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.
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. IRAP-PCR has been developed by Kalendar et al. in 1999 for retrotransposon-based genotyping and fingerprinting applications. IRAP method, can detect insertional polymorphisms by amplifying the portion of DNA between two retrotransposons. 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. 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. Although its’ aetiology remains elusive chronic infection, allergy, cystic fibrosis, and aspirin sensitivity seems to be associated with NP. The most common symptoms of NP include nasal obstruction, postnasal drip, nasal discharge, facial pain and impairment of sense of smell. 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.

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

Findings imply that polymorphisms in several genes including MMP-9, LTC4S, NOS2A, PTGDR, MET, COX-2, OSF-2 and LF may also be associated with increased risk of developing nasal polyps. 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. Furthermore, large scale research data from genome-wide association studies (GWAS) for nasal polyposis are still not available. Given the high prevalence and morbidity associated with NP, more research is required to better understand its’ aetiology and develop novel therapeutic strategies.

Previously, the association between NP and infections of DNA viruses such as Epstein-Barr virus and human herpes virus-6 infection was studied. 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. 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. 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. 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. In this context, HERV polymorphisms have gained more interest due to their potential effect on pathophysiology of certain diseases. Especially, the determination of HERV insertions in or near disease-related genes can have implications for the identification of novel biomarker candidates.

Our review of the literature revealed that IRAP-PCR technique which allows for comparison of retrotransposon polymorphisms in genomes, has not been performed for analyzing human samples to date. 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 Yenii 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:isoamylalcohol (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 microscope tubes and equal volume of chloroform:isoamyl alcohol (24:1) was added. Then tubes were 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 alcohol (24:1) was added. The reaction was completed with a final extension step at 72ºC 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.

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 “LTRharvester”. 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 ºC 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 72ºC 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

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence (5’→3’)</th>
<th>Ta (ºC)</th>
<th>NCBI Accession Number</th>
</tr>
</thead>
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<tr>
<td>HERVK-6F</td>
<td>CCTACAGGTTCACCACATCTTG</td>
<td>53</td>
<td>AF074086.2</td>
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<tr>
<td>HERVK-6R</td>
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<tr>
<td>HERVK-11F</td>
<td>CCACAGGTGGGGAGGACAACC</td>
<td>55</td>
<td>DQ112099.1</td>
</tr>
<tr>
<td>HERVK-11R</td>
<td>CACCGAGACATCCTACAGCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HERVK-L1F</td>
<td>GCAGTCTGTGCACATGTACCCAG</td>
<td>55</td>
<td>Z72519.1</td>
</tr>
<tr>
<td>HERVK-L1R</td>
<td>CCAGGTGATGATGCTCCCATCCT</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>HERVK-L2F</td>
<td>CAAAGTGCTGGGATTACAGCCGTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HERVK-L2R</td>
<td>CTGAGCAGGAGAAATGGTGAACC</td>
<td>*X</td>
<td>Z72519.1</td>
</tr>
</tbody>
</table>

* Optimization of annealing temperature was performed between 52-67ºC.
**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-Mon-tojo 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, N32005); M2 100 bp DNA ladder (Vivantis, NL1401).](image-url)
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. Furthermore, large scale research data from genome-wide association studies (GWAS) for nasal polyposis is still missing. Given the high prevalence and morbidity associated with NP, molecular genetic markers are required to better understand its’ aetiology and develop novel therapeutic strategies.

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.
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

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