



STANDARDISED MOLECULAR GENETIC MICROARRAYS FOR LABORATORY DIAGNOSTICS

By: Dr Jacqueline Gosink, Product Manager, EUROIMMUN AG, Luebeck, Germany

INTRODUCTION

Molecular genetic analysis is gaining new momentum in diagnostics as the genetic components behind many diseases become better characterised. Human leukocyte antigen (HLA) alleles, for example, exhibit extreme genetic polymorphism. Specific alleles are associated with particular autoimmune and inflammatory diseases, for example coeliac disease, psoriasis and ankylosing spondylitis, and HLA determination is now an important tool for both diagnosis and prediction of disease risk. Other well-characterised mutations, such as those in the blood coagulation factors V and II, are analysed in the management of conditions such as thrombophilia. The following examples illustrate how genetic parameters are now an integral part of routine diagnostics.

HLA-DQ2 AND DQ8 IN COELIAC DISEASE

Coeliac disease (CD) is an immune-mediated systemic disorder triggered in genetically predisposed individuals by gluten and related prolamins. It can present in many forms, including classic, silent, latent or potential variants. In addition to gastrointestinal symptoms, its manifestations include osteoporosis, neuropathies, carditis, pregnancy problems, lymphoma and Dühring's dermatitis herpetiformis. The only effective treatment is observance of a gluten-free diet.

The principle determinants of genetic susceptibility for CD are the HLA alleles DQ2 and DQ8, which are found in near to 100% of sufferers. Around 95% exhibit the DQ2 genotype, while the remainder have the DQ8 genotype. However, the presence of HLA-DQ2/DQ8 is not sufficient by itself to cause coeliac disease. Around a third of the healthy population exhibits DQ2/DQ8 alleles. Thus, the value of HLA-DQ2/DQ8 analysis lies predominantly in exclusion diagnostics.

The role of HLA-DQ2/DQ8 in coeliac disease diagnostics has gained pivotal significance with the recent publication of new guidelines from the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). HLA-DQ2/DQ8 analysis is now recommended as the first-line test for screening asymptomatic persons who have a high risk of developing CD, for example relatives of CD patients or persons with associated conditions such as type I diabetes mellitus, Down's syndrome, autoimmune thyroid or liver disease, Turner's syndrome, Williams' syndrome or selective IgA deficiency. If HLA-DQ2/DQ8 is negative, the risk of CD is negligible and no further follow up is necessary.

Determination of HLA-DQ2/DQ8 is also recommended by ESPGHAN as a confirmatory test in the diagnosis of CD in symptomatic persons. It is one of a triad of laboratory parameters, along with anti-tissue



transglutaminase antibodies and anti-endomysium antibodies that can be now employed to diagnose CD without biopsy in these individuals. HLA-DQ2/DQ8 is also a useful exclusion parameter for clarifying ambiguous cases, especially in infants and persons already on a gluten-free diet, and for differentiating CD from other intestinal diseases.

HLA-CW6 IN PSORIASIS

Psoriasis is a chronic inflammatory autoimmune disease, which manifests predominantly on the skin, but can also affect other organs such as joints, eyes and vascular system. It is one of the most common inflammatory skin diseases, and affected individuals often suffer stigmatisation and social exclusion. There is currently no cure, and treatment is based on the relief of symptoms and psychosocial therapy.

There is a strong genetic component to psoriasis. Around 40% of psoriasis cases are familial, with a concordance rate in identical twins of 62-70% and in non-identical twins of 21-23%. The most powerful genetic marker for this disease is the allele coding for the tissue antigen HLA-Cw6. Around 67% of psoriasis patients exhibit HLA-Cw6, compared to 10-20% of the general population. But not all predisposed persons develop psoriasis, as further triggers such as infections, drugs and extreme stress must also be present.

HLA-Cw6 determination is particularly important in the differential diagnosis of chronic inflammatory skin diseases, since this variant is strongly associated with type 1 psoriasis vulgaris and guttate psoriasis, but only comparatively weakly associated with type 2 psoriasis vulgaris. Since type 1 psoriasis vulgaris has a more severe course than type 2, the presence of HLA-Cw6 indicates a more serious form of disease. An accurate diagnosis can help with decisions concerning therapy and preventative measures to be implemented.

HLA-B27 IN ANKYLOSING SPONDYLITIS

Ankylosing spondylitis (Bechterew's disease) is a chronic inflammatory disease of the axial skeleton, extremity joints and tendon insertions, which affects predominantly men between the ages of 15 and 30. It is not curable, and therapy focuses on reducing symptoms and managing pain. There is a clear relationship between ankylosing spondylitis and the tissue antigen HLA-B27. Around 90% of patients are carriers of HLA-B27, compared to 6-9% of the general population. However, only 3-6% of carriers actually develop the disease. HLA-B27 is also linked to a number of other conditions, including Reiter's syndrome, inflammatory eye disorders, various forms of arthritis and chronic inflammatory bowel diseases.

HLA-B27 determination is an important component in the diagnosis of ankylosing spondylitis and associated rheumatic diseases. Further, discrimination between different HLA-B27 subtypes is critical, since some HLA-B27 subtypes are associated with disease, while others are not. Thus, a thorough genetic analysis encompassing a comprehensive differentiation of subtypes is a prerequisite.

FACTOR V AND II MUTATIONS IN THROMBOPHILIA

Thrombophilia is an increased tendency for blood coagulation. It can be fatal, with deep and superficial thrombosis and thromboembolism of the brain and coronary vessels the most frequent causes of death. The condition results from a combination of genetic and acquired factors. The latter include old age, long-term immobility, varicose veins, use of oral contraceptives and hormone replacement therapy.

The most important genetic risk factor is the factor V Leiden mutation. This is a point mutation in the factor V gene, which results in the exchange of one amino acid. The altered factor V cannot be sufficiently inactivated by activated protein C (APC) leading to an increased thrombotic tendency (APC resistance). Around 3-7% of the population has the heterozygous genotype, while homozygous mutations are relatively rare (approximately 0.2%).

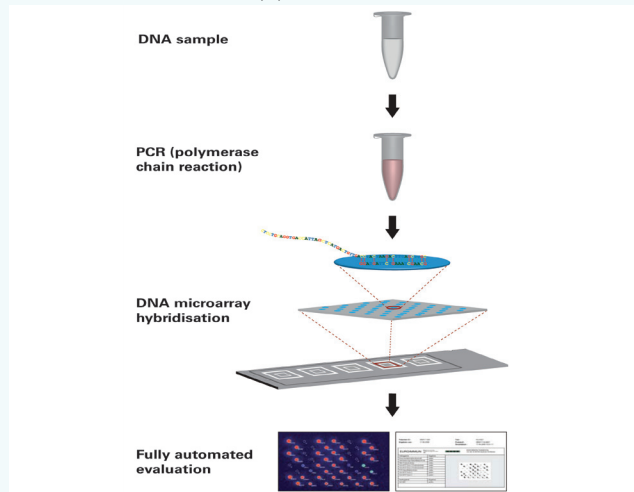
The mutation 20210G>A in the factor II (prothrombin) gene is also a major genetic risk factor. This mutation, located in the promoter region of the gene, leads to an increase in the prothrombin level in the plasma. Around 1-3% of the population is a heterozygous carrier, and less than 0.1% has the homozygous mutation. If both the factor V and factor II mutations are present, the risk of venous thrombosis is increased twenty fold, and this combination is frequently found in thrombophilia patients.

Analysis of factor V and II mutations is indicated in patients with thrombosis or embolism, particularly at a young age, at an atypical location or of unknown origin. Genetic clarification is also recommended in cases of recurring miscarriages, APC resistance, or protein C or S deficiency. Moreover, a genetic analysis is advised before giving oral contraceptives or hormone replacement therapy to women considered at risk of thrombosis or embolism.

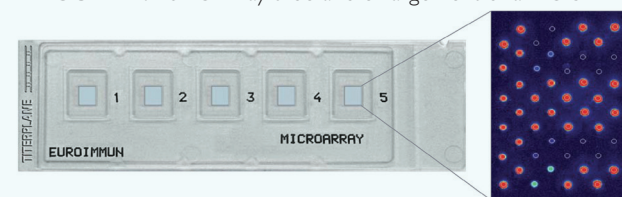
INNOVATIVE MICROARRAY TECHNOLOGY

Genetic disease factors can be analysed using microarray technology, such as the EUROArray system — a state-of-the-art molecular ►

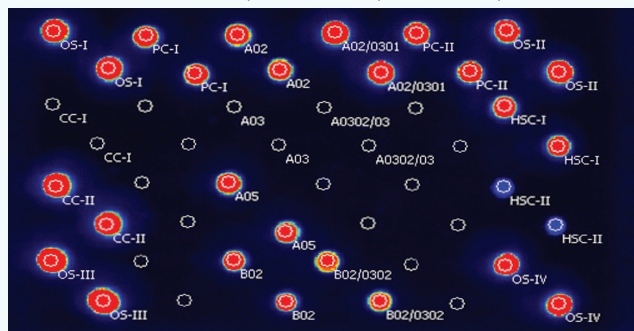
▼ **FIGURE 1:** EUROArray procedure



▼ **FIGURE 2:** EUROArray slide and enlargement of a BIOCHIP



▼ **FIGURE 3:** Microarray evaluation by EUROArrayScan

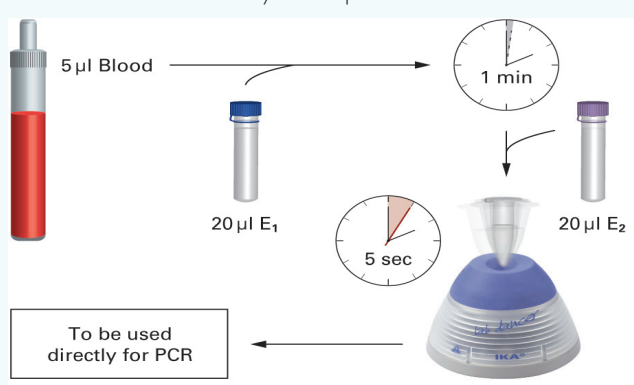


▼ **FIGURE 4:** Result documentation by EUROArrayScan

Patient ID :	110309-124	Test :	HLA-DQ2/DQ8
Result from :	11.3.2011	Protocol :	110311 HLA-DQ2/DQ8
Lot :	110225AB	Print date :	14.03.2011 10:10:38

EUROIMMUN	Medizinische Labordiagnostik AG	Automatic evaluation with the EUROMicroarray software
Partial result	Result	
Cross contamination control	valid	
Hybridisation specificity control	valid	
Positive control I	valid	
Positive control II	valid	
DQA1*02	positive	
DQA1*02/*0301	positive	
DQA1*03	negative	
DQA1*0302/03	negative	
DQA1*05	positive	
DQB1*02	positive	
DQB1*02/*0302	positive	
HLA-DQ2	positive	
HLA-DQ8	negative	
Test result	Result	
HLA-DQ2/DQ8	positive - DQ2	

▼ **FIGURE 5:** EUROArray Direct procedure for DNA extraction



genetic microarray platform which provides fast and easy determination of HLA-DQ2/DQ8, HLA-Cw6, HLA-B27, factor V Leiden and factor II 20210G>A, including fully automated data analysis.

In the simple test procedure (figure 1), disease-associated gene sections are amplified from purified genomic patient DNA samples by the polymerase chain reaction (PCR). The fluorescently labelled PCR products are then analysed using slides containing microarray BIOCHIPS (figure 2), which are composed of immobilised complementary probes. Specific binding (hybridisation) of the PCR products to their corresponding microarray spots is detected using a specialised microarray scanner system, and results are evaluated (figure 3) and documented (figure 4) using specially developed software (EUROArrayScan).

Certain parameters (currently HLA-B27, factor V Leiden and factor II 20210G>A) can be analysed directly from whole blood using the EUROArray Direct procedure (figure 5). The elimination of the DNA isolation step reduces the hands-on time dramatically, and there are no additional costs for DNA isolation kits anymore. In a typical test run with 40 samples, the hands-on time for the whole procedure from blood sample to result is reduced to 1.5 minutes per sample, without compromising the robustness of the test.

The sophisticated EUROArray constellations ensure unambiguous identification of the relevant alleles and subtypes. Highly specific primers, ready-to-use PCR components and integrated positive controls all contribute to the reliability of the analysis. Moreover, in contrast to traditionally used cytotoxicity tests, Terasaki methods or FACS analyses that are still applied in some laboratories for HLA-B27 determination, live leukocytes are not required. With microarrays, patient samples can be collected, stored for a time and analysed together as and when convenient.

DETAILED CLINICAL EVALUATION

All EUROArrays have been evaluated extensively using precharacterised samples. In a series of studies using a variety of reference methods including DNA sequencing and reference samples from the International Histocompatibility Working Group, each EUROArray demonstrated a 100% concordance with the applicable reference result (table 1).

In prevalence studies using panels of randomly selected samples, all EUROArrays yielded percentages of positivity that were in accordance with known prevalences in the population studied. This amounted to 18% for HLA-Cw6, 9.4% for HLA-B27, 37% for HLA-DQ2/DQ8, 4.2% for factor V Leiden and 1.7% for factor II 20210G>A.

Thus, the EUROArray system has demonstrated its ability to deliver highly reproducible and accurate results in the analysis of genetic determinants.

CONCLUSION

The identification of the genetic factors behind diseases such as coeliac disease, psoriasis, ankylosing spondylitis and thrombosis has paved the way for the development of diagnostic microarrays to analyse genetic susceptibility. Specialised microarrays such as the EUROArray provide highly sensitive and specific detection of disease-associated alleles without the need for any previous molecular biology knowledge. As research projects worldwide unravel more of the genome's secrets, there is enormous potential for further novel diagnostic microarrays. The possibility of preventing and managing disease by means of genetically tailored lifestyle and therapy decisions is a tantalising goal.

REFERENCES

References available on request (magazine@informa.com)