Advancements in PCR-Based Approaches for Diagnosis and Monitoring of Autoimmune Diseases: A Brief Review

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Abstract:

The diagnosis of autoimmune diseases faces various obstacles at present. The diverse nature of autoimmune disorders and the overlap of symptoms create difficulties in identifying a specific condition. Furthermore, the absence of distinct biomarkers that are unique to each disease adds complexity to the diagnostic procedure, resulting in delays and potential misclassification. While considering the diagnosis challenge, Polymerase chain reaction (PCR)-based methods have emerged as powerful tools in the diagnosis and monitoring of autoimmune diseases. This review aims to outline the recent advancements in PCR-based techniques and their applications in autoimmune disease diagnosis and monitoring. By leveraging PCR, researchers and clinicians have made substantial progress in improving the understanding, early detection, and differentiation of autoimmune disorders, thereby enhancing patient care and outcomes. PCR-based approaches have revolutionized the diagnosis and monitoring of autoimmune diseases, providing valuable insights into disease pathogenesis, patient stratification, and treatment efficacy. Continued advancements in PCR technologies hold great promise for improving the accuracy, efficiency, and accessibility of autoimmune disease diagnostics, ultimately leading to personalized and targeted therapeutic interventions.

Keywords:

Autoimmune diseases, Polymorphism, Molecular diagnosis, PCR, Biomarkers

INTRODUCTION

In the last few decades, there has been a rapid increase in the prevalence of autoimmune diseases (ADs) worldwide. This rise can be attributed to various factors, including genetic polymorphisms, infections, smoking, and environmental triggers. While the classical risk factors for ADs have long been recognized, recent advancements in sequence-based approaches have shed new light on understanding these diseases. Scientists hope to develop more effective diagnostic tools, preventive measures, and targeted therapies for autoimmune diseases by understanding the interplay between genetic factors, environmental triggers, and the immune system. The genetic-based research in this field aims to unravel the complex mechanisms underlying ADs and are able to pave new ways to improve management and treatment options for patients(Miyauchi et al., 2023; Vaitinadin et al., 2023).

Autoimmune diseases (AID) are becoming more common and encompass over 100 different clinical conditions that have a significant impact on global health. It is crucial to identify these diseases early to prevent complications and provide appropriate treatment (De Santis & Selmi, 2012). The incidence of autoimmune diseases is rapidly increasing, causing significant challenges for patients. These diseases, such as Rheumatoid arthritis (RA), multiple sclerosis (MS), Psoriasis, and Lupus (SLE), have a detrimental impact on individuals' lives, leading to disability, chronic pain, and undesirable side effects from conventional treatments. Patients constantly live in fear of worsening symptoms or experiencing new flare-ups.

Autoimmune diseases (ADs) are a leading cause of mortality in developing countries, with an estimated incidence of around 10%. Despite improvements in treatment outcomes, morbidity, and mortality rates remain high, achieving long-lasting remission remains challenging for many systemic ADs, and the focus is primarily on symptom reduction and organ damage prevention. Advances in

understanding AD immunobiology have led to the development of new drugs, but a cure or sustained remission is still elusive (Chandrashekara, 2012).

There are a growing number of case reports of various autoimmune diseases occurring after COVID-19 indicating that there should be an urgent need for new research for the better understanding of Autoimmune diseases. While no data exist on how many Indians suffer from autoimmune diseases, a global estimate shows that nearly 700 million people - that is, nearly one-tenth of the global population - suffer from some kind of an autoimmune disease, in stages ranging from mild and moderate to severe(Guo et al., 2023).

Autoantibodies play a crucial role in autoimmune diseases and are often used as diagnostic markers. High-throughput techniques such as antigen microarrays and mass spectrometry have emerged as powerful tools for studying autoimmunity. Antigen microarrays involve the immobilization of numerous antigens on a solid surface, enabling the simultaneous detection of multiple autoantibodies in patient sera. Mass spectrometry, on the other hand, allows for the identification and measurement of autoantibodies by analyzing their mass and charge properties (Plebani et al., 2009). These techniques offer advantages in terms of sensitivity, reproducibility, and the ability to analyze multiple markers simultaneously. However, challenges related to standardization and data interpretation need to be addressed before these techniques can be effectively implemented in clinical practice, and further comparisons with gene-based studies such as PCR can contribute to establishing reliable standard diagnostic methods.

The main purpose of this concise review is to examine the present and recent progressions in the identification and tracking of autoimmune disorders utilizing PCR-based methods. The review aims to give a comprehensive summary of the genetic aspects associated with the pathophysiology of autoimmune diseases and their implementation in diagnostic procedures. Moreover, the review highlights the potential advantages of PCR-based approaches, including enhanced sensitivity, specificity, and rapidity, thereby enabling prompt and precise diagnosis, as well as efficient monitoring of autoimmune diseases.

Study design

For the present review, a systemic literature search was undertaken across online databases such as PubMed, MEDLINE, Google Scholar, and ScienceDirect using the relevant keyword: "Autoimmune diseases", "Polymorphism", "Molecular diagnosis", "polymerase chain reaction" and, "Biomarkers". Journals were reviewed and relevant articles were selected with high precision.

Genetic Insights into Autoimmune Disease

Genetic markers and environmental factors both contribute to the development of autoimmune diseases. While genetic influence is less than 50%, environmental factors like particulate matter (PM) pollution play a significant role, especially in genetically susceptible individuals. PM exposure can trigger immune dysregulation and chronic inflammation, making it important to address both genetic markers and environmental factors in preventing and managing autoimmune diseases. it is important to note that genetic markers play a crucial role, as they contribute to the individual's susceptibility to environmental triggers. Therefore, efforts to reduce PM pollution should be coupled with genetic markers to effectively prevent and manage autoimmune diseases, as described in the study conducted among the normal adult population residing in a metropolitan city in India(Kumar et al., 2021).

Genetics plays a crucial role in understanding the genetic basis of ADs, as it influences genetic variation and disease risk. Natural selection, driven by factors such as pathogens, has shaped human genetic diversity, including genes related to immune function. Recent advancements in technology and large-scale projects have provided evidence for the contribution of natural selection to the heritable component of ADs. According to the researchers, the genetic regions associated with susceptibility to different ADs and the evidence for selection, shed light on functional variants and biological mechanisms. By integrating population genetics and AD susceptibility studies, we can uncover the genetic causes of human diseases and advance personalized medicine (Ramos et al., 2015).

(Gersuk & Nepom, 2009)discovered that many autoimmune diseases, like type I diabetes, multiple sclerosis, and narcolepsy, have a genetic connection to a specific gene called HLA-DQB10602. However, researchers have developed a faster and more cost-effective real-time PCR assay by using sequence-specific primers and probes and assured rapid and sensitive identification of this allele. This assay accurately detects the presence of the HLA-DQB10602 gene, allowing researchers to study it more efficiently. It has been validated with great success, showing 100% accuracy in identifying the gene in blinded and unblinded samples. This breakthrough could have significant implications for understanding and studying autoimmune diseases on a larger scale.

Wu et al., 1997 have identified a genetic variation in a protein called FcgammaRIIIA, which affects how immune cells bind to antibodies. This variation, specifically a substitution of phenylalanine with valine at position 176, was found to increase the binding of certain antibodies in individuals with the V/V genotype compared to those with the F/F genotype. Moreover, NK cells with the V/V genotype exhibited higher calcium levels, stronger activation, and faster induction of cell death when stimulated with antibodies. The research also discovered a strong association between the low-binding phenotype and systemic lupus erythematosus (SLE), particularly in patients with nephritis, while the homozygous high-binding phenotype was underrepresented. These findings suggest that the genetic variation in FcgammaRIIIA has significant implications for immune responses and may extend beyond autoimmune diseases to host defense mechanisms. This study concluded with the importance of further investigations to fully understand the underlying mechanisms and broader impact of polymorphism behind autoimmune diseases.

Molecular methods: a progressive diagnostic approach

Molecular methods like PCR can be considered a powerful tool in the diagnosis and research of autoimmune diseases, although it is not typically used as a direct diagnostic method. PCR-based techniques can help to identify genetic variations associated with autoimmune diseases, assessing an individual's susceptibility to developing the condition. Additionally, PCR can detect infectious agents that may trigger or exacerbate autoimmune diseases. It can also be used in conjunction with other techniques to detect and quantify specific autoantibodies related to autoimmune diseases, aiding in diagnosis and monitoring disease activity. However, autoimmune disease diagnosis is a complex process that relies on a combination of clinical assessment, medical history, and laboratory tests, with PCR serving as a supportive tool in the overall diagnostic approach(Berg & E.G, 2016; Castro & Gourley, 2010).

The study of non-coding RNAs (ncRNAs) holds great promise in the field of autoimmune diseases. Firstly, identifying specific ncRNAs associated with diseases can serve as biomarkers for diagnosis and prognosis. These biomarkers can be found in various accessible sites, such as blood, urine, and saliva, making them easier to detect. Secondly, understanding the mechanistic role of ncRNAs in disease processes can lead to a better understanding of the underlying mechanisms (D. Kumar et al., 2023). It is possible to detect non-coding RNAs (ncRNAs) using quantitative real-time PCR (qPCR), yet important to note that the design of specific primers and the optimization of PCR conditions are crucial steps in detecting ncRNAs accurately. Additionally, there are alternative methods available for ncRNA detection, such as RNA sequencing (RNA-seq) and northern blotting, which provide comprehensive and sensitive approaches for studying ncRNAs.

Rheumatoid arthritis (RA) is one of the chronic, inflammatory, and systemic autoimmune diseases that affect the elderly population worldwide, causing long-lasting bone and ligament damage. While there is no permanent solution for RA, advancements in next-generation sequencing (NGS), as well as PCR technology, have improved the efficacy of diagnosing the presence of rheumatoid arthritis. NGS enables the efficient sequencing of specific genomic fragments, aiding in the evaluation of RA within individuals. Biomedical research has focused on utilizing NGS and ribonucleic acid (RNA) sequencing to enhance the understanding and treatment of RA. The advanced genetic transfer capabilities offered by NGS have revolutionized the diagnosis and management of rheumatic diseases, including RA, by providing comprehensive genetic information for targeted and personalized approaches to treatment (Pati et al., 2023).

Gersuk & Nepom, 2009 has been developed a novel real-time PCR assay for the rapid and accurate identification of several autoimmune diseases. Compared to existing methods, this assay offers significant advantages including minimal hands-on time, cost savings, and avoidance of post-PCR contamination. Validation studies using blinded and unblinded samples demonstrated 100% accuracy, sensitivity, and specificity. Furthermore, analysis of a narcolepsy cohort using DNA isolated from buccal swabs confirmed the robustness and applicability of this assay as an alternative approach to traditional HLA typing methods.

The complement component C4 (C4) gene is located ~500 kb from DRB1 and DQB1, which are the genes that exhibit the strongest association with numerous autoimmune diseases. This proximity to C4 makes it a highly variable gene within the complement pathway. The relationship between C4 variation and type 1 diabetes provides significant insights into the potential role of HERV-K(C4) as a novel marker for the disease. By investigating the highly variable C4 gene Directions and its association with type 1 diabetes, the researchers reveal that individuals with the disease have fewer copies of HERV-K(C4), independent of known major histocompatibility complex class II susceptibility alleles. These findings suggest that HERV-K(C4) may contribute to functional protection against type 1 diabetes, challenging previous associations attributed to specific HLA-DQB1 alleles. This enhances the understanding of genetic factors underlying type 1 diabetes, yet further research is needed to explore the mechanisms by which HERV-K(C4) influences the disease and to validate these findings in larger and more diverse populations (Rodriguez-Calvo et al., 2015)

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) caused by AIRE gene mutations disrupts central tolerance and leads to immune attack, contributing to PCOS and infertility, characterized by ovarian autoimmune damage, is associated with reduced regulatory T cells (Tregs). However, the role of AIRE gene expression and its involvement in Treg insufficiency through the HIF1A-FOXP3 axis in PCOS remains unclear. The study of Padmanabhan et al., 2023 identified that in individuals with this autoimmune PCOS, a decrease in AIRE gene expression led to increased levels of HIF1A and reduced levels of FOXP3. This imbalance resulted in a deficiency of regulatory T cells (Tregs), which play a crucial role in immune regulation. These findings highlight the importance of the AIRE-HIF1A-FOXP3 pathway in PCOS and shed light on its impact on Treg insufficiency.

Behçet's Disease (BD) is a chronic autoimmune disease characterized by unknown causes. The role of adipokines, which regulate immune responses, is thought to be significant in the development and advancement of BD, the researchers examined the association between adiponectin and leptin gene variants and Behçet's Disease (BD). Using PCR, the researchers identified specific gene variants in BD patients, indicating a potential genetic predisposition. Additionally, elevated adiponectin levels in BD patients suggest its involvement in disease progression. PCR's importance in detecting these gene variants highlights its relevance in autoimmune disease research (Kahmini et al., 2023).

Future Directions

PCR-based approaches will continue to emerge as powerful tools for the diagnosis and monitoring of autoimmune diseases in the future. The challenges in accurately diagnosing autoimmune disorders, due to their diverse nature and overlapping symptoms, will be further addressed through advancements in PCR techniques. Researchers and clinicians will leverage PCR to make significant advancements in understanding, early detection, and differentiation of autoimmune diseases, leading to improved patient care and outcomes. PCR-based methods will revolutionize the field by providing enhanced sensitivity, specificity, and rapidity in identifying genetic variations associated with autoimmune diseases. These advancements will not only facilitate precise diagnosis but also offer valuable insights into disease pathogenesis, patient stratification, and treatment efficacy. However, further advancements in PCR technologies will be necessary to enhance the accuracy, efficiency, and accessibility of autoimmune disease diagnostics, ultimately enabling personalized and targeted diagnosing interventions.

Summary

Based on the findings and advancements discussed in this review, future directions in PCR-based approaches for autoimmune disease diagnosis and monitoring should focus on several key aspects. Firstly, further research should be conducted to identify and validate additional genetic markers and biomarkers specific to different autoimmune diseases, enabling more accurate and specific diagnostic tests. Additionally, efforts should be made to standardize PCR protocols and optimize assay conditions to ensure reproducibility and comparability across different laboratories. The integration of PCR with other molecular techniques, such as next-generation sequencing and non-coding RNA analysis, should be explored to provide a more comprehensive understanding of autoimmune disease mechanisms and potential therapeutic targets. Lastly, there is a need to enhance the accessibility and affordability of PCR-based tests, potentially through the development of simplified and cost-effective PCR platforms, to ensure widespread adoption and benefit for patients worldwide. Overall, by addressing these future directions, PCR-based approaches have the potential to further revolutionize autoimmune disease diagnostics and monitoring, paving the way for personalized and targeted therapeutic interventions.

Abbreviations

PCR - Polymerase Chain Reaction

AD - Autoimmune Disease

AID - Autoimmune Disease

RA - Rheumatoid Arthritis

MS - Multiple Sclerosis

SLE - Systemic Lupus Erythematosus

PM - Particulate Matter

NGS - Next-Generation Sequencing

RNA - Ribonucleic Acid

HLA - Human Leukocyte Antigen

HERV-K(C4) - Human Endogenous Retrovirus-   K(C4)

APECED- Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy

PCOS - Polycystic Ovary Syndrome

BD - Behçet's Disease

Conflict of interest

There is no conflict of interest.

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