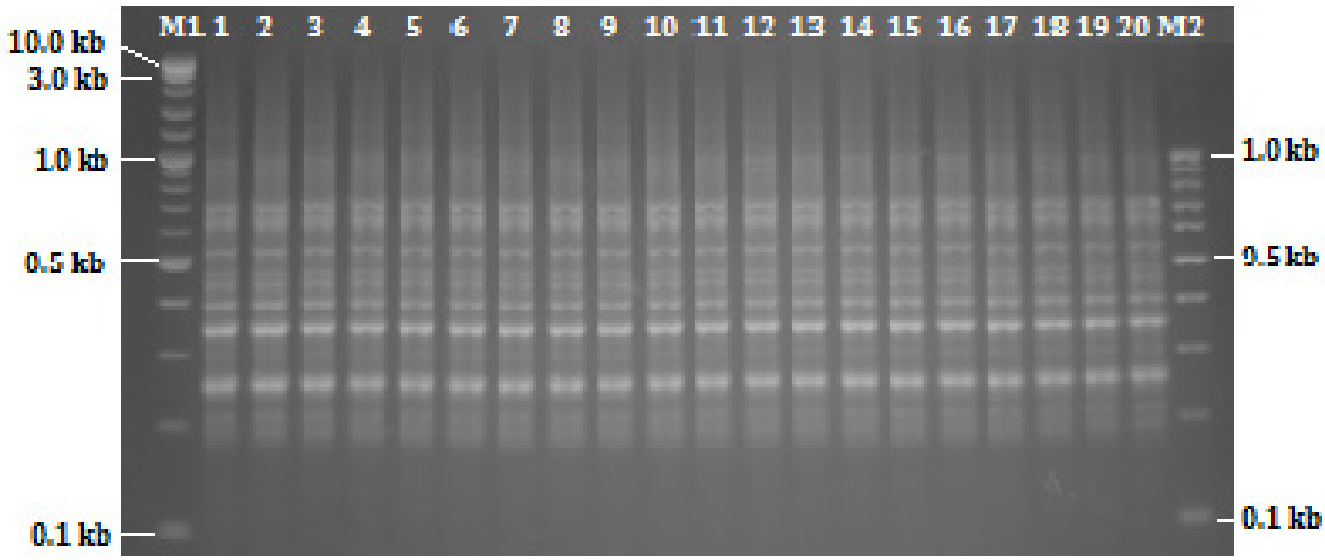

Figure 3: HERV-L1 IRAP-PCR results. In this figure only 10 patients' results are shown. Odd numbers are polyposis tissues, even numbers are nasal mucosal tissues. M1, 1kb DNA ladder (Neb, N3200S); M2 100 bp DNA ladder (Vivantis, NL1401).

Nasal polyposis (NP) is an inflammatory disease of sinonasal mucosa which triggers formation of benign polyps. Although genetic background contributing to NP pathogenesis has been investigated, these studies show limited ability for detecting genetic markers due to the heterogeneity of the mixed populations.[5] Furthermore, large scale research data from genome-wide association studies (GWAS) for nasal polyposis is still missing.[5,14] Given the high prevalence and morbidity associated with NP, molecular genetic markers are required to better understand its' aetiology and develop novel therapeutic strategies.[5,6,15]

In this study, we explored whether or not IRAP-PCR could be used for studying HERV polymorphisms in human samples. For this purpose we tested the four most common HERV sequences in human mucosal swab samples. Three out of the four HERV candidates, namely HERVs, K6, K11 and L1, yielded reproducible and highly specific band patterns (**Figures 1-3**) while HERV-L2 did

not produce specific PCR bands. The study was conducted with 16 patients. IRAP results of both NP and nasal mucosal tissues were compared for HERV insertional polymorphisms. In addition, we also tested oral and pharynx epithelium samples obtained from the same patients. Although these HERVs that were investigated in this study, do not seem to play a role in NP pathogenesis, our findings showed that nasal swab samples can be used for performing IRAP-PCR, and HERV-K6, HERV-K11, HERV-L1 primer sequences can serve as monomorphic control markers. Monomorphic primers are important components of genetic marker kits for monitoring the amplification of target sequences.[33]

In summary, we have for the first time shown that IRAP-PCR can be successfully performed in human mucosal-swab samples. This simple and inexpensive technique can have implications in studying possible links between HERV polymorphisms and the risks for developing certain diseases such as NP. On the other hand, since HERV expressions do not always result in genomic insertions, RNA analyses are also important for investigating the relation between HERVs and genetic background of diseases. Therefore, we believe that this inexpensive and effective screening method can also be combined with RNA analysis techniques to detect HERV polymorphisms.

Acknowledgements

Statement of conflict of interest: The authors declare no conflicts of interest.

Compliance with ethical standards: Ethical Approval This study was approved by the Ethics Committee of Istanbul Yeni Yüzyıl University (Approval No. 16.11.2017/044) and all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed Consent Informed consent was obtained from all individual participants included in the study.

Author contributions: Yılmaz S. and İbişoğlu M. S carried out the experiments. Yılmaz S. helped supervise the project. Kepekci A. H. provided the human materials, designed the model and the computational framework of the study. Kig C. developed the original idea, supervised the project and wrote the manuscript with support from Yılmaz S., İbişoğlu M. S. and Kepekci A. H.

References

1. Kalendar R, Flavell A, Ellis T, Sjakste T, Moisy C, Schulman AH. Analysis of plant diversity with retrotransposon-based molecular markers. Heredity 2011;106:520-30.
2. Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A. IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor Appl Genet 1999;98:704-11.
3. Naclerio RM, Bachert C, Baraniuk JN. Pathophysiology of nasal congestion. Int J Gen Med 2010;3:47-57.
4. Hedman J, Kaprio J, Poussa T, Nieminen MM. Prevalence of asthma, aspirin intolerance, nasal polyposis and chronic obstructive pulmonary disease in a population-based study. Int J Gen Med 1999;28:717-22.
5. Dinarte VRP, Santos ARDd, Araújo LFd, et al. Polymorphisms in chronic rhinosinusitis with nasal polyps-a systematic review. Braz J Otorhinolaryngol 2017;83:705-11.
6. Newton JR, Ah-See KW. A review of nasal polyposis. Ther Clin Risk Manag 2008;4:507-12.
7. Scott-Brown WG. Scott-Brown's Otolaryngology: Adult Audiology. Butterworth; 1987.
8. GreisnerIII WA, Settipane GA, editors. Hereditary factor for nasal polyps. Allergy and asthma proceedings; 1996: OceanSide Publications.
9. Delagrand A, Gilbert-Dussardier B, Burg S, et al. Nasal polyposis: is there an inheritance pattern? A single family study. Rhinology 2008;46:125-30.
10. Wang LF, Chien CY, Tai CF, Kuo WR, Hsi E, Juo SHH. Matrix metalloproteinase-9 gene polymorphisms in nasal polyposis. BMC Med Genet 2010;11:85.
11. Pescador DB, Isidoro-Garcia M, Garcia-Solaesa V, de Pedro MP, Sanz C, Hernandez-Hernandez L. Genetic association study in nasal polyposis. J Investig Allergol Clin Immunol 2012;22:331-40.
12. Sitarek P, Zielinska-Blizniewska H, Dziki L, et al. Association of the− 14C/G MET and the− 765G/C COX-2 Gene Polymorphisms with the Risk of Chronic Rhinosinusitis with Nasal Polyps in a Polish Population. DNA Cell Biol 2012;31:1258-66.
13. Zielinska-Blizniewska H, Sitarek P, Milonski J, et al. Association of the− 33C/G OSF-2 and the 140A/G LF gene polymorphisms with the risk of chronic rhinosinusitis with nasal polyps in a Polish population. Mol Biol Rep 2012;39:5449-57.
14. Bush WS, Moore JH. Genome-wide association studies. PLoS Comput Biol 2012;8:e1002822.
15. Hulse K, Stevens W, Tan B, Schleimer R. Pathogenesis of nasal polyposis. Clin Exp Allergy 2015;45:328-46.

16. Ioannidis D, Lachanas VA, Florou Z, Bizakis JG, Petinaki E, Skoulakis CE. Herpes viruses and human papilloma virus in nasal polyposis and controls. Braz J Otorhinolaryngol 2015;81:658-62.
17. Zaravinos A, Bizakis J, Spandidos DA. Prevalence of human papilloma virus and human herpes virus types 1–7 in human nasal polyposis. J Med Virol 2009;81:1613-9.
18. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860-921.
19. Jern P, Sperber GO, Ahlsén G, Blomberg J. Sequence variability, gene structure, and expression of full-length human endogenous retrovirus H. J Virol 2005;79:6325-37.
20. Küry P, Nath A, Créange A, et al. Human endogenous retroviruses in neurological diseases. Trends Mol Med 2018;24:379-94.
21. Jern P, Coffin JM. Effects of retroviruses on host genome function. Annu Rev Genet 2008;42:709-32.
22. Young GR, Stoye JP, Kassiotis G. Are human endogenous retroviruses pathogenic? An approach to testing the hypothesis. Bioessays 2013;35:794-803.
23. Heneghan H, Miller N, Lowery A, Sweeney K, Kerin M. MicroRNAs as novel biomarkers for breast cancer. J Oncol 2009;2009:950201.
24. Subramanian RP, Wildschutte JH, Russo C, Coffin JM. Identification, characterization, and comparative genomic distribution of the HERV-K (HML-2) group of human endogenous retroviruses. Retrovirology 2011;8:90.
25. Shin W, Lee J, Son SY, Ahn K, Kim HS, Han K. Human-specific HERVK insertion causes genomic variations in the human genome. PloS one 2013;8:e60605.
26. Ghatak S, Muthukumaran RB, Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech 2013;24:224-31.
27. García-Montojo M, de la Hera B, Varadé J, et al. HERV-W polymorphism in chromosome X is associated with multiple sclerosis risk and with differential expression of MSRV. Retrovirology 2014;11:2.
28. Marguerat S, Wang WY, Todd JA, Conrad B. Association of human endogenous retrovirus K-18 polymorphisms with type 1 diabetes. Diabetes 2004;53:852-4.
29. Shiroma T, Sugimoto J, Oda T, Jinno Y, Kanaya F. Search for active endogenous retroviruses: identification and characterization of a HERVE gene that is expressed in the pancreas and thyroid. J Hum Genet 2001;46:619-25.
30. Seifarth W, Frank O, Zeilfelder U, et al. Comprehensive analysis of human endogenous retrovirus transcriptional activity in human tissues with a retrovirus-specific microarray. J Virol 2005;79:341-52.
31. Krzysztalowska-Wawrzyniak M, Ostanek M, Clark J, et al. The distribution of human endogenous retrovirus K-113 in health and autoimmune diseases in Poland. Rheumatology 2011;50:1310-4.
32. Brodziak A, Ziółko E, Muc-Wierzgoń M, Nowakowska-Zajdel E, Kokot T, Klakla K. The role of human endogenous retroviruses in the pathogenesis of autoimmune diseases. Med Sci Monit 2012;18:80-8.
33. Dormoy A, Hanau D, Tongio M, Cazenave J. Development and validation of a genotyping kit for the eight principal human platelet alloantigen systems. Transfus Clin Biol 2000;7:51-62.

Please cite this article as: Kepekçi AH, İbişoğlu MS, Yılmaz S, Kığ C. Inter-retrotransposon polymorphism polymerase chain reaction as a tool for screening HERV polymorphisms in nasal mucosal swabs. ENT Updates 2019;9(2): 159-165