

Document downloaded from:

<http://hdl.handle.net/10251/78688>

This paper must be cited as:

Tortajada-Genaro, LA.; Mena-Mollá, S.; Niños Rodenes, R.; Puigmule, M.; Viladevall, L.; Maquieira Catala, Á. (2016). Genotyping of single nucleotide polymorphisms related to attention-deficit hyperactivity disorder. *Analytical and Bioanalytical Chemistry*. 408(9):2339-2345. doi:10.1007/s00216-016-9332-3.



The final publication is available at

<http://dx.doi.org/10.1007/s00216-016-9332-3>

Copyright Springer Verlag (Germany)

Additional Information

Genotyping of single nucleotide polymorphisms related to attention deficit hyperactivity disorder

Luis A. Tortajada-Genaro¹, Salvador Mena¹, Regina Niñoles¹, Marta Puigmule², Laia Viladevall², Ángel Maquieira¹

5

(1) Centro de Reconocimiento Molecular y Desarrollo Tecnológico (IDM) - Departamento de Química, Universitat Politècnica de València, Camino de Vera s/n, E46022 Valencia, Spain

(2) AB-Biotics SA (Barcelona, Spain)

10 ABSTRACT

Pharmacological treatment of several diseases, such as attention deficit hyperactivity disorder (ADHD), presents marked variability in efficiency and its adverse effects. The genotyping of specific single nucleotide polymorphisms (SNPs) can support the prediction of responses to drugs and the genetic risk of presenting comorbidities associated with ADHD. This study
15 presents two rapid and affordable microarray-based strategies to discriminate three clinically important SNPs in genes *ADRA2A*, *SL6CA2*, and *OPRM1* (rs1800544, rs5569, and rs1799971, respectively). These approaches are allele-specific oligonucleotide hybridization (ASO), and a combination of allele-specific amplification (ASA) and solid-phase hybridization. Buccal swab and blood samples taken from ADHD patients and controls were analyzed by ASO, ASA and a
20 gold-reference method. The results indicated that ASA is superior in genotyping capability and analytical performance.

Keywords: SNP genotyping, low-cost microarray technology, pharmacogenetics, attention deficit hyperactivity disorder.

25

Highlights

Pharmacogenetic genotyping easy-going tool

Microarray assay for doctor office applications

30 Screening of SNP related to a psychiatric disorder on polymer chip

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD), a neurodevelopmental disorder, is characterized by inattention, hyperactivity and impulsive behavior. This disease is a highly prevalent childhood-onset neuropsychiatric condition (about 5% in schoolchildren), and is also detected in adults [1,2]. One major clinical concern is choice of medication because therapeutic effectiveness and severe adverse events during treatment vary from patient to patient. In recent years, pharmagenomics has become a consolidated discipline. It informs physicians about molecular subtypes of diseases and which drug is more likely to effectively manage the disease [3,4]. Single nucleotide polymorphisms (SNPs) have been proposed as markers to identify the loci associated with complex diseases and their therapeutic treatment [5]. To date, pharmacogenetic research into ADHD has focused mainly on SNPs related to stimulant drugs [1,2,6]. In particular, identification in advance of patient-dependent effective drugs (nature and doses) has been correlated with reduced costs and better quality of life.

Unfortunately, the incorporation of genomic findings into health care systems is limited as the cost-effectiveness of analytical methods is the main drawback. In fact, the molecular diagnostics field is frequently limited to laborious costly methods that require significant infrastructure and skills, only available for specialized laboratory facilities [7]. The novel generation of diagnostic tools to detect SNPs from a biologic fluid sample is absolutely necessary to develop genuine personalized medicine [8]. The challenge will involve genotyping technologies that integrate conventional properties; i.e. accuracy, robustness, cost-efficiency or automation, with properties such as flexibility, portability or simplicity. Yet relatively few SNPs have been identified as being clinically relevant to treat any given disease. Therefore, the complexity of techniques could be abridged as low-throughput genotyping approaches would be suitable for most clinical scenarios.

Alternative methods to extend SNP genotyping technologies, such as Illumina and Affimetrix platforms, have been proposed and excellently reviewed [9-12]. Certain solutions lie in laboratory approaches, designed for the discrimination of only one SNP of a single patient, or which require equipment that cannot be deployed in resource-limited laboratories. Consequently, some genotyping platforms are far from being actually integrated into routine clinical practice [13].

An advance in SNP genotyping has been made and applied as a pharmacogenetic tool in ADHD treatment in decentralized medical centers. The system is based on microarray assays, performed on polycarbonate chips, and on the later colorimetric detection by a biorecognition process. The use of polymeric substrates is a good solution in research [14], but is key for commercial purposes. Polycarbonate is an excellent material given its properties: mass fabrication under high quality standards, adaptable fabrication of devices, exceptional optical

properties, wide chemical reactivity for probe anchoring [15,16]. By including similar steps to microarray assays performed on glass slides, our methodology uses a fast approach and an inexpensive non fluorescent scanner. Therefore, this method would be accessible for primary or secondary health care scenarios, and is affordable for limited health budgets.

In this study, the discrimination of polymorphisms was achieved by two low-throughput strategies. First, revised allele-specific oligonucleotide hybridization (ASO) was applied using specific probes that were immobilized on polycarbonate slides. Second, allele-specific amplification (ASA) was combined with a hybridization assay as a way to simultaneously detect amplified products.

For its use as a pharmacogenetic tool, three polymorphisms were studied. Relevant clinical evidence has been associated with ADHD drugs, such as methylphenidates and amphetamines, with two SNPs: rs1800544 in the alpha-2 adrenergic receptors (*ADRA2A*) gene and rs5569 located in the solute carrier family 6 member 2 (*SL6CA2*) gene [6,17,18]. Information about determining the risk of developing comorbidities associated with ADHD is also in demand [19]. Thus an increased risk for substance abuse has been associated with the genetic variants of polymorphism rs1799971, located in exon 1 of the mu-opioid receptor (*OPRM1*) gene. This method is examined for the resourceful integration of genetic testing into routine clinical practice and doctor office for ADHD management.

MATERIAL AND METHODS

Patients. Subjects (n=30) with ADHD symptoms and volunteers were diagnosed by specialist psychiatrists and recruited for the present study according to ethics and with informed consents. Buccal swab and blood samples were collected. The reference genotyping method was the GoldenGate assay with VeraCode Technology (Illumina), which is a high-throughput platform that combines primer extension and ligation to generate allele-specific products, followed by PCR for the amplification and hybridization to codified beads for individual readout.

DNA extraction. Extraction was performed using the PureLink Genomic DNA Mini Kit (Invitrogen), according to the manufacturer's instructions. Briefly, in order to prepare lysate from blood samples, 200 μ L of fresh blood, 20 μ L of proteinase K, 20 μ L of RNase and 200 μ L of kit buffer were added to a microcentrifuge tube. To prepare the human buccal swab lysate, brushes were placed inside the tube with 500 μ L of PBS, 20 μ L of proteinase K, 20 μ L of RNase and 500 μ L of kit buffer. After incubation at 55 °C for 15 min, 500 μ L of 96-100% ethanol were added to the tube and the lysate was loaded into the column and centrifuged at 10,000 rpm for 1 minute at room temperature. The flow-through was discarded and the column was washed twice using 500 μ L of wash buffer 1 and 2, respectively. Finally, DNA was eluted with 50 μ L of Tris-HCl, pH 8.6.

105 **Microarraying.** Each mixture of streptavidin (10 mg/L) and biotinylated-surface probe (50 nM)
in printing buffer (50 mM carbonate buffer, pH 9.6 and 1% glycerol (v/v)) was transferred to the
polycarbonate slide (25 mm×75 mm). To that end, a non contact printer (AD 1500 BioDot Inc.,
CA, USA) was used. The drop volume was 50 nL, and working temperature and relative
humidity were controlled at 25 °C and 90%, respectively. The microarray layout consisted of
110 five arrays of 6×6 dots (ASO approach), or six arrays of 4×6 dots (ASA approach). Each target
gene, positive control and negative control (immobilization and hybridization) had four
replicates with a 1-mm track pitch. The design restrictions and oligonucleotides used are
described in the Supplementary Material.

115 **ASO approach.** A triplex PCR reaction was carried out in a total volume of 12.5 µl. Each
reaction mixture contained 1x DNA polymerase buffer with 2 mM of MgCl₂ (Biotools), 200 µM
of dNTPs (Thermo Scientific), 20 µM of digoxigenin-11-dUTP (digoxigenin-X-5-aminoallyl-
2'-deoxy-uridine-5'triphosphate, Jena Bioscience), 0.4 µM of each specific primer, 0.5 units of
Taq DNA polymerase (Biotools), and 12.5 ng of the genomic DNA. Cycling conditions were:
120 initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 30 s,
primer annealing at 60 °C for 30 s, elongation at 72 °C for 30 s, and a final elongation at 72 °C
for 5 min.

A stock solution of saline sodium citrate buffer (SSC 20×) was prepared (NaCl 3 M, sodium
citrate 300 mM, pH 7). Prehybridization was performed by dispensing the prehybridization
125 solution (SSC 1.5×, formamide 30%, and Denhardt's solution 2.5×) over microarrays and
incubating slides at 50°C for 30 minutes in a conventional oven. For hybridization, 2 µL of PCR
product were mixed with 38 µL of hybridization solution (SSC 1.5×, formamide 30% and
Denhardt's solution 2.5×). The solution was denatured by heating at 95 °C for 5 min and
transferred onto the slide surface.

130 After incubation at 37°C for 1 h, slides were gently washed for 1 min with diminishing dilutions
of SSC (SSC 1×, SSC 0.5×, SSC 0.05×) and finally with water. Subsequently, a 1-mL mix of
1:2500 sheep anti-digoxigenin antibody (Abcam), and 1:300 anti-sheep antibody conjugated to
horseradish peroxidase (Abcam) in phosphate buffered saline solution with 0,05% Tween-20
detergent, was dispensed onto the slide and incubated for 30 min at room temperature in the
135 dark. After washing, 1 ml of 3,3',5,5'-tetramethylbenzidine reagent (ep(HS)TMB-mA, SDT,
Germany) was spread onto the slide surface and incubated for 8 min at room temperature. Then
slides were washed with water and read.

ASA approach. Two triplex PCR reactions (12.5 μ L) were performed by changing the forward primers for the studied polymorphisms. Hence mixture A contained primers with a lower melting temperature and mixture B contained those with a higher melting temperature. The reaction mixture contained 1 \times DNA polymerase buffer without magnesium, 3 mM of MgCl₂, 100 μ M of dNTPs, 10 μ M of digoxigenin-11-dUTP, 0.3 μ M of each specific primer, 0.5 units of DNA polymerase and 4 ng of genomic DNA. Cycling conditions were: initial denaturation at 95 $^{\circ}$ C for 5 min followed by 35 cycles of denaturation at 95 $^{\circ}$ C for 30 s, primer annealing at 64 $^{\circ}$ C (mixture A) and 66 $^{\circ}$ C (mixture B) for 30 s, elongation at 72 $^{\circ}$ C for 30 s, and final elongation at 72 $^{\circ}$ C for 5 min.

The products from both mixtures were hybridized separately in two microarrays on polycarbonate slides, but contained the same oligonucleotides, called common probes. The microarray assay was performed as described above, but the pre-hybridization step was not required and a less stringent hybridization buffer (SSC 1.5 \times , formamide 25%, and Denhardt's solution 2.5 \times) and soft washing protocol (SSC 0.1 \times solution and water) were used. Subsequently, an immunoreaction developed the hybridization products.

Detection. Chips were directly scanned (Epson Perfection 1640SU office scanner), and the variation of the reflection properties was measured given the presence of the biorecognition product. If there was no reaction product, the maximum intensity of the reflected beam was collected (background signal). If target gene-probe recognition and subsequent solid deposit formation occurred, the light would strike the product, which would attenuate beam intensity (spot signal). Then gray-scale images (Tagged Image File Format, color depth 16 bit, scale 0-65535) were generated and the optical intensity signals of each spot were quantified using in-home software. Image processing (feature gridding, addressing, segmentation and quality assurance) was automatically performed in under 5 min.

Discrimination criteria. The analysis was declared valid if the controls provided correct responses, and one or two probes of each SNP provided a signal-to-noise ratio above 5. The genotype decision rule was constructed based on the responses of specific probes for each polymorphism. A discrimination index was calculated from the signal of the wild-type (WT) and mutant (MUT) variants, according to the expression $(WT - MUT)/(WT + MUT)$.

Statistical package Statgraphics Centurion for Windows v.16 was used for the data analysis.

RESULTS

Allele-specific oligonucleotide hybridization (ASO approach)

175 A triplex PCR was run for the simultaneous amplification of all the target polymorphisms (rs1800544, rs5569, and rs1799971) using specifically designed primers. The most important variables were MgCl_2 concentration, primer concentrations, number of cycles and annealing temperature. Under the selected conditions (Table 1), the amplification factor was $(2.4 \pm 0.3) \times 10^9$, as determined by fluorescence measurements.

180 The critical step was the hybridization of the given products with allele-specific probes (wild-type and mutant) on polycarbonate slides. The optimization experiments are provided in the Supporting Material and Table 1 summarizes the best conditions. Figure 1 shows the signal-to-noise ratios (SNR) recorded by the scanner, which demonstrates the selectivity of the hybridization pattern. A detectable signal was obtained for the perfect-matched duplexes and a
185 background response (or approached) was acquired for the non matching probes. Therefore, the simultaneous genotyping of three SNPs related to ADHD was possible.

Allele-specific amplification (ASA approach)

Targeted gene regions were simultaneously amplified using allele-specific primers. For this
190 purpose, two triplex reactions (A and B) were run to obtain specific products for both variants of each polymorphism (rs1800544, rs5569, and rs1799971). The nature of the DNA polymerase, the use of modified primers, and the annealing temperature during thermocycling were optimized (see the Supplementary Material). Under the selected conditions (Table 1), the amplification of the DNA template from a homozygous patient with the incorrect primer was
195 comparable to the negative controls (t-test: p-value >0.05). The amplification factor for the correct primer was $(1.3 \pm 0.3) \times 10^9$.

The detection of ASA products was achieved with a hybridization assay run on a polycarbonate slide following a similar protocol to the ASO approach. In this case, the PCR products of reactions A and B were incubated on two microarrays with a specific immobilized probe per
200 gene (common probe independently of the genetic variant). The hybridization conditions (temperature, time, and buffer composition) were optimized by avoiding cross-reactivity between different genes. Under the selected experimental conditions (Table 1), the comparison of the hybridization patterns for both microarrays allowed SNP genotyping (Figure 1). A heterozygous patient led to effective amplification in both reaction mixtures and, consequently,
205 to positive hybridization in both microarrays (SNR > 3). Amplification occurred in a single reaction mixture for a homozygous patient, with a positive result obtained only in the corresponding microarray.

Method features for SNP genotyping

210 Different experiments were performed to establish the capabilities of both methods (ASO and
ASA) for the genotyping of the three ADHD-related SNPs. Sensitivity was studied by preparing
heterozygous mixtures with decreasing percentages of mutant-type DNA compared to the wild
type. Mutant DNA was detected up to 4% (ASO) and 2% (ASA), which indicates that the
system was capable of discriminating both genotypes selectively. Equimolar mixtures of
215 genomic DNA from the WT and MUT homozygous patients were prepared and analyzed. For
both approaches, microarray intensities were comparable to the heterozygous genotypes for the
three polymorphisms (test t: $p > 0.1$). Intra-day reproducibility and inter-day reproducibility,
expressed as the relative standard deviation of spot intensities for triplicate assays, were lower
than 15% and 22%, respectively.

220 The analytical performances, shown in Table 2, demonstrated that both developed methods were
adequate as SNP genotyping tools. In fact, the ASO results showed a notably shorter analysis
time and lower cost compared to other published or commercially available allele-specific
hybridization-based methods [6,9,20]. These platforms need long protocols, e.g. overnight
hybridization incubations, and also expensive detection equipment. The method developed
225 herein to detect ASA products is much simpler and has a greater multiplexing capability than
previously reported methods [8,21,22]. In these studies, allele-specific products were detected
by electrophoresis or fluorescent dye (real-time or post-amplification). This is also a simpler
approach than previous methods that use hybridization assays on glass chips as it avoids
chemical activation of the surface, surface blocking and using a fluorescent detector [12].

230 After comparing both the methods developed herein, we found that most assay performances
were similar. However, the number of simultaneously analyzed samples was larger in the ASO-
based method, while the amount of DNA template required was smaller or the assay time was
shorter in the ASA-based method. The most marked difference was the efforts made to set-up
the multiplex-assay, particularly detection in a microarray format. The design of
235 oligonucleotides and hybridization buffer composition were more critical in the ASO approach.
The intrinsic characteristics of ASA genotyping involved a less difficult process to select
optimal conditions. Hence the capabilities of the ASA-based method seems less limited than the
ASO-based method to extend this genotyping tool to other pharmacogenomic applications.

240 Analysis of ADHD patients

The discrimination index was calculated for each studied SNP using the signal-to-noise
responses obtained from the three variants (both homozygous and heterozygous individuals).
Although different human samples brought about slight variations in the concentration of the

245 PCR products, and consequently scored different optical intensities, the discrimination index remained nearly constant for intra-groups (ANOVA test: $p\text{-value} > 0.05$). A clear difference was found among the three groups, which allowed the unequivocal genotype assignment of the investigated SNP locus. The heterozygous genotypes produced an intermediate discrimination factor (between -0.3 and +0.3), whereas the homozygous ones led to discrimination factors above 0.3 (wild-type) and under -0.3 (mutant), respectively.

250 The method was applied to identify the three loci related to ADHD drugs (rs1800544, rs5569, and rs1799971) in the blinded buccal swab and blood samples. Figure 2 illustrates an example of the microarray images for two patients with a known genotype. As we can see, positive and negative controls provided detectable and background responses, respectively. In all cases, positive optical signals were observed according to the specific polymorphism in the
255 corresponding probes. Nevertheless, the reported genotyping was based on the discrimination factors calculated for each studied SNP, and three populations were perfectly distinguished (Figure 3).

The accuracy of the genotype calls by the microarray-based methods was verified by analyzing the same genomic DNA extracts from ADHD patients using the Illumina Goldengate platform
260 (Supplementary Material). Only five polymorphisms were genotyped erroneously by ASO. The coincidence percentages fell between 90 % (rs5569) and 100 % (rs1800544) for the ASO approach, and were 100 % for the ASA approach. The obtained values were comparable to other low-throughput simple methods that used a single primer/probe per loci [23,24]. The reliability of the ASO approach can be enhanced by increasing the number of probes by SNP or
265 including stem probes [6-9]. The comparison to other performances revealed the potential of the technique presented herein. Like other high-throughput platforms, the Goldengate bead-array technology was able to analyze large numbers of SNPs per patient. However, the time required to perform the entire automated process takes about 3 days and involves extremely expensive equipment. In contrast, the developed methods provided genotyping information tailored for the
270 specific disease, e.g. ADHD, in 3.5 h with equipment that is accessible to, and can be afforded by, almost any laboratory (thermocycler, heating block, and an office scanner or optical microscope).

CONCLUSIONS

275 The pharmacogenetics applied to personalized medicine aims to identify patterns of genetic variations with cost-effective tools. These systems would make diagnoses in decentralized laboratories, which would streamline management and patient care. This paper addresses this aim developing two fast simple and cheap genotyping methods. Both are based on a simple array assay performed on a planar polycarbonate support for so-called individualized therapy in

280 ADHD. Although the ASO approach gave adequate results, the ASA approach, based on
hybridization with a common probe immobilized on a solid support, yielded better results. The
experiments confirmed that the SNP genotyping of ADHD patients, supported by analyzing
their genetic code from biological fluids, is actually possible. Nevertheless the present study
must be considered the initial step to develop integrated cheap devices, such as lab-on-a-chips,
285 fabricated in plastics and combined with a low-cost detection system.

Acknowledgements

This research has been funded through projects FEDER MINECO INNPACTO IPT-2011-1132-
010000, CTQ/2013/45875R and PrometeoII/2014/040 (GVA).

290

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. Informed consent was obtained from
all individual participants included in the study.

REFERENCES

- 295 1. Cortese S (2012) *Eur J Paediatr Neurol* 16:422-433
2. Contini V, Rovaris DL, Victor MM, Grevet EH, Rohde LA, Bau CH (2013) *Eur Neuropsychopharmacol* 23:555-560
3. Gardiner SJ, Begg EJ (2006) *Pharmacol Rev* 58(3): 521-590
4. Abul-Husn NS, Obeng AO, Sanderson SC, Gottesman O, Scott SA (2014)
300 *Pharmacogenomics Pers Med* 7:227
5. Altman RB, Flockhart D, Goldstein DB (Eds.) (2012) *Principles of pharmacogenetics and pharmacogenomics*. Cambridge University Press.
6. Hawi Z, Cummins TDR, Tong J, Johnson B, Lau R, Samarrai W, Bellgrove MA (2015). *Mol Psychiatry*. 20:289-297
- 305 7. Limaye N (2013). *Appl Transl Genomics* 2:17-21
8. Manolio TA, Chisholm RL, Ozenberger B, Roden DM, Williams MS, Wilson R, *et al.* (2013) *Genet Med* 15:258-267
9. Kim S, Misra A (2007) *Annu Rev Biomed Eng* 9:289-320
10. Lucarelli F, Tombelli S, Minunni M, Marrazza G, Mascini M (2008) *Anal Chim Acta*
310 609:139-159
11. Knez K, Spasic D, Janssen KP, Lammertyn J (2014) *Analyst* 139: 353-370
12. Choi JY, Kim YT, Byun JY, Ahn J, Chung S, Gweon DG, *et al.* (2012) *Lab Chip* 12:5146-5154
13. Ragoussis J (2009) *Annu Rev Genomics Hum Genet* 10:117-33
- 315 14. Sethi D, Gandhi RP, Kuma P, Gupta KC (2009) *Biotech J* 4:1513-1529
15. Bañuls MJ, García-Piñón F, Puchades R, Maquieira A (2008) *Bioconjugate Chem.* 19: 665-672
16. Tortajada-Genaro LA, Rodrigo A, Hevia E, Mena S, Niñoles R, Maquieira A (2015) *Anal Bioanal Chem* 407:7285-7294
- 320 17. Kieling C, Genro JP, Hutz MH, Rohde LA (2010) *Pharmacogenomics* 11:407-419
18. Kim, B. N., Kim, J. W., Cummins, T. D., Bellgrove, M. A., Hawi, Z., Hong, S. B., *et al.* (2013) *J Clin Psychopharmacol* 33:356-362
19. Carpentier, P. J., Arias Vasquez, A., Hoogman, M., Onnink, M., Kan, C. C., Kooij, J. J. S., *et al.* (2013) *Eur Neuropsychopharmacol* 23:448-457
- 325 20. Zhang Y, Haraksingh R, Grubert F, Abyzov A, Gerstein M, Weissman S, Urban AE (2013) *Child Dev* 84:34-48

21. Asari M, Watanabe S, Matsubara K, Shiono H, Shimizu, K (2009) *Anal Biochem* 386:85-90
22. Choi JY, Kim YT, Ahn J, Kim KS, Gweon DG, Seo TS (2012) *Biosens Bioelectron*, 35:327-334
23. Konstantou JK, Ioannou PC, Christopoulos TK (2009) *Eur J Hum Genet* 17:105-111
24. Sebastian T, Cooney CG, Parker J, Qu P, Perov A, Golova J B., *et al.* (2014) *Clin Chim Acta*, 429:198-205

330

335

List of Figures

Figure 1. Comparison of microarray-based ASO and ASA approaches. Signal-to-noise ratios (SNR) recorded in each probe for a patient with genotypes: GC for rs1800544, GG for rs5569, and AG for rs1799971. WT: wild-type allele-specific probe. MUT: Mutant allele-specific probe. Parentheses indicates the allelic nucleotide.

Figure 2. Microarray images obtained for two patients. (a) ASO probes: 1= NC, 2=DPC, 3=HPC, 4=rs5569 (WT), 5=rs1800544 (WT), 6=rs1799971 (WT), 7=rs5569 (MUT), 8=rs1800544 (MUT), 9=rs1799971 (MUT). (b) ASA probes: 1=NC, 2=DPC, 3=HPC, 4=rs5569, 5=rs1800544, 6=rs1799971. Patient 1 genotypes: AG for rs5569, GC for rs1800544, and GG for rs1799971; Patient 2 genotypes: AG for rs5569, GG for rs1800544, and AA for rs1799971. Abbreviations: NC= Negative control; DPC: Development positive control; HPC: Hybridization positive control; WT: Wild-type allele-specific probe; MUT: Mutant allele-specific probe.

Figure 3. Discrimination of population groups based on ASA results. Boxplot of discrimination index for wild-type homozygous (left), heterozygous (central) and mutant homozygous (right) patients: (a) rs1800544, (b) rs5569, and (c) rs1799971.

List of Tables

360 Table 1. Optimal experimental conditions for SNP genotyping by both microarray-based methods.

Table 2. Analytical performances of both microarray-based methods.

365