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SINGLE NUCLEOTIDE POLYMORPHISMS (SNP) AND ITS APPLICATIONS IN TREATING HUMAN DISEASES

S. Abdolhamid Angaji*, K. Akashi , S. Darvishani and M. Sabeti Azad

Department of Biology, Tarbiat Moallem University, Tehran, Iran
Corresponding author, email: Angaji@tmu.ac.ir

ABSTRACT

Single nucleotide polymorphism (SNP) is a single base mutation in DNA. SNPs are the simplest form and also the most common sources of genetic polymorphism in the human genome. They have been used in homogeneity testing, plant genetics, pharmacogenetic studies and so on. They have also been used to identify and map complex and common diseases such as high blood pressure, diabetes, and heart disease and etc. Application of SNP in human diseases is studied in this work. There are several methods for SNP genotyping as classified in four categories in this work: Hybridization-based, PCR-based, Restriction site cleavage and Combinations methods. The diseases-associated SNP which are detected by any of these methods is investigated. Finally, the application of some specific methods in detecting SNP for a certain disease is explained. These include the application of SNP array in diabetes disease, the application of Pyrosequencing in Schizophrenia disease, the application of DHPLC in breast cancer disease and the application of melting curve in Parkinson disease.

Key Words: Single base mutations, Genotyping, Hybridization.

INTRODUCTION

A Single Nucleotide Polymorphism is a source variance in a genome (Gobbi *et al*., 2006) which was discovered by Dr. Steve Ligget. A SNP (ßnip") is a single base mutation in DNA. SNPs are the most simple form and most common source of genetic polymorphism in the human genome (Gobbi *et al*., 2006). SNPs are not uniformly distributed over the entire human genome, neither over all chromosomes and nor within a single chromosome. There are one third as many SNPs within coding regions as non-coding region SNPs (Gobbi *et al*., 2006). Current estimates are that SNPs occur as frequently as every 100-300 bases (Perkinelmer, 2005). There are variations between human populations, so a SNP allele that is common in one geographical or ethnic group may be much rarer in another. Some SNPs may be specific for a certain population. In addition, groups of SNPs that are associated with each other (linkage disequilibrium (LD) block) are not the same in different populations (Wang and Moult, 2001).

Single nucleotides may be changed (substitution), removed (deletion) or added (insertion) to a polynucleotide sequence. SNPs are associated with diversity in the population, individuality, susceptibility to diseases, and individual response to medicine. Recently, it has been suggested that SNPs can be used for homogeneity testing and pharmacogenetic studies and to identify and map complex, common diseases such as high blood pressure, diabetes, and heart disease etc (Shastry, 2002; Chanock, 2003).

There are several methods for SNP genotyping. In this study, we try to explain some of these methods which were used to detect SNP-associated diseases. The applications of four techniques in four diseases are also explained.

Applications of different methods in detecting SNP-associated diseases

Genotyping provides a measurement of the genetic variation between members of a species. Single nucleotide polymorphisms (SNP) are one of the most common types of genetic variation. Because SNPs are evolutionarily conserved, they have been proposed as markers for use in quantitative trait loci (QTL) analysis and in association studies in place of microsatellites. SNPs can also provide a genetic fingerprint for use in identity testing. The increase in interest in SNPs has been reflected by the furious development of a diverse range of SNP genotyping methods (Wikipedia). SNPs present a potentially vast arena for the detection of genetic alterations that seem to relate to medically important differences in disease susceptibility and drug response (Lohman *et al.* 2000).

In this part some SNP genotyping methods which have been arranged in four groups; Hybridization-based methods, PCR-based methods, Restriction site cleavage methods and Combinations methods and their applications in human diseases are studied.

In the Hybridization based methods (Table 1), specify of sequences are determinant. Several applications have been developed that interrogate SNPs by hybridizing complementary DNA probes to the SNP site through hybridization based methods. The challenge of this approach is reducing cross-hybridization between the allele-specific probes. This challenge is generally overcome by manipulating the hybridization stringency conditions and alleles can be distinguished by hybridizing complementary oligonucleotide sequence to a target sequence (Rapley

and Harbron, 2004). There are several kinds of methods which are classified as Hybridization based methods. In this study, we explained the application of SNP Array, Allele specific oligonucleotide ligation, Allele specific hybridization, Array hybridization and fluorescence detection, Single-feature polymorphism (SFP), Invader probe (Invader PCR) and MutS protein binding assays methods, in human diseases, as subclasses of Hybridization based methods.

In the PCR-based methods (Table 2), the methods operate on the basis of the specific amplification of a target allele by the polymerase chain reaction with extension primers designed such that their 3' end is placed at the mutation site. When this base is complementary to that of the specific allele, the DNA segment is amplified; when it is not complementary, the polymerase chain reaction cannot proceed (9). There are several kinds of methods which are classified as PCR-based methods. In this study, we explained the application of Primer extension, Minisequencing, Single base primer extension (SBE) method, Single base primer extension (SBE) method, Pyrosequencing and Allele-specific extension methods, in human diseases, as subclasses of PCR-based methods.

Restriction site cleavage methods (Table 3) are marker techniques that rely on the recognition site of a specific restriction enzyme that can maximally detect all the corresponding restriction sites within a genome. In contrast, every SNP in context with its surrounding genomic sequence is unique. SNPs can mark functionally important allelic differences and SNPs that flag individual alleles of known genes have been used widely as molecular markers (Angaji, 2009). This technique is used when an SNP occurs at a restriction endonuclease recognition sequence, and one allele preserves the sequence while the other destroys it. If we consider any DNA fragment with three adjacent restriction sites, with the middle one containing an SNP, then digestion of amplified genomic DNA with the appropriate restriction endonuclease will produce either a single large fragment (if the central restriction site is absent) or two smaller fragments (if the central restriction site is present and cleavage occurs) (Twyman, 2005). There are several kinds of methods which are classified in the restriction site cleavage methods. In this study, we explained the application of Invasive cleveage, CFLP, DGGE, DHPLC, SSCP and Chemical cleavage mismatch methods, in human diseases, as subclasses of Restriction site cleavage methods.

In combinations methods (Table 4), methods have been gathered, centered on a statistical approach (Kirk *et al.*, 2002; Suha and Vijg, 2005; Winkler *et al.*, 2006; Tokuhiro *et al.*, 2003; Gail, 2008; Hawn *et al.*, 2005; Jiang *et al*., 2004; Toyota *et al*., 2000; Pham *et al.*, 2005; Pont-Kingdon and Lyon, 2003) or followed through multiple approach and instruments (Gaylord *et al.*, 2005) that finally result in detecting single nucleotide polymorphisms in genome. In statistical approach first, with one detecting method SNPs are identified in genome and then the results are analyzed with statistic analysis. There are several kinds of methods which are classified as combinations methods. In this study, we explained the application of Constructing haplotypes in Linkage- Disequilibrium-block (LD block), Case-Control association study, Melting curve analysis and Peptide nucleic acid (PNA) probes, an optically amplifying conjugated polymer (CP), and S1 nuclease enzyme methods, in human diseases, as subclasses of Combinations methods.

Case Studies

Here the applications of four methods in detecting SNP-associated diseases are discussed. From each of Hybridization-based methods, PCR-based methods, Restriction site cleavage methods and Combinations methods groups, one method is chosen and its application in a certain diseases is studied.

An SNP array is a useful tool to study the whole genome. The most important application of SNP array is in determining disease susceptibility and consequently, in pharmacogenomics by measuring the efficacy of drug therapies specifically for the individual (Wikipedia). Framingham Heart Study (FHS) family members were genotyped on the Affymetrix 100K single nucleotide polymorphism (SNP) array and examined for association with incident diabetes and six diabetes-related quantitative traits (Florez *et al.*, 2007).

In another study a dense genomewide linkage search of the family was undertaken using a first generation 10K single nucleotide polymorphism chip containing 10,044 markers. A region of homozygosity harboring the neonatal diabetes disease gene on chromosome 10p12.1-p13 was identified. It is likely that chromosome 10p12.1-p13 may harbor maturity-onset diabetes of the young or type 2 diabetes genes (Sellick *et al.*, 2003).

Type 2 diabetes, a complex disease that is characterized by insulin resistance and impaired β-cell function, represents a serious global public health problem, with more than 100 million people affected worldwide. In another study DNA from 124 type 2 diabetic case subjects and 295 control subjects with normal glucose tolerance were genotyped on the Affymetrix 100K single nucleotide polymorphism (SNP) array. Recently, four GWAS studies of type 2 diabetes have identified variants at several novel loci, including *SLC30A8*, *IGF2BP2*, *CKDAL1*, *CDKN2A/CKDN2B*, and *HHEX/IDE*, that show strong replicated association with type 2 diabetes (Rampersaud *et al.*, 2007).

Table 1. Hybridization-based methods and their applications in treating human diseases

Technique	Disease
1.SNP Array	Breast cancer (Gerhardus *et al.,* 2007), Wilson disease (Gupta *et al.,* 2007), Neonatal diabetes, Craniosynostosis, Inherited renal dysplasia, Inherited neonatal diabetes (Wikipedia, Sellick *et al.,* 2004; Florez *et al*., 2007; Sellick *et al*., *2003; Rampersaud et al*., 2007), Chronic mucocutaneous candidiasis, Thyroid disease (Matsuzaki *et al.,* 2004), Lung carcinoma cell line, Lung tumor (Zhao *et al.,* 2004), Rheumatoid arthritis, Prostate cancer, Gastric cancer, Liver cancer (Wikipedia).
1.1. Allele specific oligonucleotide ligation	Hereditary diseases and Somatic mutations of oncogenes (Tozaki *et al.,* 2002), Cancer and congenital diseases (Tozaki *et al.,* 2002), Breast cancer (Gerhardus *et al.,* 2007).
2. Allele specific hybridization	Breast cancer (Gerhardus *et al.,* 2007).
2.1. Array hybridization and fluorescence detection	In human major histocompatibility complex (MHC) (Mark *et al.,* 2002), Drug resistance in Plasmodium falciparum (Zhang *et al.,* 2008).
3. Single-feature polymorphism (SFP)	Disease resistance genes, genes involved in secondary metabolism (Borevitz *et al.,* 2003).
4. Invader probe (Invader PCR)	Venous thrombosis (Patnail *et al.,* 2004), Diabetes (Hulme *et al.,* 2004).
5. MutS protein binding assays	Cystic fibrosis (Lishanski *et al.* 1994), Mutation of p53 gene (resulted in many cancers) (Behrensdorf *et al.,* 2002).

Trace amines and their receptors may be implicated in the pathogenesis of psychiatric disorders. Previous studies have reported association of the *trace amine associated receptor 6* (*TAAR6*) genes with susceptibility to schizophrenia. In one study in Korean patients, five single nucleotide polymorphisms (SNPs: rs4305745; rs8192625; rs7452939; rs6903874 and rs6937506) were genotyped in the *TAAR6* gene and in the 3′ regulatory region, using pyrosequencing. SNP rs6903874 was significantly associated with schizophrenia ($p = 0.012$) and bipolar disorder ($p = 0.004$). A three SNP haplotype consisting of alleles GCT from SNPs rs7452939, rs6903874 and rs6937506, respectively, was significantly over-represented in patients with schizophrenia ($p = 0.0003$) and bipolar disorder ($p = 0.00002$). A second three SNP haplotype (GTT) derived from the same SNPs was significantly under-represented in patients with bipolar disorder ($p = 0.001$).These findings strongly support association of the *TAAR6* gene with susceptibility to both schizophrenia and bipolar disorder in Korean patients (Pae *et al.,* 2008).

In another study, they found a highly significant association between schizophrenia and a COMT haplotype ($P=9.5\times10^{-8}$). To test the COMT-schizophrenia association, they examined 12 SNPs in the COMT gene using the quantitative pyrosequencing technology. Of the 12 SNPs studied, 4 were found not to be polymorphic in the population they studied, and an additional SNP failed to undergo PCR amplification. Among the remaining seven SNPs, they found significant allele-frequency differences between patients and control individuals for five SNPs. Using 12 SNPs distributed in the 27 kb of the COMT gene and testing 7 of them with a very large sample of Ashkenazi Jews, they have been able to confirm a complex association between the COMT gene and schizophrenia. Their results also suggest the possibility of more than one functional polymorphism affecting susceptibility to

schizophrenia at the COMT locus. This is supported by the finding that SNP rs165599 affects primarily women, whereas rs737865 affects both sexes, although in different ways (Shifman *et al.*, 2002).

Table 2. PCR-based methods and their applications in treating human diseases

Technique	Disease
1. Primer extension	Coronary artery disease and diabetes (Broadbent *et al.*, 2008).
1.1. Minisequencing	Imbalanced allelic expression in foetal liver or kidney tissues (Liljedahl *et al.*, 2004), Detection of alternatively spliced transcripts in leukemia cell lines (Milani *et al.*, 2006), Chronic obstructive pulmonary (Hersh *et al.*, 2006)
1.2. Pyrosequencing	Inflammatory bowel disease (Palmieri *et al.*, 2006)**,** Schizophrenia (Bogaert *et al*., 2003; Pae *et al*., *2008; Shifman et al*., 2002; Deng *et al.*, 2004; Saviouk *et al.*, 2005), Chronic hepatitis B virus (HBV) infection (Lindstrom *et al.*, 2004).
1.3. Allele-specific extension	Breast cancer (Li *et al*., 2007).
2. single base primer extension (SBE) method	Broad range of analytical necessities: single-cell analysis, molecular diagnosis of monogenic diseases, forensic mitochondrial DNA analysis on highly degraded human remains, high-throughput SNP screening for population studies (Podini and Vallone, 2009).

In another study, they genotyped 100 Japanese schizophrenics and 100 controls recruited from the Kyushu area for 11 single nucleotide polymorphism (SNP) markers distributed in the *SLC1A2* region using the direct sequencing and pyrosequencing methods, and examined allele, genotype and haplotype association with schizophrenia.They found significant differences in genotype and allele frequencies of SNP2 between cases and controls (P = 0.013 and 0.008, respectively).After Bonferroni corrections, the two significant differences disappeared. They tested haplotype associations for all possible combinations of SNP pairs. SNP2 showed significant haplotype associations with the disease ($P = 9.4 \times 10^{-5}$, P = 0.0052 with Bonferroni correction, at the lowest) in 8 combinations. They concluded that at least one susceptibility locus for schizophrenia is probably located within or nearby *SLC1A2* in the Japanese population (Deng *et al.*, 2004).

In another study, using restriction fragment length polymorphism and pyrosequencing methods, they genotyped two *TNFA* gene promoter SNPs (-G308A, -G238A) and analyzed the haplotype structure in 24 Canadian families of primarily Celtic origin. Their results demonstrate that after correction for multiple testing based on simulations of 10 000 replicates of unlinked/unassociated data, there is evidence for association (P=0.026) of a specific haplotype (-308A, -238G) with schizophrenia and schizophrenia spectrum disorders with a family-based trimmed haplotype linkage disequilibrium test (Saviouk *et al.*, 2005).

In another study, they have investigated the gene for dystrobrevin-binding protein 1 (DTNBP1), or dysbindin, which has been strongly suggested as a positional candidate gene for schizophrenia, in three samples of subjects with schizophrenia and unaffected control subjects of German (418 cases, 285 controls), Polish (294 cases, 113 controls), and Swedish (142 cases, 272 controls) descent. They analyzed five single-nucleotide polymorphisms (P1635, P1325, P1320, P1757, and P1578) and identified significant evidence of association in the Swedish sample but not in those from Germany or Poland. Their results suggest that genetic variation in the dysbindin gene is particularly involved in the development of schizophrenia in cases with a familial loading of the disease (Bogaert *et al.*, 2003).

Table 3. Restriction site cleavage methods their applications in treating human diseases

Technique	Disease
1. Invasive cleveage	10 mutations in the cystic fibrosis transmembrane conductance regulator gene (Chen *et al.,* 2005).
2. DGGE	AIDS (Petersen *et al.,* 2005).
3. DHPLC	Hereditary non-polyposis colorectal cancer (HNPCC) (Meldrum *et al.,* 2003), Motor neurone disease (MND) (Yu *et al.,* 2005), Papillary renal carcinomas (PRC) (Nickerson *et al.,* 2000),Ovary cancer (Gross *et al.,* 2001), Breast cancer (Gerhardus *et al.,* 2007; Ona *et al.,* 2006; Atencio *et al.,* 2001; Wagner *et al.,* 1999), Familial hypertrophic cardiomyopathy (FHC) (Yu *et al.,* 2005).
4. SSCP	AIDS (Lalonde *et al.,* 2007), Crohn's disease (Klein *et al.,* 2004), Breast cancer (Gerhardus *et al.,* 2007).
5. CFLP	Congenital adrenal hyperplasia (CAH) (Vilain, 1998; Kileen *et al.,* 1998; Wei and Kileen, 1998)**,** Breast cancer (Gerhardus *et al.,* 2007; Casadei *et al.,* 2001), X-linked nephrolithiasis, or Dent's disease (Hoopes *et al.,* 1998), Mitochondrial DNA Mutations (Chen *et al.,* 1999), focal glomerulosclerosis (FGS) in Negroid (Gaillard *et al.,* 1999).
6. Chemical cleavage mismatch	Cystic fibrosis (Jones *et al.,* 1992), Multiple endocrine neoplasia type 2 (Kambouris *et al.,* 1996), Fanconi anemia (Gibson *et al.,* 1996), Tay—Sachs (Akli *et al.,* 1993), X chromosome-linked diseases (Grompe *et al.,* 1989), Mutation of p53 gene (resulted in many cancers), , Mutation of K-ras gene(resulted in Leukemias, colon cancer, pancreatic cancer, and lung cancer), Mutation of APC gene (resulted in colorectal cancer), Mutation of VHL gene (resulted in VHL syndrome), breast cancer, chorionic villi biopsy (Buzdin and Lukyayanov, 2007).

Table 4. Combinations methods their applications in treating human diseases

Technique	Disease
1. Constructing haplotypes in Linkage-Disequilibrium-block (LD block)	Parkinson's, Alzheimer's, psoriasis, migraine, type II diabetes and Crohn's disease (Kirk *et al.,* 2002), Schizophrenia (Suha and Vijg, 2005), Rheumatoid arthritis (Tokuhiro *et al.,* 2003).
2. Case-Control Association Study	Prostate cancer (Gail, 2008), Legionnaires' disease (Hawn *et al.,* 2005).
3. Melting curve analysis	Parkinson disease (PD) (Winkler *et al.,* 2006; Jian *et al.,* 2004), Bipolar disorder susceptibility loci (Toyota *et al.,* 2000), Newcastle disease (ND) (Pham *et al.,* 2005), Aneuploidies, trisomy 21 (Pont-Kingdon and Lyon, 2003).
4. Peptide nucleic acid (PNA) probes, an optically amplifying conjugated polymer (CP), and S1 nuclease enzyme	Neurodegenerative disease (Gaylord *et al.,* 2005).

Breast cancer predisposition genes identified to date (e.g., BRCA1 and BRCA2) are responsible for less than 5% of all breast cancer cases. Many studies have shown that the cancer risks associated with individual commonly occurring single nucleotide polymorphisms (SNPs) are incremental. However, polygenic models suggest that

multiple commonly occurring low to modestly penetrant SNPs of cancer related genes might have a greater effect on a disease when considered in combination (Ona *et al.*, 2006).

Ten studies included report on 12 different scanning methods for the detection of BRCA1 and BRCA2 mutations. Among them, only DHPLC can be considered to be used as a scanning test in a routine diagnostic setting. The DHPLC is a chromatography-based method and is able to distinguish between homoduplices – in the case of two wild-type alleles – and heteroduplices in the case of a heterozygous sequence alteration (Wikipedia). The advantage of DHPLC is that it enables the rapid, sensitive, and accurate identification of polymorphisms and mutations in an automated fashion (Atencio *et al.*, 2001). It is used to detect mutations with a high sensitivity in BRCA2 and BRCA1 (Wikipedia).

In one study 71 breast and breast-ovarian cancer (HBC/HBOC) families along with 95 control individuals from a wide range of ethnicities were analyzed by means of denaturing high-performance liquid chromatography (DHPLC) and direct sequence analysis. In the coding (10 257 bp) and non-coding (2799 bp) sequences of *BRCA2*, 82 sequence variants were identified (Wagner *et al.*, 1999).

Indirect evidence suggests that, in some cases, the mitochondrial dysfunction in idiopathic Parkinson disease (PD) may be due to mutations in mitochondrial DNA (mtDNA). A single nucleotide polymorphism (SNP) leading to alterations in complex I activity may play a role in the pathogenesis of PD. The activity of complex I is reduced within brains of patients with PD. Related groups of polymorphic sites in mtDNAs are called haplogroups. Genotyping of SNPs by using fluorescent probes offers several advantages. This technology should prove useful for population-based studies on the interaction between genetic factors and environmental exposures and the risk of PD. In one study, an assay system for rapidly genotyping and haplogrouping mitochondrial polymorphisms based on hybridization probe technology and melting curve analysis was developed. The result was that, in comparison to individuals carrying the most common haplo-group, haplogroup H, individuals classified as haplogroup J and K demonstrate less risk of developing PD. This protective effect is strongly associated with the SNP 10398G that defines haplogroups J and K. The absence of 10398G characterizes haplogroup U and may be associated with an increased risk of PD. SNP 9055A may be associated with a protective effect for women (Jiang *et al.*, 2004).

In the another study To investigate the H1-SNP 13, they designed variant-specific probes and carried out a melting curve analysis on the LightCycler system with the melting temperature for the G allele of 63°C and for A of 54°C. As a result, an association of the H1 haplotype and subhaplotypes in the microtubule-associated protein Tau (*MAPT*) gene with PD has been reported (Winkler *et al.*, 2006).

REFERENCES

Akli, S., J.C. Chomel, J.M. Lacorte, L. Bachner, Axel and L. Poenaru (1993). Ten novel mutations in the HEXA gene in non-Jewish Tay — Sachs patients. *Hum.Mol.Genet*., 2 (1): 61-67.

Angaji, S. A. (2009). Single nucleotide polymorphism genotyping and its application on mapping and marker-assisted plant breeding. *African Journal of Biotechnology*, 8(6): 908-914.

Atencio, D.P., C.M. Iannuzzi, S. Green, R.G. Stock, J.L. Bernstein and B.S. Rosenstein (2001). Screening Breast Cancer Patients for ATM Mutations and Polymorphisms by Using Denaturing High-Performance Liquid chromatography. *Environ. Mol. Mutagen.*, 38 (2-3): 200-208.

Behrensdorf, H.A., M.Pignot, N. Windhab and A. Kappel (2002). Rapid parallel mutation scanning of gene fragments using a microelectronic protein–DNA chip format. Nucleic Acids Research., 30: e64.

Bogaert, A.V. D., J. Schumacher, T.G. Schulze, A.C. Otte, S. Ohlraun, S. Kovalenko, T. Becker, J. Freudenberg, E.G. Jönsson, M. Mattila-Evenden, G.C. Sedvall, P.M. Czerski, P. Kapelski, J. Hauser, W. Maier, M. Rietschel, P. Propping, M.M. Nöthen and S. Cichon (2003). The DTNBP1 (Dysbindin) Gene Contributes to Schizophrenia, Depending on Family History of the Disease. *Am. J. Hum. Genet*., 73(6): 1438-1443.

Borevitz, J.O., D. Liang, D. Plouffe, H.S. Chang, D. Weigel, C.C. Berry, E. Winzeler and J. Chory (2003). Large-Scale Identification of Single-Feature Polymorphisms in Complex Genomes. *Genome Res*., 13(3): 513-523.

Bray, M.S., E. Boerwinkle and P.A. Doris (1999). High-Throughput Multiplex SNP Genotyping With MALDI-TOF Mass Spectrometry: Practice, Problems and Promise. *Hum Mol Genet*., 8(3): 413-23.

Broadbent , H.M., J.F. Peden, S. Lorkowski, H.Ongen, F. Green, R.Clarke, R. Collins, M.G. Franzosi, G. Tognoni, U. Seedorf, S. Rust, P. Eriksson, A. Hamsten, M. Farrall and H. Watkins (2008). Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. *Hum Mol Genet*., 17(6): 806-814.

Buzdin, A.A. and S. Lukyanov (2007). *DNA hybridization in solution for Mutation Detection*. Springer., Nucleic Acids Hybridization: 211-239.

Casadei, S., L. Cortesi, V. Pensotti, P. Radice, M. Pierotti, D. Amadori and D. Calistri (2001). Detection of germline BRCA1 mutations by Multiple-Dye Cleavase Fragment Length Polymorphism (MD-CFLP) method. *J. Cancer*., 85(6): 845–849.

Chanock, S. (2003). Genetic variation and hematology: single-nucleotide polymorphisms, haplotypes and complex disease. *Semin Hematol*., 40(4): 321-328.

Chen, T.J., R. G. Boles and L.J.C. Wong (1999). Detection of Mitochondrial DNA Mutations by Temporal Temperature Gradient Gel Electrophoresis. *Clinical Chemistry*., 45: 1162-1167.

Chen, Y., M. R. Shortreed, M. Olivier and L.M. Smith (2005). Parallel Single Nucleotide Polymorphism Genotyping by Surface Invasive Cleavage with Universal Detection. *Anal Chem*., 77(8): 2400-2405.

Deng, X., H. Shibata, H.Ninomiya, N.Tashiro, N. Iwata, N. Ozaki and Y. Fukumaki (2004). Association study of polymorphisms in the excitatory amino acid transporter 2 gene (SLC1A2) with schizophrenia. BMC Psychiatry., 4: 21.

Perkinelmer (2005). SNP and Genotyping Overview**.** http://las.perkinelmer.com/content/snps/genotyping.asp.

Florez, J.C., A.K. Manning, J. Dupuis, J. McAteer, K. Irenze, L. Gianniny, D.B. Mirel, C.S. Fox, L.A. Cupples and J.B. Meigs (2007). A 100K Genome-Wide Association Scan for Diabetes and Related Traits in the Framingham Heart Study. *Diabetes*., 56 (12): 3063-3074.

Gail, M.H., (2008). Probability of detecting disease-associated single nucleotide polymorphisms in case-control genome-wide association studies. *Biostat* . 9 (2): 201-215.

Gaillard, M.C., R. Mahadeva and D.A. Lomas (1999). Identification of DNA polymorphisms associated with the V type α_1-antitrypsin gene. Biochimica et Biophysica Acta (BBA). *Gene Structure and Expression*, 1444: 166-170.

Galas, D.J. and S. Joseph McCormack (2002). *Genomic technologies: present and future*. Horizon Scientific Press., pp: 418.

Gaylord, B.S., M.R. Massie, S.C. Feinstein and G.C. Bazan (2005). SNP detection using peptide nucleic acid probes and conjugated polymers: Applications in neurodegenerative disease identification. *PNAS*., 102 (1): 34-39.

Gerhardus, A., H. Schleberger, B. Schlegelberger and D. Gadzicki (2007). Diagnostic accuracy of methods for the detection of BRCA1 and BRCA2 mutations: a systematic review. *Eur J Hum Genet*., 15 (6): 619-627.

Gibson, R.A., N.V. Morgan, L. H. Goldstein, I.C. Pearson, I.P. Kesterton, N.J. Foot, S.Jansen, C.Havenga, T. Pearson, T.J.D. Ravel, R.J. Cohn, I.M. Marques, I.Dokal, I.Roberts, J.Marsh, S. Ball, R.D.Milner, J.C. Llerena Jr, E.Samochatova, S.P. Mohan, P. Vasudevan, F.Birjandi, A.Hajianpour, M. Murer-Orlando, C.G. Mathew (1996). Novel mutation and polymorphisms in the Fanconi anemia group C gene. *Human Mutation*., 8: 140–148.

Gobbi, M.D., V. Viprakasit, J.R. Hughes, C. Fisher, V.J. Buckle, H. Ayyub, R.J. Gibbons, D. Vernimmen, Y. Yoshinaga, P.D. Jong, J.F. Cheng, E.M. Rubin, W.G. Wood, D. Bowden and D.R. Higgs (2006). A Regulatory SNP Causes a Human Genetic Disease by Creating a New Transcriptional Promoter. *Science*, 312(5777): 1215-1217.

Grompe, M., D.M. Muzny and C.T. Caskey (1989). Scanning detection of mutations in human ornithine transcarbamoylase by chemical mismatch cleavage. *Proc. Natl. Acad. Sci*. U.S.A., 86(15): 5888–5892.

Gross, E., M. Kiechle and N. Arnold (2001). Mutation analysis of p53 in ovarian tumors by DHPLC. *J. Biochem.Biophy. Methods*, 47: 73-81.

Gupta, A., M. Maulik, P. Nasipuri, I. Chattopadhyay, S.K. Das, P.K. Gangopadhyay and K. Ray (2007). Molecular Diagnosis of Wilson Disease Using Prevalent Mutations and Informative Single-Nucleotide Polymorphism Markers. *Clin Chem*., 53(9): 1601-1608.

Hawn, T.R., A. Verbon, M. Janer, L. Ping Zhao, B. Beutler and A. Aderem (2005). Toll-like receptor 4 polymorphisms are associated with resistance to Legionnaires' disease. *Proc Natl Acad Sci*. U S A., 102(7): 2487-2489.

Hersh, C.P., D.L. DeMeo, R. Lazarus, J.C. Celedon, B. A. Raby, J.O. Benditt, G. Criner, B. Make, F.J. Martinez, P.D. Scanlon, F.C Sciurba, J.P. Utz, J.J Reilly and E.K. Silverman (2006). Genetic Association Analysis of Functional Impairment in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*., 173(9): 977-984.

Hoopes Jr, R.R., P. A. Hueber, R. J. Reid Jr, G. L. Braden, P.R. Goodyer, A. R. Melnyk, J. P. Midgley, D. I. Moel, A. M. Neu, S. K. VanWhy and S. J. Scheinman (1998). CLCN5 chloride-channel mutations in six new North American families with X-linked nephrolithiasis. Kidney International., 54: 698–705.

Hulme, J. S., B.J. Barratt, R.C.J. Twells, J.D. Cooper, C.E. Lowe, J. M.M. Howson, A.C. Lam, L. J. Smink, D.A. Savage, D.E. Undlien, C. Guja, C. Ionescu-Tîrgovişte, E. Tuomilehto-Wolf, J. Tuomilehto and J. A. Todd,

(2004). Association Analysis of the Lymphocyte-Specific Protein Tyrosine Kinase (LCK) Gene in Type 1 Diabetes. *Diabetes*., 53 (9): 2479-2482.

Jiang, Y., T. Ellis and A.R. Greenlee (2004). Genotyping Parkinson Disease-Associated Mitochondrial Polymorphisms. *Clin Med Res*., 2(2): 99–106.

Jones, C.T., l. Mclntosh, M. Keston, A. Ferguson and D. J. H. Brock (1992). Three novel mutations in the cystic fibrosis gene detected by chemical cleavage: analysis of variant splicing and a nonsense mutation. *Hum.Mol.Genet*., 1(1): 11-17.

Kambouris, M., C.E. Jackson and G.L. Feldman (1996). Diagnosis of multiple endocrine neoplasia (MEN) 2A, 2B and familial medullary thyroid cancer (FMTC) by multiplex PCR and heteroduplex analyses of RET proto-oncogene mutations. *Human Mutation*., 8: 64–70.

Killeen, A.A., R.R. Jiddou and K.S. Sane (1998). Characterization of frequent polymorphisms in intron 2 of CYP21: application to analysis of segregation of CYP21 alleles. *Clinical Chemistry*., 44: 2410-2415.

Kirk, B.W., M. Feinsod, R. Favis, R.M. Kliman and F. Barany (2002). Single nucleotide polymorphism seeking long term association with complex disease. *Nucl. Acids Res*., 30 (15): 3295-3311.

Klein, W., A. Tromm, C. Folwaczny, M. Hagedorn, N. Duerig, J.T. Epplen, W.H. Schmiegel and T. Griga (2004). A polymorphism of the NFKBIA gene is associated with Crohn's disease patients lacking a predisposing allele of the CARD15 gene. *Int J Colorectal Dis*., 19(2): 153-156.

Lalonde, M. S., R.M. Troyer, A.R. Syed, S. Bulime, K. Demers, F. Bajunirwe and E.J. Arts (2007). Sensitive Oligonucleotide Ligation Assay for Low-Level Detection of Nevirapine Resistance Mutations in Human Immunodeficiency Virus Type 1 Quasispecies. *J Clin Microbiol.*, 45(8): 2604-2615.

Li, X., Y. Huang, Y. Guan, M. Zhao and Y. Li (2007). A novel one cycle allele specific primer extension—Molecular beacon displacement method for DNA point mutation detection with improved specificity. *Anal Chim Acta*., 584(1): 12-18.

Liljedahl , U., M. Fredriksson, A. Dahlgren and A.C. Syvänen (2004). Detecting imbalanced expression of SNP alleles by minisequencing on microarrays. *BMC Biotechnolog*., 4: 24.

Lindström, A., J. Odeberg and J. Albert (2004). Pyrosequencing for Detection of Lamivudine-Resistant Hepatitis B Virus. *Journal of Clinical Microbiology*., 42(10): 4788-4795.

Lishanski, A., E.A. Ostrander and J. Rine (1994). Mutation detection by mismatch binding protein, MutS, in amplified DNA: application to the cystic fibrosis gene. *Proc. Natl. Acad. Sci*. U.S.A., 91(7): 2674–2678.

Lohmann, S., L. Lehmann and K. Tabiti (2000). Fast and Flexible Single Nucleotide Polymorphism (SNP) Detection with the Light Cycler System. *BioChemica*., 4: 23-28.

Mark, Z.G., S. Gatterman, L. Hood, J.A. Hansen and E. W. Petersdorf (2002). Oligonucleotide Arrays for High-Throughput SNPs Detection in the MHC Class I Genes: HLA-B as a Model System. *Genome Res*., 12(3): 447-457.

Matsuzaki, H., H. Loi, S. Dong, Y.Y. Tsai, J. Fang, J. Law, X. Di, W.M. Liu, G. Yang, G. Liu, J. Huang, T. B. Ryder, G.C. Kennedy, G.A. Marcus, P.S. Walsh, M.D. Shriver, J.M. Puck, K.W. Jones and R. Mei (2004). Parallel Genotyping of Over 10,000 SNPs Using a One-Primer Assay on a High-Density Oligonucleotide Array. *Genome Res*., 14(3): 414-425.

Meldrum, C. J., M. McPhillips, R. Crooks, L. Thomas, T. Edkins, R. Creegan, E. Miller, M. Agrez and R.J. Scott (2003). A Comparison Between Denaturing Gradient Gel Electrophoresis and Denaturing High Performance Liquid Chromatography in Detecting Mutations in Genes Associated with Hereditary Non-Polyposis Colorectal Cancer (HNPCC) and the Identification of 9 New Mutations Previously Unidentified by DGGE. *Hered Cancer Clin. Pract*., 1(1): 39–48.

Milani, L., M. Fredriksson and A.C. Syvänen (2006). Detection of Alternatively Spliced Transcripts in Leukemia Cell Lines by Minisequencing on Microarrays. *Clinical Chemistry*., 52: 202-211.

Nickerson, M.L., G. Weirich, B. Zbar and L.S. Schmidt (2000). Signature-based analysis of MET proto-oncogene mutations using DHPLC. *Hum Mutat*., 16(1): 68-76.

Ona, V.Ü., L. Briollais, J.A. Knigh, E. Shi, Y. Wang Sean Wells, H. Li, I. Rajendram, I.L. Andrulis and H. Ozcelik, (2006). SNP-SNP interactions in breast cancer susceptibility. *BMC Cancer*., 6: 114.

Pae, C.U., H.S. Yu, D. Amann, J. J. Kim, C.U. Lee, S.J. Lee, T.Y. Jun, C.Lee, I. H. Paik, A. A. Patkar and B. Lerer, (2008). Association of the trace amine associated receptor 6 (TAAR6) gene with schizophrenia and bipolar disorder in a Korean case control sample. *J. Psychiatr Res*., 42(1): 35-40.

Palmeiri, O., S. Toth, A. Ferraris, A. Andriulli, A. Latiano, V. Annese, B. Dallapiccola, M. Vecchi, M. Devoto, S. Surrey and P. Fortina (2003). CARD15 Genotyping in flammatory bowel disease patients by multiplex Pyrosequencing. *Clinical Chemistry*, 49: 1675-1679.

Patnaik, M., J.S. Dlott, R.N. Fontaine, M.T. Subbiah, M. J. Hessner, K.A. Joyner, M.R. Ledford, E.C. Lau, C. Moehlenkamp, J. Amos, B. Zhang and T.M. Williams (2004). Detection of Genomic Polymorphisms Associated with Venous Thrombosis Using the Invader Biplex Assay. *JMD*., 6(2): 137.

Petersen, D.C., A. Laten, M.D. Zeier, A. Grimwood, E. Janse, V. Rensburg and V.M. Hayes (2005). Novel mutations and SNPs identified in CCR2 using a new comprehensive denaturing gradient gel electrophoresis assay. *Mutantion Research*, 573: 41-53.

Pham, H.M., S. Konnai, T. Usui, K.S. Chang, S. Murata, M. Mase, K. Ohashi and M. Onuma (2005). Rapid detection and deferention of Newcastle disease viruse by real-time PCR with melting-cruve analyze. *Archive of virology*, 150: 2429-2438.

Podini, D. and P. M. Vallone (2009). SNP Genotyping Using Multiplex Single Base Primer Extension Assays. *Methods Mol Biol*., 578: 379-391.

Pont-Kingdon, G. and E. Lyon (2003). Rapid Detection of Aneuploidy (Trisomy 21) by Allele Quantification Combined with Melting Curves Analysis of Single-Nucleotide Polymorphism Loci. *Clinical Chemistry*, 49: 1087-1094.

Rampersaud, E., C.M. Damcott, M. Fu, H. Shen, P. McArdle, X. Shi, J. Shelton, J. Yin, Y.P.C. Chang, S.H. Ott, L. Zhang, Y. Zhao, B.D. Mitchell, J.O'Connell and A.R. Shuldiner (2007). Identification of Novel Candidate Genes for Type 2 Diabetes from a Genome-Wide Association Scan in the Old Order Amish. *Diabetes*., 56(12): 3053-3062.

Rapley, R. and S. Harbron (2004). *Molecular Analysis and Genome Discovery*. Chichester. John Wiley & Sons Ltd.

Saviouk, V., E.W.C. Chow, A.S Bassett and L.M. Brzustowicz (2005). Tumor necrosis factor promoter haplotype associated with schizophrenia reveals a linked locus on 1q44. *Molecular Psychiatry*., 10: 375–383.

Sellick, G.S., C. Garrett and R.S. Houlston (2003). A Novel Gene for Neonatal Diabetes Maps to Chromosome 10p12.1-p13. Diabetes., 52 (10): 2636-2638.

Sellick, G.S., C. Longman, J. Tolmie, R. Newbury-Ecob, L. Geenhalgh, S. Hughes, M. Whiteford, C. Garrett and R.S. Houlston (2004).Genomewide linkage searches for Mendelian disease loci can be efficiently conducted using high-density SNP genotyping arrays. *Nucl. Acids Res.*, 32 (20): e164.

Shastry, B.S. (2002). SNP alleles in human disease and evolution. *Journal of Human Genetics*, 47(11): 561-566.

Shifman, S., M. Bronstein, M. Sternfeld, A. Pisanté-Shalom, E. Lev-Lehman, A. Weizman, I. Reznik, B. Spivak, N. Grisaru, L. Karp, R. Schiffer, M. Kotler, R. D. Strous, M. Swartz-Vanetik, H. Y. Knobler, E. Shinar, J. S. Beckmann, B. Yakir, N. Risch, N.B. Zak and A. Darvasi (2002). A Highly Significant Association between a COMT Haplotype and Schizophrenia. *Elsevier*, 71: 1296-1302.

SNP array, Wikipedia. http://en.wikipedia.org/wiki/SNP_array.

Sobrino, B., M. Brio´n and A. Carracedo (2005). SNPs in forensic genetics: a review on SNP typing methodologies. *Forensic Sci Int.*, 154(2-3): 181-194.

Suha, Y. and J. Vijg (2005). SNP discovery in associating genetic variation with human disease phenotypes. *Mutat Res.*, 573(1-2): 41-53.

Tokuhiro, S., R. Yamada, X. Chang, A. Suzuki, Y. Kochi, T. Sawada, M. Suzuki, M. Nagasaki, M. Ohtsuki, M. Ono, H. Furukawa, M. Nagashima, S. Yoshino, A. Mabuchi, A. Sekine, S. Saito, A. Takahashi, T. Tsunoda, Y. Nakamura and K. Yamamoto (2003). An intronic SNP in a RUNX1 binding site of *SLC22A4*, encoding an organic cation transporter, is associated with rheumatoid arthritis. *Nature Genetics*., 35: 341 – 348.

Toyota, T., A. Watanabe, H. Shibuya, M. Nankai, E. Hattori, K. Yamada, A. Kurumaji, J.D. Karkera, S.D. Detera-Wadleigh and T. Yoshikawa (2000). Association study on the DUSP6 gene, an affective disorder candidate gene on 12q23, performed by using fluorescence resonance energy transfer-based melting curve analysis on the Light Cycler. *Mol Psychiatry*, 5(5):461, 489-494.

Tozaki, T., N. H. Choi-Miura, M. Taniyama, M. Kurosawa and M. Tomita (2002). SNP analysis of the inter-alpha-trypsin inhibitor family heavy chain-related protein (IHRP) gene by a fluorescence-adapted SSCP method. *BMC Medical Genetics*, 3: 6.

Twyman, R.M. (2005). Single nucleotide polymorphism (SNP) *Genotyping Techniques. Encyclopedia of Diagnostic Genomics and Proteomics*. Marcel Dekker Inc, pp:1600.

Vilain, E. (1998). CYPs, SNPs, and Molecular Diagnosis in the Postgenomic Era. *Clinical Chemistry*, 44 (12): 2403-2404.

Wagner, T.M.U., K. Hirtenlehner, P.Shen, R. Moeslinger, D. Muhr, E. Fleischmann, H. Concin, W. Doeller, A. Haid, A.H. Lang, P. Mayer, E. Petru, E. Ropp, G. Langhauer, E. Kubista, O. Scheiner, P. Underhill, J. Mountain, M. Stierer, C. Zielinski and P. Oefner (1999). Global Sequence diversity of BRCA2: Analysis of 71 breast cancer families and 95 control individuals of worldwide populations. *Hum. Kol. Genet*, 8 (3): 413-423.

Wang, Z. and J. Moult (2001). SNPs, Protein Structure, and Disease. *Hum Mutat*., 17(4): 263-270.

Wei, W.L. and A.A. Killeen (1998). Analysis of four common salt-wasting mutations in CYP21 (steroid 21-hydroxylase) by cleavase fragment length polymorphism analysis and characterization of a frequent polymorphism in intron 6. *Molecular Diagnosis*., 3: 171-178.

Winkler, S., I.R. König, K. Lohmann-Hedrich, P. Vieregge, V. Kostic and C. Klein (2006). Role of ethnicity on the association of *MAPT* H1 haplotypes and subhaplotypes in Parkinson's disease. *Hum. Mol. Genet*., 15(10): 1640-1649.

Yu. B., N.A. Sawyer, M. Caramins, Z.G. Yuan, R.B. Saudderson, R. Pamphlett, D.R. Richmond, R.W. Jeremy and R.J. Tren (2005). Denaturing high performance liquid chromatography: high throughput mutation screening in familial hypertrophic cardiomyopathy and SNP genotyping in motor neurone disease. *J Clin Pathol*., 58: 479-485.

Zhang, G.Q., Y.Y. Guan, H.H. Sheng, B. Zheng, S. Wu, H.S. Xiao and L.H. Tang (2008). Multiplex PCR and Oligonucleotide Microarray for Detection of Single-Nucleotide Polymorphisms Associated with *Plasmodium falciparum* Drug Resistance. *Journal of Clinical Microbiology*., 46(7): 2167-2174.

Zhao, X., C. Li, J.G. Paez, K. Chin, P.A. Jänne, T.H. Chen, L. Girard, J. Minna, D. Christiani, C. Leo, J. W. Gray, W.R. Sellers and M. Meyerson (2004). An Integrated View of Copy Number and Allelic Alterations in the Cancer Genome Using Single Nucleotide Polymorphism Arrays. *Cancer Res*., 64: 3060.

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