J. Microbiol. Biotechnol. (2009), **19**(7), 635–646
doi: 10.4014/jmb.0803.226
First published online 23 October 2008

REVIEW

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Applications of DNA Microarray in Disease Diagnostics

Yoo, Seung Min[1], Jong Hyun Choi[1], Sang Yup Lee[1,2], and Nae Choon Yoo[3]*

[1]*Department of Chemical and Biomolecular Engineering (BK21 Program), BioProcess Engineering Research Center, Center for Systems and Synthetic Biotechnology, and Institute for the BioCentury, KAIST, Daejeon 305-701, Korea*
[2]*Department of Bio and Brain Engineering, and Bioinformatics Research Center, KAIST, Daejeon 305-701, Korea*
[3]*Department of Internal Medicine, Department of Oncology and Cancer Center, Yonsei University College of Medicine, Seoul 120-752, Korea*

Received: March 26, 2008 / Revised: August 28, 2008 / Accepted: September 8, 2008

Rapid and accurate diagnosis of diseases is very important for appropriate treatment of patients. Recent advances in molecular-level interaction and detection technologies are upgrading the clinical diagnostics by providing new ways of diagnosis, with higher speed and accuracy. In particular, DNA microarrays can be efficiently used in clinical diagnostics which span from discovery of disease-relevant genes to diagnosis using its biomarkers. Diagnostic DNA microarrays have been used for genotyping and determination of disease-relevant genes or agents causing diseases, mutation analysis, screening of single nucleotide polymorphisms (SNPs), detection of chromosome abnormalities, and global determination of posttranslational modification. The performance of DNA-microarray-based diagnosis is continuously improving by the integration of other tools. Thus, DNA microarrays will play a central role in clinical diagnostics and will become a gold standard method for disease diagnosis. In this paper, various applications of DNA microarrays in disease diagnosis are reviewed. Special effort was made to cover the information disclosed in the patents so that recent trends and missing applications can be revealed.

Keywords: DNA microarray, diagnosis, biomarker, genetic disorder, single nucleotide polymorphism

Diagnostic assays are playing an increasingly important role in the subsequent treatment of diseases. A rapid, accurate, and reliable diagnostic method is very important, as it allows identification of the disease for suitable therapy, which consequently can reduce the mortality rate and also the cost of treatment. The recently developed molecular diagnostic assays based on the hybridization of probe nucleic acids with target nucleic acids from clinical samples are allowing effective detection of various diseases with high speed, sensitivity, and specificity. In addition, the availability of the complete genome sequences of disease-causing organisms and of the human is changing the way of designing probes for the accurate diagnosis of diseases caused by them.

A DNA microarray is generally composed of a solid support onto which multiple DNA probes with known identities are fixed for molecular hybridization with clinical samples (J. P. Fruehauf *et al.* 2008: US7323300; M. J. Heller *et al.* 1998: US5849486A1; M. Chee *et al.* 1998: US5837832A1; E. A. Hubbell *et al.* 1999: US5856101A1; M. Chee *et al.*

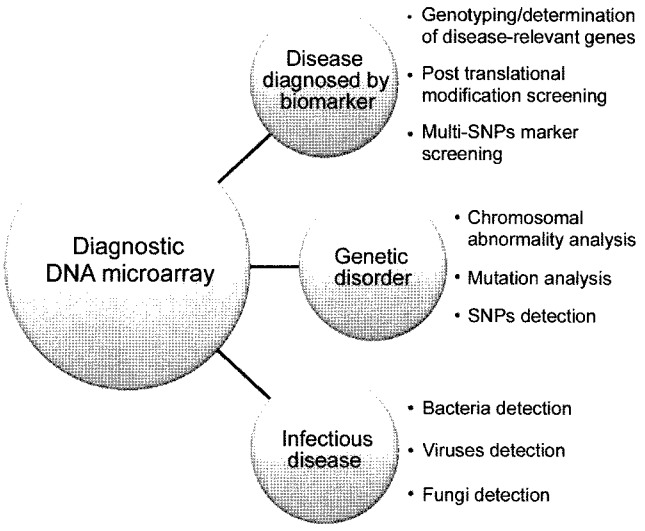

Fig. 1. Use of DNA microarrays for the diagnosis of diseases.
DNA microarrays can be used for diagnosing various diseases *via* genotyping and determination of genes involved in the diseases, detection of infectious pathogens from the samples of patients, detection of chromosome abnormalities and mutations causing genetic disorders, SNPs screening, and global determination of posttranslational modifications associated with diseases.

*Corresponding author
Phone: +82-11-9163-7604; Fax: +82-2-393-3652;
E-mail: ync0011@yumc.yonsei.ac.kr

1999: US5861242A1; K.-K. Wong *et al.* 1999: US5925522A1). It has been utilized in various fields including gene discovery, disease diagnosis, drug discovery, and toxicological research [26]. The DNA microarray allows more rapid, reliable, efficient, reproducible, precise, and high-throughput detection of diseases or disease-causing agents compared with the other techniques used to date. Several applications of DNA microarrays for diagnosing specific diseases have been reported (Fig. 1): genotyping and determination of disease-relevant genes or disease-causative agents, mutation analysis using relatively low-density DNA microarrays, single nucleotide polymorphisms (SNPs) screening using high-density DNA microarrays, analysis of copy number changes at the level of chromosome using comparative genomic hybridization DNA microarrays (arrayCGH), and global determination of posttranslational modifications including methylation, acetylation, and alternative splicing.

This review focuses on the applications of DNA microarrays for diagnosing diseases. Application of DNA microarrays for diagnosing and studying cancer is not covered in this paper as there are numerous papers for the readers to consult on. As the intellectual properties can guide the direction of new research, we reviewed the patents on the applications of DNA microarrays for disease diagnosis (Table 1). First,

Table 1. Patents on the applications of DNA microarrays in disease diagnosis.

Classification	Patent No.	Target disease or disease-causative agent	Target genes or regions	Features
Diagnosis using disease-related biomarker	US6905827	Chronic inflammatory diseases (especially, systemic lupus erythematosis)	Whole genomic DNAs Disease-relevant gene	• Using genes obtained from microarray analysis for the expression of sequences in clinical samples
	US20040077020A1	Inflammatory bowel disease, Crohn's disease and ulcerative colitis	Acidic calponin, Arp 2/3 protein complex subunit 34-Arc, human antigen CD36, human epidermal growth factor receptor substrate, mRNA for Hrs, serine threonine kinase 11, mRNA for SHPS-1	• Microarray analysis for the expression of 2,400 sequences in peripheral blood mononuclear cells from patient and healthy • Development of diagnostic microarray consisting of gene sequence obtained cluster analysis • Confirmation of the expression of disease-related genes by using real-time PCR
	US20070292857A1	Type 1 diabetes	Histone H3-K4, H3-K9, H3-K79, H3-K36	• Chromatin immunoprecipitation (ChIP) and cDNA microarray analysis of dimethylation, trimethylation, and acetylation of target genes in THP-1 monocytes cell • ChIP and cDNA microarray analysis of dimethylation of target genes in THP-1 monocytes cell under high glucose *vs.* normal glucose conditions • ChIP and cDNA microarray analysis of dimethylation of target gene in normal vs. patients with Type 1 diabetes
Chromosome abnormalities detection	US20070048742A1	Down's syndrome, Patau syndrome, Edward syndrome, Turner syndrome, Klinefelter syndrome, Alpha-Thalassemia Retardation-16, Charcot-Marie-Tooth neuropathy 1A, Cri-du-chat syndrome, hereditary neuropathy with liability to pressure palsies disease, Prader-Willi syndrome, Rubinstein-Taybi syndrome, Williams syndrome, and Wolf-Hirschhorn syndrome	Disease-relevant chromosome	• Fabrication of microarray comprising specific chromosome-relevant BAC clone reconfirmed by *fluorescence in situ hybridization* (FISH) • Enable to detect the chromosome abnormality on genome of the organism or in intron region • Enable to investigate the relationship between specific disease and the detected chromosome and detect the genomic mutation aspect caused by endogeneous or exogeneous factor

Table 1. Continued.

Classification	Patent No.	Target disease or disease-causative agent	Target genes or regions	Features
Monogenic disease-related mutation detection	US6979542	Autosomal recessive disorders (ataxia telangiectasia, cystic fibrosis, sickle cell anemia, Tay-Sachs disease, Phenylketonuria, oculocutaneuos albinism, hereditary hemochromatosis, AAT deficiency, ADA deficiency, beta-thalassemia, alpha-1 antitrypsin deficiency, spinal muscular atrophy, Friedreich's atoxia, congenital adrenal hyperplasia)	Whole genomic DNAs, Disease-relevant gene	• Analysis of a genetic signature for heterozygous carriers of autosomal recessive disorders
	WO07083928A1	Corneal dystrophy	*βIGH3* gene	• Enable to detect the mutation associated with Avellino dystrophy (R124H), lattice type I dystrophy (R124C), Reis-Bucklers I (R124L), and granular type (R555W) • Showing 100% accuracy (n=98) compared with DNA sequencing
	US20040132015A1	Genetic disorder (*e.g.*, Wilson disease), single nucleotide polymorphisms (SNPs)	Disease-relevant gene (*ATP7B* gene)	• Identification of disease-associated mutation using codon-scanning algorithm in which allele-specific probes are designed by changing three consecutive bases of codon sequence of gene related to disease
	US20030073082A1	Congenital adrenal hyperplasia	21-Hydroxylase gene (exon 1, intron 2, exon 4)	• Immobilization of tandem cDNA fragments amplified from 7 sets of constructed vectors containing target genes
SNPs detection	US20060263815A1	Cardiovascular diseases	Disease-relevant gene	• Diagnosis of cardiovascular disease by detecting multiple single nucleotide polymorphisms in disease-relevant genes, yielding the distinct patterns of genotypes
	US20070196845A1	SNPs	Disease-relevant gene	• Detection of SNPs through four steps; hybridization, cleavage of mismatch sites, denaturation of hybridized dimer, and detection of four detection probes with dsDNA
Bacteria detection	US20020187490A1	*Pseudomonas fluorescens*, *P. chlororaphis*, *P. putida*, *P. aeruginosa*	Whole genomic DNAs	• Detection of arrayed probes to produce a hybridization pattern
	US20080020379A1	*Mycoplasma*, *Bordetella pertussis*, *Chlamydia pneumoniae*	Host genes expressed specifically by pathogen exposure	• Providing gene expression profiles caused by respiratory infection • Using gene expression markers from blood that are indicative of host response to exposure, response, and recovery of pathogen infections

the development of DNA microarrays containing the specific biomarkers for diagnosing various diseases including metabolic, autoimmune, and inflammatory diseases is reviewed. Then, DNA microarrays developed for the detection of mutations associated with genetic disorders are described. Finally, DNA microarrays developed for the detection and identification of infectious agents are reviewed. In particular, those for detecting pathogenic microorganisms causing infectious diseases such as sepsis, bacteremia, and pneumonia are reviewed in detail.

Table 1. Continued.

Classification	Patent No.	Target disease or disease-causative agent	Target genes or regions	Features
	WO03095677A1	*Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Peptostreptococcus anaerobius*, *Fusobacterium necrophorum*, *Enterobacter cloacea*, *Enterobacter aerogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Burkholderia cepacia*, *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Legionella pneumoniae*	23S rRNA, 16S-23S ITS	• Detection of 44 bacterial and 2 fungal pathogens (*Candida albicans*, *C. glabrata*) using species-specific probes • Using 7 bacteria universal probes that consist of sequences existing in all bacteria • Validation of species- and bacterial-specific probes with 300 clinical isolates
	US20030091991A1	*Bacillus*, *Bordetella*, *Branhamella*, *Chlamydia*, *Corynebacterium*, *Haemophilus*, *Mycobacterium*, *Orientia*	16S rDNA, 18S rDNA, toxin-related gene, specific antigen gene, antigenic gene, virulence-related gene,	• Using DNA microarray comprising specific target gene amplicons with species-specific primers
	US20060194223A1	*Staphylococcus*, *Shigella*, *Mycobacterium*, *Listeria*, *Campylobacter*, *Lactococcus*, *Bacillus*	23S rDNA	• Combined with zipcode oligonucleotides and DNA microarray, allowing identifying the specific species
	US20050136432A1	*Mycobacterium*	16S rDNA, drug resistance-relevant gene	• Enable to discriminate mycobacterial species from other pathogens and detect the resistance of antibiotics
	US6924094	*Mycobacterium*	*rpoB* gene	• Detection of mycobacterial species (*M. avium*, *M. gordonae*, *M. chelonae*, *M. kansasii*, *M. scrofulaceum*, *M. xenopi*, *M. intracelluare*, and *M. tuberculosis*) by using species-specific probes designed by tiling strategy for sequencing determination, which show distinct hybridization patterns
Viruses detection	US6759193	Hepatitis B virus	Surface antigen mutant 145 (glycine to arginine)	• Using probes detectable of the mutation change (G→A) of amino acid 145 of human hepatitis B virus surface antigen in serum samples
	US20030186222A1	Enterovirus	Enteroviral 5'untranslated region (5'-UTR)	• Combined nucleic acid sequence-based amplification, DNA microarray technology, and electrochemiluminescence • Enable to detect Poliovirus (1, 2, 3), Coxsackievirus (A9, A16, A21, A24, B1, B3, B4, B5), Echovirus (5, 6, 11, 12, 70, 71)
	US7301015	Human papillomavirus (HPV)	L1 region	• Enable to detect 22 HPV genotypes including 15 high-risk types (HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 69) and 7 low-risk types (HPV type 6, 11, 34, 40, 42, 43, 44) • Showing 100% sensitivity (n=213) and 98% specificity compared with Hybrid Capture II assay

Table 1. Continued.

Classification	Patent No.	Target disease or disease-causative agent	Target genes or regions	Features
	US20070248968A1	HPV	L1, E6, E7 region	• Enable to detect 40 HPV genotypes including 20 high-risk (16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 57, 58, 66, 67, 68, 70), 17 low-risk (6, 7, 10, 11, 27, 32, 40, 42, 44, 54, 55, 59, 61, 62, 72, 73, 83), and 3 middle-risk types (82/MM4, 84/MM8, CP8304/8) • Showing 100% accuracy (n=20) compared with DNA sequencing
	US20070031826A1	HPV	L1 region	• Enable to detect 24 HPV genotypes including 16 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68) and 8 low-risk (6, 11, 34, 40, 42, 43, 44, 70) types • Showing 91.1% accuracy (n=282) compared with DNA sequencing
	US20070207456A1	HPV	E1 region	• Providing 66 primers covering 89 HPV types • Validation of HPV type-specific primers with cervical cancer cell line (SiHa, CaSki, C4I, ME-180, SW765), and vulvar carcinoma samples
	US20060147905A1	Orthopoxvirus	*crmB* gene	• Enable to subdivide orthopoxvirus into five groups (Variola virus, Monkeypox virus, Cowpox virus, Vaccinia virus, Rabbitpox virus) by using species-specific probes of the *crmB* gene that show distinct hybridization patterns
Fungi detection	US20050260584A1	*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Penicillium marneffei*, *Cryptococcus neoformans*, *Pneumocystis carinii*, *Penicillium citrinum*, *Penicillium purpurogenum*	ITS (internal transcribed spacers) 2 coding region (5.8S-28S)	• Using universal fungal probes, probes for endemic dimorphic and species-specific probes
	US7052836	*Aspergillus lavus*, *A. fumigates*, *A. niger*, *A. terreus*, *A. nidulans*, *Fusarium solani*, *F. moniliforme*, *Mucor ouxii*, *M. racemosus*, *M. plumbeus*, *M. indicus*, *M. circinilloides f. circinelloides*, *Rhizopus oryzae*, *R. microspores*, *R. circinans*, *R. stolonifer*, *Rhizomucor pusillus*, *Absidia corymbifera*, *Cunninghamella elegans*, *Pseudallescheria boydii*, *Penicillium notatum*, *Sporothrix schenkii*	ITS2 coding region (5.8S-28S)	• Providing ITS2 region sequences of target fungal species (*Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Rhizomucor* species)
	US7291465	*Stachybotrys-chartarum*, *Aspergillusersicolor*, *A. fumigates*, *Penicillium spinulosum*, *P. chrysogenum*	Species-specific genes	• Using multiple pairs of primers specific for the same species of fungi • Enable to detect multiple species simultaneously

Diagnostic DNA Microarray Using Disease-Relevant Biomarkers

The discovery of new specific biomarkers related to a particular disease is very important for its accurate diagnosis, drug discovery, and toxicity test.

The conventional methods such as the hypothesis-based approaches to biomarker discovery usually consider that only one to several potential markers are investigated at a time. Thus, these methods result in a relatively low rate of successful discovery. On the other hand, the biomarker discovery using the DNA microarray can be carried out by analyzing global expression levels under different genotypic, phenomic, and environmental conditions, thus allowing global-scale candidate identification (D. D. Shoemaker *et al.* 2004: US6713257). It allows simultaneous identification of candidate biomarkers by analyzing differentially expressed genes under comparative conditions such as healthy *vs.* diseased states (J. P. Fruehauf *et al.* 2008: US7323300; P. Tamayo *et al.* 2008: US7324926; J. D. Shaughnessy *et al.* 2005: US20050112630A1; E. Wang, 2007: US7157227; A. Slominski *et al.* 2007: US20070059711A1). By carefully applying clinical samples at various stages or conditions to the DNA microarray, those genes that are specifically associated with different disease stages or conditions can be revealed [8].

DNA microarray-based approaches for biomarker discovery have been applied for studying several chronic diseases including diabetes [7], arthritis [8], and cardiovascular disease [33]. For example, biomarkers for diagnosing systemic lupus erythematosis (SLE) and rheumatoid arthritis (RA), which are chronic autoimmune and inflammatory disorders, can be screened by expression profiling in leukocytes because the differential gene expression in leukocytes is clearly relevant to SLE and RA. Osteoarthritis, the degenerative joint disease that can be confused with RA, can also be diagnosed by using the expression profiling of leukocytes [21]. Based on these facts, Wohlgemuth and coworkers (J. Wohlgemuth *et al.* 2007: US6905827) developed a method for diagnosing and monitoring an autoimmune or chronic inflammatory disease, particularly SLE, based on the differentially expressed genes. The selected genes were studied further by comparing them with the clinical data. The nucleotide sequences of the selected genes were determined and employed for the diagnosis of these diseases.

Crohn's disease, ulcerative colitis, and inflammatory bowel disease (IBD) could also be diagnosed by using the biomarker genes selected from gene expression profiling using DNA microarray (E. Mannick *et al.* 2004: US20040077020A1). Specifically, the diagnosis of IBD with high prevalence rate and morbidity is important for prognosis evaluation and treatment [9, 23, 25]. Mannick and colleagues fabricated the diagnostic chip for these diseases based on the sequences of over- and under-expressed genes selected from Affymetrix GeneChip experiments (E. Mannick *et al.* 2004: US20040077020A1). As a result, in IBD patients, 25 sequences were identified as IBD-relevant genes, resulting in a sensitivity of 84% and a specificity of 100%. In Crohn's disease, from those with ulcerative colitis, 36 genes were identified as Crohn's disease-relevant genes with ulcerative colitis, resulting in a sensitivity of 89% and a specificity of 80%.

Natarajan and Miao developed a DNA microarray for mapping histone modification in gene coding regions involved in gene regulation and expression (R. Natarajan *et al.* 2007: US20070292857A1). They compared the histone modification patterns within the coding regions of disease-specific gene(s) by genome-wide location analysis using chromatic immunoprecipitation linked to cDNA microarrays. Those genes showing up (over 2-fold) and down (under 0.5-fold) expressed genes under specific conditions were selected. This patent suggested a method for determining a risk of developing the disease, by examining the presence or absence of histone modification in genes (H3-K9 dimethylation) associated with type 1 diabetes, which is an autoimmune disorder that can be followed by complications such as retinopathy, neuropathy, and nephropathy.

DNA Microarray for the Diagnosis of Genetic Disorders

Genetic disorders are obviously caused by the inherited genetic abnormality. Chromosome abnormality and mutations in the DNA sequence are the two types of disorders. The latter can cause changes in the amino acid sequence in proteins, lack of protein production, and/or different splicing, causing disease phenotypes. Other than these, SNPs also turned out to be important clues for tracking disease genes, in particular those genes causing disease development at different stages of life.

Conventional methods for mutation detection include gel-based sequencing of polymerase chain reaction (PCR) amplified material, restriction fragment length polymorphism (RFLP), and single-strand conformation polymorphism (SSCP). These sequencing procedures, in spite of their effectiveness, are time-consuming and laborious. To satisfy the needs for more effective mutation screening, conformation-sensitive gel electrophoresis (CSGE) [1, 30] and capillary electrophoresis (CE) [4] are being extensively used to screen large number of genes for studying sequence variations and genetic diseases. Furthermore, DNA microarray has been developed and applied to various diagnostic fields including genetic counseling, and SNP screening and typing. In practice, the improved microarray techniques have provided much advantage to analysis of genetic

mutations and SNPs in a high-throughput manner, and thus accumulation of large amounts of data is ongoing for their use in disease diagnosis.

DETECTION OF CHROMOSOMAL ABNORMALITIES

Genetic disorder can be caused by a chromosome abnormality such as deletion or duplication of a chromosome, deletion or duplication of a part of chromosome, or a break, translocation, or inversion in the chromosome. The diseases caused by chromosome abnormalities include several types such as Down's syndrome (associated with chromosome 21), Edwards syndrome (chromosome 18), Patau syndrome (chromosome 13), Turner syndrome (XO), and Klinefelter syndrome (XXY), leading to irreversible physical and mental abnormalities and death, and even fetal death.

Kang and coworkers developed a DNA microarray for detecting chromosomal abnormalities causing various genetic disorders consisting of Down's syndrome, Patau syndrome, Edward syndrome, Turner syndrome, Klinefelter syndrome, alpha-thalassemia retardation-16, Charcot-Marie-Tooth neuropathy 1A, Cri-du-chat syndrome, hereditary neuropathy with liability to pressure palsies (HNPP) disease, Prader-Willi syndrome, Rubinstein-Taybi syndrome, Williams syndrome, and Wolf-Hirschhorn syndrome (J.-J. Kang *et al.* 2007: US20070048742A1). They used the bacterial artificial chromosome chip (BAC chip) to diagnose chromosomal abnormalities.

DETECTION OF MUTATIONS CAUSING MONOGENIC DISEASES

Monogenic diseases are largely divided into two types according to etiological classification: dominant and recessive disorders. Corneal dystrophy caused by mutation in the gene coding for βIG-H3 is one of the autosomal dominant diseases. It begins with a blurry symptom in the center of the cornea and ends up with vision loss as a patient gets older. In the case of Avellino corneal dystrophy, heterozygous mutation causes severe loss of vision as one gets older, whereas homozygous mutation brings out complete loss of vision much sooner. In recent years, this disease having 1/340-1/1,000 prevalence rates in Korea (in the case of heterozygote) is coming into the spotlight because of LASIK surgery [2, 17, 24, 29, 42]. If a patient with heterozygous Avellino corneal dystrophy receives LASIK surgery, the opacity of the cornea starts to develop aggressively and eventually results in vision loss after 2 years [27]. A group of scientists developed a DNA microarray for the diagnosis of corneal dystrophy by detecting the mutations in exons 4 and 12 of the βIG-H3 (S. Y. Lee *et al.* 2007: WO07083928) [55]. This chip was optimized with various factors for high specificity and sensitivity: the capture probe concentration (100, 50, 30, and 10 μM), the length of capture probe ranging from 11 to 17 mers, and hybridization time (1, 2, 3, 4, 6, and 24 h). This DNA microarray was validated for its performance using the blood samples from 98 patients, resulting in 100% of accuracy.

In general, heterozygous mutation does not cause a disease. However, carriers of ataxia telangiectasia (AT), which is a recessive disorder, show reduced life span, high risk for cancer (especially breast cancer), and elevated sensitivity to ionizing irradiation [6, 44, 45]. Unfortunately, the AT-relevant gene is large (about 150 kb) and has no common mutations, so it is difficult to diagnose AT [10, 14, 20, 54]. Thus, to identify heterozygous carriers of autosomal recessive disorders, Cheung *et al.* invented a cDNA microarray that exhibits different genetic signatures from the heterozygous mutation carriers and normal individuals (V. G. Cheung *et al.* 2005: US6979542). Wilson disease (WD) also has a similar problem of the difficulties in searching and detecting mutations in the WD-relevant gene (*ATP7B*) spanning more than 80 kb. Thus, the development of a more efficient method for detecting WD mutations has been in urgent need [19, 38, 39, 46, 47, 49]. Lee *et al.* (S. Y. Lee *et al.* 2004: US20040132015A1) developed a diagnostic DNA microarray for detecting the mutations causing WD by using a codon scanning algorithm (COSA), which facilitates an oligonucleotide probe design. COSA is a probe design method in which allele-specific probes are designed by changing three consecutive bases in the codon sequence of the gene related to the disease. Using this method, a DNA microarray was fabricated to cover all reported 16 substitution mutations causing WD. It was used to successfully diagnose nine WD patients, while distinguishing three normal persons.

In another report, a diagnostic kit for congenital adrenal hyperplasia (CAH) was developed by Jin (D. K. Jin. 2003: US20030073082A1). In humans, CAH is one of the most common innate defects of metabolism (cortisol biosynthesis) and observed in a rather high frequency of 1 in 10,000. As infants with CAH suffer from the loss of salts, hyperkalemic acidosis and dehydration occur and infants can finally die unless treated with steroid hormone replacement. Thus, early diagnosis is critical [35, 41, 53]. Jin employed 7 sets of tandem cDNA fragments of normal and patient's 21-hydroxylase gene (exon 1, intron 2, and exon 4) for detecting point mutations in this gene (D. K. Jin. 2003: US20030073082A1).

Detection of Polymorphic Changes

Polymorphic changes (so-called SNPs) are genetic variations occurring at a frequency of about one in every 1,000 bases in the genome. The SNPs can exhibit an individual's susceptibility to a particular disease. Recently, much effort has been made to find disease-relevant markers using an SNP

microarray, so-called multi-SNP markers (S.-H. Choi *et al.* 2006: US20060263815A1). High-throughput screening of SNPs using DNA microarrays may make it possible to predict responses to a specific medicine and disease diagnosis, leading to its ultimate use towards developing personalized medicine. However, the lack of a suitable discriminative power in detecting one base difference using DNA microarrays makes its use as a detection tool difficult. Negishi tried to enhance the detection accuracy of the DNA chip by removing the unhybridized probes (M. Negishi. 2007: US 20070196845A1). Probes immobilized on the slide were designed with a flanking sequence around SNPs and different bases at SNP sites. These probes were hybridized with target DNAs having the flanking sequence around the SNPs in the gene. Unhybridized probes (three of four probes) were cleaved off by the single-stranded DNA (ssDNA)-cleaving enzyme and denatured in ssDNA form. This invention enhanced the discriminative power of the DNA microarray for SNPs detection employing specific strand cleaving enzymes, and thus is suitable for high-throughput screening of SNPs with high accuracy.

DNA Microarray for the Diagnosis of Infectious Diseases

Human beings have been continuously fighting with pathogens causing infectious diseases. Infectious diseases have been a major cause of deaths in the 20the century and is still so [36]. Recently, the emergence of infectious diseases has become more serious, as represented by new pathogens such as the viruses causing acquired immune deficiency syndrome (AIDS), Nipah virus encephalitis, avian influenza, dengue fever, and West Nile encephalitis; re-emerging pathogens such as those causing malaria, measles, foodborne pathogens; and antibiotic-resistant superbacteria such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *S. aureus*, and multidrug-resistant *Mycobacterium tuberculosis* [36]. To make the situation worse, it has become more difficult to diagnose and treat these infectious diseases because of the abuse of antibiotics, overuse of immunosuppressants in transplantation, and overdose of drugs as anticancer therapy. Rapid detection and accurate identification of pathogenic agents are critical to treating the patients with appropriate therapeutic agents. Various methods have been developed and used for the detection and identification of pathogenic microbes. Several techniques such as culture-based, serological [40], and immunological [37] assays have been developed, and remarkable progress has been made in the area of nucleic acid-based assays (*e.g.*, PCR-based assays [31]). However, the culture-based assay, which is an automatic system coupled with biochemical test for the identification of microbial pathogens, has a low success rate especially when antibiotics are administrated to patients. Serological and immunological methods often generate nonspecific adsorption, resulting in high false-positive values, poor reproducibility, low speed, and intensive labor. On the other hand, recent advances in DNA microarray analysis have revolutionized molecular approaches towards detection of microbial pathogens by not only enhancing assay capabilities (sensitivity and specificity), but also allowing high-throughput detection. Most of all, the compatibility of DNA microarray with miniaturized devices can result in enhanced speed, sensitivity, and portability, which are important factors in the field of diagnostics.

Detection of Pathogenic Bacteria

There have been several reports on the development of microarrays for the detection of bacteria (J. Tiedje *et al.* 2002: US20020187490A1; B. K. Agan *et al.* 2008: US20080020379A1; B. K. Agan *et al.* 2006: US20060210967A1). Anthony and colleagues [5] developed a microarray for the identification of bacteria by using universal PCR primers capable of amplifying bacterial 23S rRNA. They evaluated the microarray with blood cultured samples from bacteremia patients. Wang and coworkers [50, 51] developed a 16S rRNA-based microarray for the detection of intestinal bacteria in fecal samples. The predominant bacterial species in the human fecal samples are anaerobic bacteria that often require at least a 5-day-long culture for their detection. In this study, 20 predominant human intestinal bacterial species from fecal samples were directly detected by microarray-based assay. However, in contrast with the cases of using sterile specimens such as blood, the existence of other bacteria in nonsterile specimens such as feces makes it difficult to accurately identify the diseases-causing pathogens. On the other hand, Cleven *et al.* [12] detected bacteremia-causing bacterial species such as *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* by employing bacterial species-specific genes, including housekeeping genes, virulence factors, and antibiotic resistance determinants, instead of ribosomal RNA sequences. Here, the gene-specific PCR products were immobilized on the slide glass as capture probes and subsequently used for the characterization of the bacteria in terms of antibiotics resistance and virulence. Recently, Lee and his colleagues reported design strategies and probe sequences for the detection of 44 pathogenic bacteria, which were selected based on their high prevalence in infectious states and/or difficulty of cultivation (S. Y. Lee *et al.* 2003: WO03095677A1). The thus-developed DNA microarray, named PathoChip™, was evaluated by applying a variety of clinical isolates from blood, sputum, stool, cerebral spinal fluid, pus, and urine. Based on the 23S rDNA and 16S-23S rDNA ISR sequence analysis, the bacteria- and bacterial species-specific probes were designed by considering relations between the probe properties and hybridization efficiency. Specific nucleotide sequences solely present in the microbe of interest were

identified by performing multiple alignment of nucleotide sequences derived from all microorganism species. The specificity and sensitivity of the candidate probes were first confirmed by comparison with the sequences in public databases by BLAST analyses and then reconfirmed by hybridization studies with clinical samples. Adding bacteria-specific probes as well as species-specific probes to this system enabled detection of all bacteria. The selected probes provided relatively high sensitivity and specificity in microarray-based assay with reference bacteria and clinical isolates from various specimens. Takayuki reported the DNA microarray composed of DNAs amplified with 35 kinds of primers for detecting pathogens causing respiratory infectious disease (T. Ezaki. 2003: US20030091991A1). Thirty-five kinds of primers were used to amplify 16S-18S sequences, specific antigen, toxin, and virulent genes of pathogens.

Detection of pathogens in food samples was also carried out by using DNA microarrays. Andreoli and colleagues reported the method for identifying the contaminating microorganisms in large amounts of foodstuff (P. Andreoli *et al.* 2006: US20060194223A1). This method employed unique and distinct oligonucleotides (ZIP-code) in manufacturing a DNA microarray to overcome the disadvantages of whole-genome DNA-DNA hybridization. This diagnostic microarray was also combined with a ligase detection reaction (LDR), which is suitable for identifying SNPs and point mutations, as well as pathogen detection because of its powerful discriminatory power of DNA bases.

Among many bacterial species, mycobacteria have been receiving particular attention as this bacterium is the world's most notorious infectious killer, causing tuberculosis. Although mycobacterial vaccines are available, it is still prevalent in the undeveloped countries. According to the report of the Center for Disease Control, mycobacterial disease has more than 15 thousand new cases (about 70% of the total announced number of infectious diseases) and 15 hundred deaths (five times that of the other infectious diseases) annually. The microarray for the detection of mycobacteria has been developed by several researchers (K.-S. Lu *et al.* 2005: US20050136432A1; T. R. Gingeras *et al.* 2005: US6924094). In addition, considering the importance of drug resistance of *Mycobacterium* as a serious problem encountered in the clinical treatment of the disease, probes for detecting drug resistance have also been reported (K.-S. Lu *et al.* 2005: US20050136432A1).

Detection of Pathogenic Viruses

A virus is a small infectious agent, much smaller than a fungus or bacterium, and must invade a living cell to replicate. Viral infection causes various symptoms such as sore throat, sinusitis, and common cold. Viral infection can be dangerous to the community owing to fast contagiousness. Thus, accurate and rapid detection is crucial for the protection against viral diseases. Viruses generally contain specific nucleic acid sequences that are different by more than 30% in the same species or even serotypes. Therefore, it is not too difficult to detect them by nucleic acid-based assays such as the microarray-based assay.

Hepatitis virus is responsible for chronic liver disease, cirrhosis, and hepatocellular carcinoma. It can be detected from a patient's serum through various techniques such as quantitative microarray and microarray employing nanogold-silver stain [28, 32]. The use of gold nanoparticles treated with silver ($AgNO_3$) for signal amplification allowed enhanced sensitivity, which consequently contributed to lowering the detection limit of the assay up to 100 fM of amplicon, corresponding to 1 copy of virus per liter of serum. Oon and colleagues used DNA probes for detecting human hepatitis B virus surface antigen mutant 145 (glycine to arginine) in serum samples and developed a DNA microarray using its unique sequence (C.-J. Oon *et al.* 2004: US6759193).

Enterovirus is one of the most common viral infectious agents in humans, causing an estimated 10–15 million infections a year in the United States [43]. A majority of enteroviral infections result in mild illness; however, enteroviruses can cause different diseases affecting many organs. For example, neurologic (polio, aseptic meningitis, encephalitis), respiratory (common cold, tonsillitis, pharyngitis, rhinitis), and cardiovascular (myocarditis, pericarditis) diseases can be caused by enteroviral infection. Detection of enteroviruses has several problems: misdiagnosis confused by bacterial or herpes virus infections in enteroviral meningitidis, and a high degree of serological cross-reactivity amongst the more than 70 known enteroviruses. Therefore, the development of a system for the accurate and rapid detection of enteroviruses is very important and urgent to the health and welfare of those with increased potential of exposure. Paul reported a technology for alternate amplification and detection of enteroviruses (J. H. Paul III. 2003: US20030186222A1). Target DNAs were prepared by nucleic acid sequence based amplification (NASBA). NASBA is started by attaching a primer containing a T7 RNA polymerase promoter at the 3' end of mRNA. Reverse transcriptase generates a RNA/DNA hybrid and then the RNA of the hybrid is specifically cleaved by RNAse H. T7 RNA polymerase recognizes the T7 RNA polymerase promoter, producing an antisense RNA product. Then, DNA/RNA hybrid synthesis by reverse transcriptase, RNA degradation from the RNA/DNA hybrid, duplex DNA generation, and cycling of antisense RNA are performed. Target DNAs amplified by NASBA are hybridized with enteroviral-specific probes on a microarray, and viral presence is detected by electrochemiluminescence.

Human papilloma virus (HPV), having more than 120 different types, is also an important pathogenic virus, which infects through the skin and mucous membranes. HPV is considered to be an etiological agent of invasive cervical

cancer, which represents 450,000 new cases worldwide annually. Over 70 genotypes of HPV have been identified since the recognition of HPV as the main etiological factor for cervical cancer [11]. Several researchers have reported specific probes for HPV detection (R. C. McGlennen *et al.* 2007: US20070238100A1; U. Gyllensten *et al.* 2007: US20070037137A1; S. A. Norman *et al.* 2006: US20060121516A1; S.-W. Yoon et al. 2007: US7301015; L.-J. Van Doorn *et al.* 2003: US20030165821A1; M. A. Cohenford *et al.* 2003: US20030108866A1; C. Wheeler *et al.* 2003: US20030059806A1; P. E. Kroeger *et al.* 2002: US20020137021A1; C. Jeney *et al.* 2007: US7294488). Moreover, several companies have developed DNA microarrays for HPV detection. Yoon and colleagues developed a HPV detection chip composed of probes for prevalent HPV types including 15 high-risk types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 69) and 7 low-risk types (HPV types 6, 11, 34, 40, 42, 43, and 44) (S.-W. Yoon *et al.* 2007: US7301015). This chip showed 100% relative sensitivity and 98% relative specificity compared with the conventional assay. Moon and colleagues also developed a DNA chip containing the probes for analyzing 40 types of HPV (W.-C. Moon *et al.* 2005: US20070248968A1). The probes were designed on the basis of the sequences of L1, E6, and E7 genes of 35 types of HPV obtained from cervical cell specimens of 4,898 Korean adult women and tissue specimens of 68 cervical cancer cases, in addition to information based on American and European cases. This chip allowed genotyping of HPV with high sensitivity and specificity of up to 100%. Lee and colleagues developed a similar DNA chip, allowing genotyping of 24 HPVs (D.-J. Lee *et al.* 2007: US20070031826A1). Pourmand and colleagues developed a diagnostic DNA microarray for distinguishing 89 genotypes of HPV (N. Pourmand *et al.* 2007: US20070207456A1).

In another study, Ezaki introduced 12 kinds of primers for detecting viruses causing respiratory infectious diseases, by using the information on specific antigen, capsid, and virulence factors (T. Ezaki, 2003: US20030091991A1). The specific primer sets for detecting 64 strains of RNA virus, 16 strains of DNA virus, and 15 species of *Salmonella* belonging to the group E were also disclosed. Mirzabekov developed a DNA microarray for diagnosing orthopoxvirus (A. D. Mirzabekov *et al.* 2006: US20060147905A1).

Detection of Pathogenic Fungi

The infectious diseases caused by pathogenic fungi have been increasing because of the increase of chemotherapy for hematological malignancies, high-dose corticosteroid treatment for organ transplant recipients, and the spread of AIDS over the past decade [3, 34]. For example, *Penicillium marneffei* is the third most common cause of opportunistic infections in patients with AIDS in Thailand [48]. Additionally, *P. chrysogenum* and *P. citrinum* have been well recognized as the cause of human disease. Infection by *Aspergillus*, *Rhizopus*, and *Mucor* species can be fatal in immunocompromised patients [52]. Specifically, aspergillosis has a high mortality rate of up to 90% [16,18].

Fungi can be diagnosed by cultivation, serological, and histopathological assays [22]. However, these conventional methods for detecting pathogenic fungi may require several weeks, owing to slow growth. Specifically, in the case of immunosuppressed patients, it is very difficult to detect circulating antifungal antibodies in blood by serological tests. Although the histopathological test is relatively rapid and available to diagnosis of invasive fungal disease, atypical morphological features cause problems in accurate identification (M. D. Lindsley *et al.* 2005: US20050260584A1).

Most of the diagnostic tests are directed to genus- and species-specific or single-copy genes. Several groups introduced the broad-spectrum detection system with probes designed from internal transcribed spacer (ITS) regions of the rRNA genes. Lindsley *et al.* (M. D. Lindsley *et al.* 2005: US20050260584A1) suggested the methods of detecting a dimorphic fungus, including differentiating a dimorphic fungus from other fungi. They identified the presence or absence of fungi in blind samples by using a dimorphic fungal probe, species-specific probe, microbe-specific probe, and a nucleic acid sequence corresponding to the ITS region of fungal rDNA.

Morrison and colleagues reported oligonucleotide probes for detecting *Aspergillus* species and other filamentous fungi (C. J. Morrison *et al.* 2006: US7052836). The unique ITS regions allowed design of specific nucleic acid probes for five *Aspergillus*, three *Fusarium*, four *Mucor*, two *Penicillium*, five *Rhizopus*, one *Rhizomucor* species, *Absidia corymbifera*, *Cunninghamella elegans*, *Pseudallescheria boydii*, and *Sporothrix schenkii*. They also suggested the genus-specific probes for *Aspergillus*, *Fusarium*, and *Mucor*.

In another study, α-aminoapidate reductase, which is a highly conserved protein in fungi, was employed as a target gene for designing probes (D. K. R. Karaolis, 2007: US7291465). This allowed enhanced detection sensitivity and specificity. They designed the nucleic acid hybridization probes for *Candida albicans* causing candidosis, a common nosocomial infection seriously causing problems to both immunosuppressed and postoperative patients.

Future Prospects

During the past two decades, DNA microarray-based techniques have been predominantly applied to identification of biomarkers, detection of pathogens, and identification of disease-causative agents. In many cases, they allowed accurate and rapid results, but their wide-spread use has been hampered for the limited identification capability. To

increase its practical usage in diagnosis, it should become more affordable, convenient to practice, accurate, and maybe portable. One example is on-site pathogen and SNPs detection (C.-B. Chae *et al.* 2008: US20080008990A1), such as lateral flow assays (W. Gumbrecht *et al.* 2007: US20070264630A1) that can simply and rapidly detect pathogens and SNPs. This will enable sensitive on-site diagnosis of specific diseases in clinical or environmental samples. Another interesting alternative of microarrays in medical diagnostics is the lab-on-a-chip type system, which integrates several processes (from DNA extraction to DNA analysis) within a single, portable, small, and fully automated instrument (S.-H. Paek *et al.* 2008: US20080019866A1). Additionally, DNA microarray systems may be combined with other techniques such as CpG island detection or chromatin immunoprecipitation [13, 15]. Indeed, much progress is being made on the use of the DNA microarray platform for disease diagnosis. For the successful diagnosis of diseases, it is of utmost importance to have specific probes, which can be considered as the contents of chips! Genome projects on many infectious organisms are generating unprecedentedly large amounts of sequence information, which can be used to design more specific probes for the diagnosis of more diverse infectious agents. With all these advances, it is expected that the DNA microarray or its sister technologies will play an increasingly important role in disease diagnosis in the near future.

Acknowledgments

Our work described in this review paper was supported by Medigenes Co. The work at KAIST has also been partially supported by the Korean Systems Biology Research Project from the Ministry of Education, Science and Technology, and LG Chem Chair Professorship.

References

1. Acquila, M., F. Bottini, A. Valetto, D. Caprino, P. G. Mori, and M. P. Bicocchi. 2001. A new strategy for prenatal diagnosis in a sporadic haemophilia B family. *Haemophilia* **7:** 416–418.
2. Afshari, N., J. Mullaly, M. Afshari, R. F. Steinert, A. P. Adamis, D. Azar, and J. Talamo. 2001. Survey of patients with granular, lattice, Avellino, and Reis-Bucklers corneal dystrophies for mutations in the *BIGH3* and gelsolin genes. *Arch. Ophthalmol.* **119:** 16–22.
3. Ampel, N. M., D. G. Mosley, B. England, P. D. Vertz, K. Komatsu, and R. A. Hajjeh. 1998. Coccidioidomycosis in Arizona: Increase in incidence from 1990 to 1995. *Clin. Infect. Dis.* **27:** 1528–1530.
4. Andersen, P. S., C. Jespersgaard, J. Vuust, M. Christiansen, and L. A. Larsen. 2003. Capillary electrophoresis-based single strand DNA conformation analysis in high-throughput mutation screening. *Hum. Mutat.* **21:** 455–465.
5. Anthony, R. M., T. J. Brown, and G. L. French. 2000. Rapid diagnosis of bacteremia by universal amplification of 23S ribosomal DNA followed by hybridization to an oligonucleotide array. *J. Clin. Microbiol.* **38:** 781–788.
6. Athma, P., R. Rappaport, and M. Swift. 1996. Molecular genotyping shows that ataxia telangiectasia heterozygotes are predisposed to breast cancer. *Cancer Genet. Cytogenet.* **92:** 130–134.
7. Baelde, H. J., M. Eikmans, P. P. Doran, D. W. Lappin, E. de Heer, and J. A. Bruijn. 2004. Gene expression profiling in glomeruli from human kidneys with diabetic nephropathy. *Am. J. Kidney Dis.* **43:** 636–650.
8. Barnes, M. G., B. J. Aronow, L. K. Luyrink, M. B. Moroldo, P. Pavlidis, M. H. Passo, *et al.* 2004. Gene expression in juvenile arthritis and spondyloarthropathy: Pro-angiogenic ELR+ chemokine genes relate to course of arthritis. *Rheumatology* **43:** 973–979.
9. Becker, K. G., R. M. Simon, J. E. Bailey-Wilson, B. Freidlin, W. E. Biddison, H. F. McFarland, and J. M. Trent. 1998. Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. *Proc. Natl. Acad. Sci. USA* **95:** 9979–9984.
10. Broeks, A., J. H. Urbanus, A. N. Floore, E. C. Dahler, J. G. Klijn, E. J. Rutgers, *et al.* 2000. ATM-heterozygous germline mutations contribute to breast cancer-susceptibility. *Am. J. Hum. Genet.* **66:** 494–500.
11. Chen, S. L., C. P. Han, Y. P. Tsao, J. W. Lee, and C. S. Yin. 2006. Identification and typing of human papillomavirus in cervical cancers in Taiwan. *Cancer* **72:** 1939–1945.
12. Cleven, B. E., M. Palka-Santini, J. Gielen, S. Meembor, M. Krönke, and O. Krut. 2006. Identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray. *J. Clin. Microbiol.* **44:** 2389–2397.
13. Collas, P. and J. A. Dahl. 2008. Chop it, ChIP it, check it: The current status of chromatin immunoprecipitation. *Front. Biosci.* **13:** 929–943.
14. Concannon, P. and R. A. Gatti. 1997. Diversity of ATM gene mutations detected in patients with ataxia-telangiectasia. *Hum. Mutat.* **10:** 100–107.
15. Delgado, I. J., D. S. Kim, K. N. Thatcher, J. M. LaSalle, and I. B. Van den Veyver. 2006. Expression profiling of clonal lymphocyte cell cultures from Rett syndrome patients. *BMC Med. Genet.* **7:** 61.
16. Denning, D. W. 1996. Diagnosis and management of invasive aspergillosis. *Curr. Clin. Top. Infect. Dis.* **16:** 277–299.
17. Dolmetsch, A. M., F. A. Stockl, R. Folberg, I. Gensini, and M. N. Burnier Jr. 1996. Combined granular lattice dystrophy (Avellino) in a patient with no known Italian ancestry. *Can. J. Ophthalmol.* **31:** 29–31.
18. Fridkin, S. K. and W. R. Jarvis. 1996. Epidemiology of nosocomial fungal infections. *Clin. Microbiol. Rev.* **9:** 499– 511.
19. Forbes, J. R. and D. W. Cox. 1998. Functional characterization of missense mutations in *ATP7B*: Wilson disease mutation or normal variant? *Am. J. Hum. Genet.* **63:** 1663–1674.
20. Gilad, S., A. Bar-Shira, R. Harnik, D. Shkedy, Y. Ziv, R. Khosravi, *et al.* 1996. Ataxia-telangiectasia: Founder effect among North African Jews. *Hum. Mol. Genet.* **5:** 2033–2037.
21. Gladkevich, A., S. A. Nelemans, H. F. Kauffman, and J. Korf. 2005. Microarray profiling of lymphocytes in internal diseases with an altered immune response: Potential and methodology. *Mediators Inflamm.* **2005:** 317–330.

22. Hamilton, A. J. 1998. Serodiagnosis of histoplasmosis, paracoccidioidomycosis and penicilliosis marneffei; current status and future trends. *Med. Mycol.* **36:** 351–364.
23. Hampe, J., S. Schreiber, S. H. Shaw, K. F. Lau, S. Bridger, A. J. Macpherson, *et al.* 1999. A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. *Am. J. Hum. Genet.* **64:** 808–816.
24. Holland, E. J., S. M. Daya, E. M. Stone, R. Folberg, A. A. Dobler, J. D. Cameron, and D. J. Doughman. 1992. Avellino corneal dystrophy: Clinical manifestations and natural history. *Ophthalmology* **99:** 1564–1568.
25. Hugot, J. P. and G. Thomas. 1998. Genome-wide scanning in inflammatory bowel diseases. *Dig. Dis.* **16:** 364–369.
26. Iida, K. and I. Nishimura. 2002. Gene expression profiling by DNA microarray technology. *Crit. Rev. Oral Biol. Med.* **13:** 35–50.
27. Jun, R. M., H. W. Tchah, T. I. Kim, D. R. Stulting, S. E. Jung, K. Y. Seo, D. H. Lee, and E. K. Kim. 2004. Avellino corneal dystrophy after LASIK. *Ophthalmology* **111:** 463–468.
28. Kawaguchi, K., S. Kaneko, M. Honda, F. H. Kawai, S. Yukihiro, and K. Kenichi. 2003. Detection of hepatitis B virus DNA in sera from patients with chronic hepatitis B virus infection by DNA microarray method. *J. Clin. Microbiol.* **41:** 1701–1704.
29. Kennedy, S. M., M. McNamara, M. Hillery, C. Hurley, L. M. Collum, and S. Giles. 1996. Combined granular lattice dystrophy (Avellino corneal dystrophy). Br J *Ophthalmol.* **80:** 489–490.
30. Korkko, J., I. Kaitila, L. Lonnqvist, L. Peltonen, and L. Ala-Kokko. 2002. Sensitivity of conformation sensitive gel electrophoresis in detecting mutations in Marfan syndrome and related conditions. *J. Med. Genet.* **30:** 34–41.
31. Kotilainen, P., J. Jalava, O. Meurman, O. P. Lehtonen, E. Rintala, O. P. Seppälä, E. Eerola, and S. Nikkari. 1998. Diagnosis of meningococcal meningitis by broad-range bacterial PCR with cerebrospinal fluid. *J. Clin. Microbiol.* **36:** 2205–2209.
32. Liu, H. H., X. Cao, Y. Yang, M. G. Liu, and Y. F. Wang. 2006. Array-based nano-amplification technique was applied in detection of hepatitis E virus. *J. Biochem. Mol. Biol.* **39:** 247–252.
33. Ma, J. and C. C. Liew. 2003. Gene profiling identifies secreted protein transcripts from peripheral blood cells in coronary artery disease. *J. Mol. Cell. Cardiol.* **35:** 993–998.
34. McNeil, M. M., S. L. Nash, R. A. Hajjeh, M. A. Phelan, L. A. Conn, B. D. Plikaytis, and D. W. Warnock. 2001. Trends in mortality due to invasive mycotic diseases in the United States, 1980–1997. *Clin. Infect. Dis.* **33:** 641–647.
35. Morel, Y. and W. L. Miller. 1991. Clinical and molecular genetics of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Adv. Hum. Genet.* **20:** 1–68.
36. Morens, D. M., G. K. Folkers, and A. S. Fauci. 2004. The challenge of emerging and re-emerging infectious diseases. *Nature* **430:** 242–249.
37. Mulligan, M. E., L. R. Peterson, R. Y. Kwok, C. R. Clabots, and D. N. Gerding. 1988. Immunoblots and plasmid fingerprints compared with serotyping and polyacrylamide gel electrophoresis for typing *Clostridium difficile*. *J. Clin. Microbiol.* **26:** 41–46.
38. Payne, A. S., E. J. Kelly, and J. D. Gitlin. 1998. Functional expression of the Wilson disease protein reveals mislocalization and impaired copper-dependent trafficking of the common H1069Q mutation. *Proc. Natl. Acad. Sci. USA* **95:** 10854–10859.
39. Petrukhin, K., S. Lutsenko, I. Chernov, B. M. Ross, J. H. Kaplan, and T. C. Gilliam. 1994. Characterization of the Wilson disease gene encoding a P-type copper transporting ATPase: Genomic organization, alternative splicing, and structure/function predictions. *Hum. Mol. Genet.* **3:** 1647–1656.
40. Rennie, R. P., C. E. Nord, L. Sjoberg, and I. B. R. Duncan. 1978. Comparison of bacteriophage typing, serotyping, and biotyping as aids in epidemiologic surveillance of *Klebsiella* infections. *J. Clin. Microbiol.* **8:** 638–642.
41. Speiser, P. W., B. Dupont, P. Rubinstein, A. Piazza, A. Kastelan, and M. I. New. 1985. High frequency of nonclassical steroid 21-hydroxylase deficiency. *Am. J. Hum. Genet.* **37:** 650–667.
42. Stewart, H. S., A. E. Ridgway, M. J. Dixon, R. Bonshek, R. Parveen, and G. Black. 1999. Heterogeneity in granular corneal dystrophy: Identification of three causative mutations in the *TGFBI* (*BIGH3*) gene - lessons for corneal amyloidogenesis. *Hum. Mutat.* **14:** 126–132.
43. Strikas, R. A., L. Anderson, and R. A. Parker. 1986. Temporal and geographic patterns of isolates of nonpolio enteroviruses in the United States, 1970-1983. *J. Infect. Dis.* **153:** 346–351.
44. Swift, M., D. Morrell, E. Cromartie, A. R. Chamberlin, M. H. Skolnick, and D. T. Bishop. 1986. The incidence and gene frequency of ataxia-telangiectasia in the United States. *Am. J. Hum. Genet.* **39:** 573–583.
45. Swift, M., D. Morrell, R. B. Massey, and C. L. Chase. 1991. Incidence of cancer in 161 families affected by ataxia-telangiectasia. *N. Engl. J. Med.* **325:** 1831–1836.
46. Thomas, G. R., O. Jensson, G. Gudmundsson, L. Thorsteinsson, and D. W. Cox. 1995. Wilson disease in Iceland: A clinical and genetic study. *Am. J. Hum. Genet.* **56:** 1140–1146.
47. Thomas, G. R., P. C. Bull, E. A. Roberts, J. M. Walshe, and D. W. Cox. 1994. Haplotype studies in Wilson disease. *Am. J. Hum. Genet.* **54:** 71–78.
48. Vanittanakom, N. and T. Sirisanthana. 1997. *Penicillium marneffei* infection in patients infected with human immunodeficiency virus. *Curr. Top. Med. Mycol.* **8:** 35–42.
49. Waldenstrom, E., A. Lagerkvist, T. Dahlman, K. Westermark, and U. Landegren. 1996. Efficient detection of mutations in Wilson disease by manifold sequencing. *Genomics* **37:** 303–309.
50. Wang, R. F., M. L. Beggs, L. H. Robertson, and C. E. Cerniglia. 2002. Design and evaluation of oligonucleotide-microarray method for the detection of human intestinal bacteria in fecal samples. *FEMS Microbiol. Lett.* **213:** 175–182.
51. Wang, R. F., S. J. Kim, L. H. Robertson, and C. E. Cerniglia. 2002. Development of a membrane-array method for the detection of human intestinal bacteria in fecal samples. *Mol. Cell. Probes.* **16:** 341–350.
52. Warnock, D. W. 1995. Fungal complications of transplantation: Diagnosis, treatment, and prevention. *J. Antimicrob. Chemother.* **36(Suppl B):** 73–90.
53. White, P. C., M. I. New, and B. Dupont. 1987. Congenital adrenal hyperplasia. *N. Engl. J. Med.* **316:** 1519–1524.
54. Wright, J., S. Teraoka, A. Onengut, S. A. Tolun, R. A. Gatti, H. D. Ochs, and P. Concannon. 1996. A high frequency of distinct ATM gene mutations in ataxia-telangiectasia. *Am. J. Hum. Genet.* **59:** 839–846.
55. Yoo, S. Y., T.-I. Kim, S. Y. Lee, E. K. Kim, K. C. Keum, N. C. Yoo, and W. M. Yoo. 2007. Development of DNA chip for the diagnosis of most common corneal dystrophies caused by mutations in the betaigh3 gene. *Br. J. Opthalmol.* **91:** 722–727.