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MALDI-TOF mass spectrometry screening of cholelithiasis risk markers in the gene of *HNF1alpha* ☆

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ABSTRACT

In recent years MALDI-TOF MS gained importance for high-throughput DNA analysis. In the present study this technique was used for the pathogenetic analysis of gallstone disease. The intestinal apical sodium-dependent bile acid transporter (ASBT) shows a genetic association with gallstone disease. *ASBT* has 3 binding sites in its 5′UTR for hepatocyte nuclear factor 1alpha (HNF1alpha). We hypothesized that genetic alterations in the *HNF1alpha* gene could influence ASBT expression. The gene *HNF1alpha* was sequenced in 46 Stuttgart random samples, composed of 16 controls and 30 gallstone patients. Subsequently, two independent cohorts (Stuttgart: 67 gallstones carriers, 109 controls, Leutkirch: 112 gallstone carriers, 99 controls) were screened by MALDI-TOF MS. The subjects were further divided by gender and weight. 24 known polymorphisms and two novel SNPs in the 3′UTR of *HNF1alpha* were detected (*c.*220G>A* and *c.*1151G>A*). After gender-specific sub-division of the pooled cohorts, 4 SNPs resulted in significant differences between male gallstone carriers and male controls (Stuttgart/Leutkirch: *rs2255531* OR=2.78; p=0.006, *rs1169288* OR=2.13; p=0.032, *rs7310409* OR=2.34; p=0.025 and *rs1169294* OR=2.13; p=0.031). Two novel variants in the 3′UTR of *HNF1alpha* were detected and four SNPs of *HNF1alpha* show a significant association to cholelithiasis in male gallstone patients. This article is part of a Special Section entitled: Understanding genome regulation and genetic diversity by mass spectrometry.

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1. Introduction

As specific genetic variants gain more and more interest in the pathogenesis of several diseases, a rapid and cost-efficient method to establish genotyping approaches and to detect genetic aberrations is of major interest. Hereby matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a rapid and reliable method for nucleic acid-based analysis since a few years ago.

1.1. MALDI-TOF MS

MALDI-TOF MS is a method where biomolecules such as proteins or nucleic acids are ionized and analyzed according to their molecular mass. The biomolecules are embedded in

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an aromatic matrix characterized by a low molecular weight depending on the analyte. In most cases this matrix is composed of 4-methoxy cinnamic acid or 3-hydroxypicolinic acid [1]. The use of a laser with a defined wavelength results in desorption and ionization of the molecules which are then accelerated in an electric field into the time-of-flight (TOF) device [1]. The mass-to-charge ratio is calculated by a detector at the end of the tube and is limited by the resolution and ionization capabilities of the molecules.

First, Karas and Hillenkamp and Tanaka developed MALDI-TOF MS in 1988 for the identification and characterization of proteins [2,3]. In 2002 Tanaka was honored with the Nobel Prize in Chemistry for the advancement of methods to investigate protein structures.

1.2. MALDI-TOF MS and DNA

However, during the early 1990s the potential of MALDI-TOF MS in the DNA sequence analysis could also be proven [4,5]. With the optimization of a suitable matrix, Nordhoff et al. succeeded in sequencing a 24-base long oligothymidylic acid as well as small ribonucleic acids [6]. Further progress was made in the application of 3-hydroxypicolinic acid (HPA) as a matrix for longer DNA sequence analysis in a positive ion mode in combination with an optimized laser wavelength [7–9]. At the same time, Pieles et al. showed that treatment of the matrix with ammonium ions increases the desorption efficiency of natural and modified oligonucleotides. Degradation of the oligonucleotides by 5′- and 3′-exonucleases, combined with a subsequent analysis by MALDI-TOF MS results in the sequence determination of DNA and RNA [10,11]. Moreover, lower levels of chemical noise, reduction of the matrix background and an improvement in the mass resolution were achieved by the integration of the delayed extraction into the MALDI device [12]. These improvements made MALDI-TOF MS capable of detecting single-based variations at specific locations within the DNA sequence.

1.3. Different applications

1.3.1. Hybridization assay

Meanwhile various applications exist that generate allele-specific products which were subsequently analyzed by MALDI-TOF MS. The first one is the hybridization assay which uses the main principle of a perfect and therefore stronger binding of two complementary sequences in contrast to sequences paired with a mismatch. The mass of the hybridized probe is higher than the non-hybridized probe and can be identified by MALDI-TOF MS [13].

1.3.2. Oligonucleotide ligation assay

Another application combined MALDI-TOF MS with oligonucleotide ligation. Two oligonucleotides were linked to each other by a DNA ligase when binding complementarily adjacent to a genetic variation on a single-stranded template. Subsequent ligation steps cause an exponential enrichment of the ligation products. Polymorphic alleles cannot be ligated and can therefore be distinguished by MALDI-TOF MS [14].

1.3.3. Cleavage assay

Specific recognition and cleavage of an overlapping structure with two oligonucleotides, which bind next to each other on a single DNA strand, characterizes a cleavage assay. An upstream oligonucleotide (invader) and a downstream oligonucleotide (probe), which contains a tail of an unpaired sequence, form a DNA triplex structure. Thereby invader and probe overlap at one nucleotide in the complementary sequence and the probe is cleaved at the 5′ end. The unpaired sequence tail accumulates linearly and can then be detected by MALDI-TOF MS [15].

1.3.4. Primer extension assay

A simple application to distinguish two alleles for polymorphism genotyping and point mutation is the primer extension method [16]. An oligonucleotide binds complementarily adjacent to a polymorphism and is extended by a DNA polymerase through the variant. The extension products differ exactly at this position and have a different mass, which is analyzed by MALDI-TOF MS. So far numerous primer extension assays are devised.

1.3.4.1. PinPoint assay. In 1997, Haff and Smirnov developed the PinPoint assay, which combines the use of MALDI-TOF MS with the single-base primer extension reaction in the presence of four dideoxynucleotide triphosphates (ddNTP) to detect a single nucleotide polymorphism. Thereby, a specific primer anneals adjacent to a polymorphic site. In the presence of a DNA polymerase and four ddNTPs, the primer is extended by a single nucleotide complementary to the variant. The mass difference of the extended and non-extended primer is measured by MALDI-TOF MS and the addition can be clearly defined [17].

1.3.4.2. GOOD assay. In 2000, Sauer developed the GOOD assay, which increases the sensitivity of DNA detection by chemical modification. The modification step is implemented in the primer extension reaction and transfers the DNA products either in a positive or negative charge which increases the sensitivity of MALDI to approximately 100-fold. All reaction steps are carried out in a single tube to simplify handling [18,19].

1.3.4.3. PROBE assay. Braun et al. performed the primer extension reaction with three deoxynucleotide triphosphates (dNTP) and one dideoxynucleotide triphosphate (ddNTP). The consequence is a termination of the reaction at the position of the incorporated ddNTP. The different number of bases added to the primer distinguishes the different alleles of a heterozygous polymorphism and results in a mass difference of at least 300 Da (Dalton). This *p*rimer *o*ligo *b*ase *e*xtension (PROBE) assay was applied in the detection of several gene mutations [20].

1.3.4.4. VSET assay. The *v*ery *s*hort *e*x*t*ension (VSET) assay is a modification of the PROBE assay whereby the primer extension reaction is carried out in the presence of three dideoxynucleotide triphosphates (ddNTP) and one deoxynucleotide triphosphate (dNTP). Hereby one allele is extended by one nucleotide whereas the other allele is extended by two

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