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Review

High-throughput single-strand conformation polymorphism analysis by capillary electrophoresis

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Abstract

Mutation detection plays a great role in genetic and medical research and clinical diagnosis of inherited diseases and particular cancers. Single-strand conformation polymorphism (SSCP) analysis is one of the most popular methods for detection of mutations. Recently, automated capillary electrophoresis (CE) systems have been used in SSCP analysis instead of conventional slab gel electrophoresis. SSCP analysis in combination with CE is a rapid, simple, sensitive and high-throughput mutation screening tool, and has been successfully applied for mutation detection involving human tumor suppressor genes, oncogenes and disease-causing genes. The new technique has a great potential for mutation screening of large numbers of samples in clinical diagnosis. This review discusses basic issues about the methodology of SSCP analysis based on CE and summarizes several key applications.

Keywords: Reviews; Single-strand conformation polymorphism; Mutation detection; Polymerase chain reaction

Contents

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1. Introduction

A number of mutations in genomes probably affect the expressions of genes or the functions of gene products, which are thought to be the main causes of inherited diseases and most human cancers [1–3]. Various methods for mutation detection, which mainly include denaturing gradient gel electrophoresis, single-strand conformation polymorphism, chemical/enzymatic mismatch cleavage, restriction fragment length polymorphism, allele-specific oligonucleotide hybridization, allele-specific amplification and DNA sequencing, have been developed and used in basic research, and clinical diagnosis of genetic disorders and certain cancers [4–6]. Compared to these methods, SSCP analysis is considered to be the most popular method for detection of mutations [7–10]. Its broad use is related to its simplicity, speed, feasibility and low cost.

SSCP analysis is based on the principle that single-strand DNA (ssDNA) fragments with a single base substitution (or a small insertion or deletion) often get an unique conformation (secondary structure) because of different intramolecular interaction, and therefore migrate differently in nondenaturing gel electrophoresis. In the conventional protocol of SSCP analysis [11–13], a target sequence of genomic DNA is amplified by polymerase chain reaction (PCR) using a pair of specific primers and then the amplified DNA fragments are denatured and separated in polyacrylamide gel. A comparison of the migration pattern of unknown DNA samples with reference DNA samples, makes it possible to determine samples containing potential mutation by the presence of shifted bands and the change in the band number. In spite of its simplicity the method remains both labor-intensive and time-consuming because of the need for radioisotopes and large polyacrylamide gels.

Capillary electrophoresis (CE) has become an attractive alternative to slab gel electrophoresis for DNA analysis. CE can be automated and is characterized by short analysis time, small sample and reagent requirements, high separation efficiency and, when coupled to laser-induced fluorescence (LIF) detection, unsurpassed sensitivity [14–18]. An additional important advantage of CE is that the running temperature can be precisely controlled to allow SSCP analysis with high resolution and good reproducibility using either a liquid or air cooling system, while accurate temperature control is difficult when using conventional electrophoretic techniques. CE in entangled polymer solutions has high resolving power and can differentiate single base substitutions. The application of CE to SSCP analysis instead of slab gel electrophoresis was first reported by Kuyper et al. [19]. The SSCP analysis of the *p53* gene mutations was completed in about 30 min using CE in replaceable polyacrylamide solution, while the conventional SSCP analysis required several hours (sometime even 16 h) [10]. The genotypes were identified by the comparison of the electropherograms of the unknown samples and known genotype samples (references). This primary work showed that SSCP analysis in combination with CE was a promising alternative method for mutation detection.

To date, CE has successfully been used for SSCP analysis instead of slab gel electrophoresis. Its applications cover a wide range from human tumor suppressor genes and oncogenes to some disease-causing genes [20–27]. This new technique is simple, rapid and well suited to screening of large numbers of clinical samples.

2. Methodology

2.1. DNA sample preparation

Polymerase chain reaction (PCR) has become a powerful tool available for in vitro syntheses of millions or billions of copies of DNA fragments [28]. Applications of PCR range from genetic analysis to disease diagnosis and forensic identification [29]. Most DNA samples used in SSCP analysis are prepared by PCR amplification in the presence of the

specific up- and down-stream primers and DNA polymerase using genomic DNA from whole blood, tissues and culture cells as templates. The salts in PCR product solution may affect the CE separation efficiency and sample loading by electrokinetic injection, which is the most commonly used injection method for CE in polymer solution, especially when using a poor sensitivity detector or low concentration of PCR products. Some purification methods, such as ethanol precipitation, spin column separation and membrane dialysis, can be used to efficiently remove the salts from PCR products.

UV absorption is usually used for detection of dsDNA and ssDNA fragments in CE separation since nucleotides can absorb 260 nm wavelength light [19,30,31]. However, this detection has low sensitivity and PCR products frequently need to be concentrated by the use of, e.g., ethanol precipitation.

LIF detection has high sensitivity (100–1000 times more than UV detection) and selectivity, and is widely applied for DNA analysis by CE without purification of DNA samples such as PCR products and enzymatic digests in most instances [32,33]. Since they have no native fluorescence, DNA fragments need to be labeled with fluorescent molecules. DNA fragments can be labeled simultaneously during PCR amplification using fluorescent-labeled primers that are usually synthesized in solid-phase using a commercial DNA synthesizer with a fluorescent label attached to the 5′-end of the molecule. This labeling is convenient and widely applied for DNA and SSCP analyses. However, we observed occasionally that PCR products using fluorescent labeled primers contained some fluorescent materials that interfered with SSCP analysis when analyzing short DNA fragments. We found that the fluorescent materials could be efficiently removed using QIAquick PCR Purification Kit [23].

An alternative method is post-PCR product labeling based on the 3′-terminal exchange reaction catalyzed by the Klenow fragment of DNA polymerase I, in which the 3′-terminal base of DNA fragments can be exchanged by the fluorescent nucleotides. Originally, the labeling procedure covered several steps involving ethanol precipitation to isolate the reaction products [34]. The modified protocol does not require purification of PCR products and the whole labeling can be performed in one tube. Excess of fluorescent deoxynucleotide from the labeling reaction may interfere with SSCP analysis and may need to be removed by dephosphorylation of the phosphase. This method was used for labeling of PCR products in SSCP analysis [35,36].

Both sense and antisense strands of dsDNA fragments are usually labeled with the same color fluorescent dyes because most commercial capillary electrophoresis instruments are equipped with a single channel detector. The labeling of both single strands of dsDNA fragments with two different fluorescent dyes can be used in a multiple channel detection system, which gives more information on SSCP analysis and may increase its sensitivity [20].

Fluorescein (or FAM) is a widely used label and its excitation wavelength matches well the emission wavelength of the argon ion laser source; JOE, ROX HEX and TAMRA are compatible with He–Cd or He–Ne lasers. Infrared dyes, such as thiazine-, oxazine-, and cyanine-type compounds, match semiconductor lasers with low cost and small size, and may become potential reagents for labeling of DNA fragments [33,37].

Before CE analysis, dsDNA fragments need to be melted into ssDNA fragments. Heat melting is a commonly used method for SSCP analysis based on CE and slab gel electrophoresis. The melted ssDNA samples should be immediately placed in ice water to avoid reannealing of ssDNA into dsDNA which may interfere with SSCP analysis. A novel method, called on-line melting, was applied to obtain ssDNA in the capillary coupling with a heating device [25]. A very small part of the capillary (4 mm) was heated to 95°C using this heating device. When dsDNA fragments migrated through the heated part of the capillary they melted into ssDNA fragments. The on-line melting method was applied for SSCP analysis of single point mutations in the *N-ras*, *K-ras* and *p53* genes. The technique may contribute more automated analysis of SSCP by CE.

2.2. Sieving media

The early technique for DNA separation by CE was based on filling the capillary with a polyacrylamide gel similar to that used in slab gel electrophoresis [38–41]. The gel column was used in SSCP analysis, and was prepared by polymerizing

acrylamide in situ in the capillary [22]. However, this approach was far from optimal. The capillary gel columns were difficult to prepare, the polymerization of the acrylamide inside the capillary often resulted in bubble formation and gel shrinkage, and the lifetime of the column was usually short (about five injections) [42].

A major step forward was the introduction in recent years of replaceable polymer solutions as separation media, including linear polyacrylamide [14,18,43,44], cellulose derivatives (HEC, HMC and HPMC) [17,45] and poly(ethylene oxide) [16], which are commercially available. These advances in matrix preparation have promoted the application of CE in DNA analysis, such as mutation detection [20,23,46–50], forensic genotyping [51,52], DNA sequencing [16,41,44] and measurement of gene expression [53–55]. Of these polymers, polyacrylamide (PA) is a widely used sieving medium for DNA and SSCP analysis because it has extremely high resolution [20,44]. However, long-chain PA has high viscosity and capillary filling with this medium and medium replacement need high pressure. It is inconvenient to use this highly viscous polymer solution in commercial capillary electrophoresis instruments. The viscosity of the sieving medium can be lowered markedly by the use of a low-molecular weight polymer. We synthesized short-chain PA using isopropanol as chain transferring agent to control the molecular mass of the polymer [46]. Capillary filling of this low-viscosity medium and medium replacement were easily carried out on commercial capillary electrophoresis instruments, and a good reproducibility of migration time and high resolution were obtained, which was central to SSCP analysis. This medium has been successfully used for detection of single point mutations in the *MTHFR*, *factor V* and *CBS* genes by SSCP analysis [23,46].

Commercial products, such as GeneScan and POP polymers (Perkin-Elmer), were also successfully used as sieving media in SSCP analysis [24,26,56,57].

2.3. Modification of capillary

In order to perform SSCP analysis in capillaries filled with a polymer solution, the inner surface first has to be modified, usually by covalent bonding of hydrophilic polymers, which thereby suppress the electroosmotic flow and the adsorption of DNA to the capillary surface, and improve the reproducibility of the migration time. The polyacrylamide-coated capillary is easily prepared in the laboratory and is widely used in DNA and SSCP analysis. This coating procedure, which was developed by Hjertén [58], involves two chemical reaction steps that the capillary wall is first silicified with 3-methacryloxypropyltrimethoxysilan and then bonded with PA by polymerization of the acrylamide. A variety of coated capillaries (e.g., polyethylene glycol, polysiloxane, DB-17 and epoxy coating) are also commercially available for DNA analysis [33,59]. We examined the performance of the DB-17 commercial column in SSCP analysis and found that at least 100 successive analyses could be performed in the same capillary [23].

The dynamic coating is an alternative promising method to modify capillary. Certain polymer solutions adsorb to the capillary inner surface and form a stable dynamic coating, which reduces the electroosmotic flow to a negligible level, and limits the DNA–capillary wall interaction. This has been reported for poly(ethylene oxide) [60], poly(vinylpyrrolidone) [61] and polydimethylacrylamide (PDMA) [62,63], which have been successfully used for DNA sequencing and genotyping. These polymers have two functions that act as dynamic coating reagents and sieving media. The stability of the dynamic coating dramatically depends on the pH of the sieving medium. We found that the PDMA dynamic coating at pH 7.8 was more stable than that at pH 8.3. Furthermore, we successfully carried out SSCP analysis on uncoated capillaries using PDMA as sieving medium [63].

2.4. Optimization of SSCP analysis

The sieving medium, the electrophoretic conditions and the length of DNA fragments largely influence the SSCP analysis.

The running temperature is an important factor affecting the conformation and the electrophoretic mobility of ssDNA. This effect was widely investigated [20,22,23,26,27,64]. Arakawa et al. [22] examined the effect of temperature on the SSCP

analysis of the *divE 42* gene mutations and found that the four ssDNA fragments from a mixture of wild and mutant DNA were clearly differentiated at 20 and 25°C; but at 35°C the resolution of ssDNA fragments was dramatically decreased and three peaks from four ssDNA fragments were observed. Atha et al. systematically explored the temperature effect on the SSCP analysis of *p53* mutations from 16 to 50°C. They found that each mutation has a unique temperature profile and the optimal temperature was related to the particular mutation [26]. We systematically examined the influences of the temperature on the SSCP analyses of eight mutations in the *MTHFR, factor V* and *CBS* genes using a Beckman CE instrument with a liquid cooling system [23]. Fig. 1 shows the temperature effect on the SSCP analysis of the *MTHFR* A1298C gene mutation. Our results and others [27] indicated that a lower running temperature was beneficial for SSCP analysis. This effect is probably related to altered conformation of ssDNA fragments which attain a more folded structure at lower temperature.

The components and pH of the sieving medium and the buffer have marked impacts on SSCP analysis. We compared various sieving media and found that HPMC and PEO were unsuitable for SSCP analysis, due to inadequate resolution or high viscosity at the concentration required for adequate resolution. We found that short-chain PA has high resolution of ssDNA in SSCP analysis. The concentration of sieving medium also affects the resolution of ssDNA. In the SSCP analysis of the C677T mutation in the *MTHFR* genes, we found that the resolution was largely dependent on the short-chain PA concentration (Fig. 2.) and 6% short-chain PA concentration was an optimal, which was probably related to the change in pore size of the sieving medium with increase of the polymer concentration [46].

TBE (about pH 8.3) is a commonly used buffer in SSCP analysis based on slab gel electrophoresis or CE since it has low conductivity and good buffer power. Some new buffers, such as *HEPES*, MES and TAPS, were used in DNA separation and SSCP analysis [63]. In order to improve the separation of ssDNA fragments and increase the sensitivity of

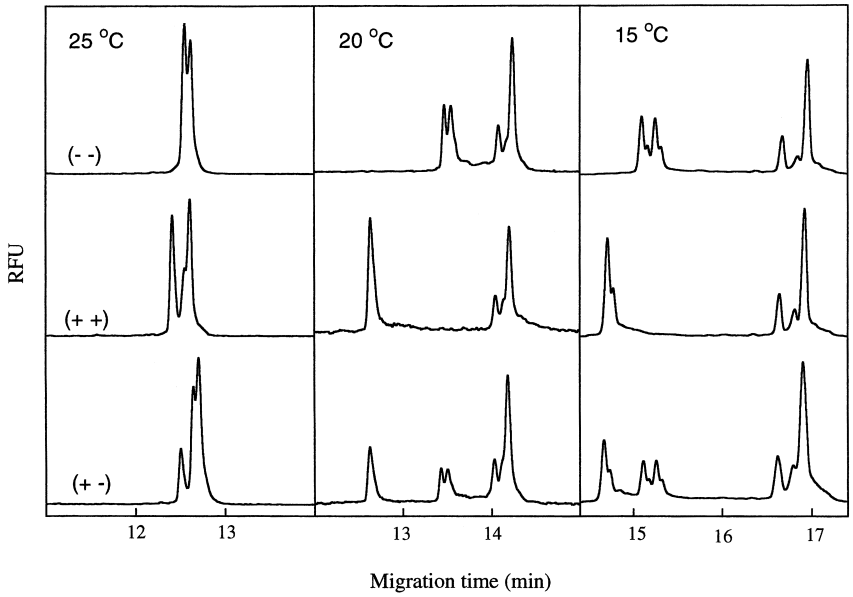

Fig. 1. Temperature effect on the SSCP analysis of the *MTHFR* A1298C gene mutation. Electropherograms of wild type (−−), homozygous mutant (++) and heterozygous (+−) samples, obtained at 15, 20 and 25°C are shown. The electrophoresis medium is 89 m*M* Tris–borate, pH 8.3, containing 2 m*M* EDTA, and 6% short-chain PA. The applied voltage is −12 kV. RFU, relative fluorescence units. Reprinted from Ref. [23].

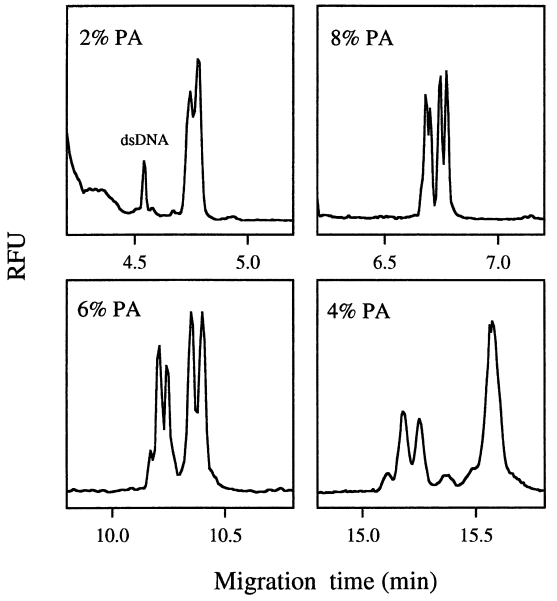

Fig. 2. Separation of ssDNA components of a MTHFR C677T heterozygous sample using various concentrations of polyacrylamide (PA) as sieving matrix. Electrophoretic conditions: buffer, 1×TBE; temperature, 25°C; applied voltage, −20 kV. RFU, relative fluorescence units. Reprinted from Ref. [46].

SSCP analysis, some researchers added glycerol to sieving medium and buffer initiating the conventional SSCP analysis [20,24,26,36,56,57,65]. In fact, the effect of glycerol was complicated, and negative effects were observed in some instances [66]. We found that glycerol reduced the resolution of ssDNA fragments in the SSCP analysis of the C677T mutation in the *MTHFR* gene [46]. This effect remains unclear and is probably related to lowering of the buffer pH as a result of the reaction of glycerol and borate, which is similar to the pH effect of the sieving medium (see below).

Kukita et al. recently found that the sensitivity of SSCP analysis was improved at low pH on slab gel electrophoresis [67]. They explained the effect of low pH as being due to suppression of the charge of the phosphate backbone, leading to reduced intramolecular repulsion and increased involvement of base interaction in stabilization of the ternary structure. We systematically studied the influence of pH on the SSCP analysis based on a CE system [23]. The results are shown in Fig. 3. Seven of nine single point mutations (in *factor V*, *CBS* and *MTHFR* genes) were detected at the ordinary pH of 8.3, whereas the *CBS* T833C mutation was discriminated at extreme pH values of 9.0 and 6.4, and the *CBS* G797A mutation could not be detected at any pH value within the range 6.4–9.0. This emphasized the importance of the pH of the sieving medium and buffer in detecting certain mutations by SSCP analysis. The mutation specificity suggests additional mechanisms related to the differential dissociation constant of A, G, C and T bases. The enolate forms in hetero aromatic rings of G and T dissociate within pH 6.4–9.0, which may influence the conformation and the charges of ssDNA fragments.

The effects of electric field on the separation of DNA fragments and SSCP analysis have been noted. The extremely high electric field will produce serious Joule heating. This increases the temperature in the center of the capillary, which may alter the conformation of ssDNA, increase diffusion, and thereby cause peak broadening and insufficient resolution. However, low electric field will prolong the migration time of ssDNA fragments, which will increase sample zone diffusion and lose resolution. We investigated the influence of electric field strength on the SSCP analysis of the C677T mutation in the *MTHFR* gene (Fig. 4), and obtained an optimal resolution at an electric field strength of −500 V/cm [46]. The choice of electric field should be made according to the electrophoretic current that is dependent on the dimensions of the capillary and the conductivity of the buffer. We suggest that the electrophoretic current should be below 20 μA.

The sensitivity of SSCP analysis is associated with the size of the DNA fragments [10]. It has been reported that the sensitivity significantly decreased with large DNA fragments in conventional slab gel electrophoresis. So far, we have not seen any systematic study on the effect of the DNA fragment length on the sensitivity of SSCP analysis based on CE. But, we observed that most amplified DNA fragments, which are usually applied in SSCP analysis based on slab gel electrophoresis and CE, are shorter than 300 bp. Notably, it has been found that CE was successfully used for the SSCP analysis of single point mutations in large DNA fragments of 741 and 1233 bp in length [27,36].

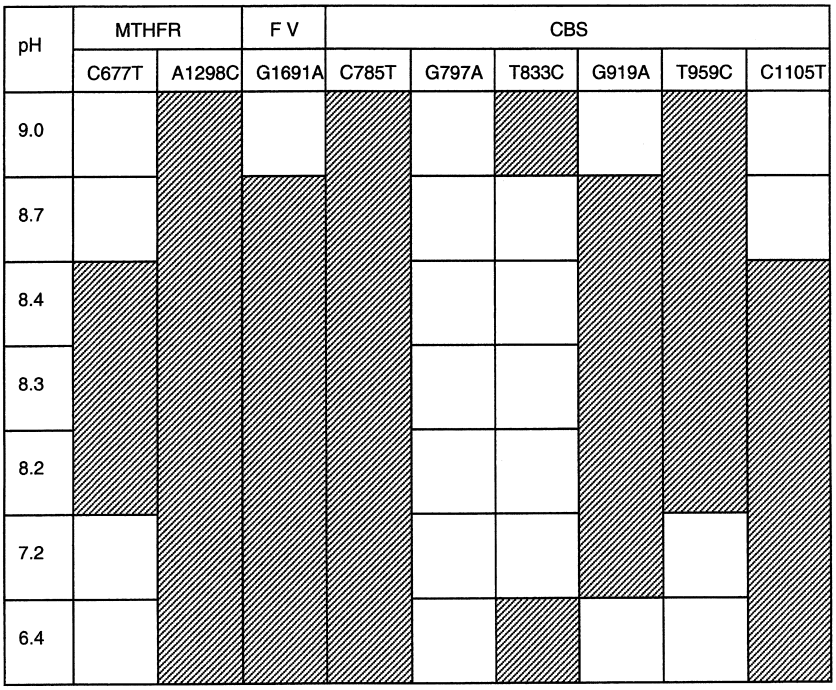

Fig. 3. The sensitivity of SSCP analysis at different pH values. The various mutations investigated are listed in the upper horizontal column and the pH in the left row. The electrophoresis medium is 6% short-chain PA; applied voltage, −12 kV; and temperature, 15°C. The shaded area indicates that the SSCP profile allows the identification of the actual genotype. Reprinted from Ref. [23].

3. Applications

SSCP analysis in combination with CE technique has been successfully applied for oncogenes, tumor suppressor genes and some disease-causing genes. Selected applications are summarized in Table 1.

3.1. p53 and ras genes

Mutations in cellular oncogenes and tumor suppressor genes play an important role in the formation of most human cancers. The altered forms of these genes have already been shown to be potential prognostic markers in particular cancers [68,69]. SSCP analysis has become a widely used method for detecting the mutations of the *p53* tumor suppressor gene and ras oncogenes [10]. Kuypers et al. first reported SSCP analysis of the *p53* gene mutations using CE system [19]. The PCR amplified DNA fragments with 372 bp were from normal white blood cell and heterozygous cell line CEM which contained the *p53* point mutations, and from bone marrow cells of two multiple myeloma patients known to have a *p53* point mutation. The normal and mutated DNA samples were distinguished using 4% PA as a separation matrix. Katsuragi et al. developed the SSCP analysis of the mutations in exon 7 of the *p53* gene from tissue specimens of lung tumors using an in-house built CE system with fluorescent detection in which a 100-W I_2 lamp was used as excitation source [21]. The target DNA fragments were amplified by PCR using fluorescent labeled primers. Four mutations, including a 6-bp deletion at codons 247–248, a 1-bp deletion at codon 260, a 1-bp deletion and a G–C substitution at codon 244, were differentiated by SSCP analysis using 2% PA sieving medium with 5% glycerol (the partial results are shown in Fig. 5). Atha et al. applied a multiple fluorescent detection system in the SSCP analysis of the *p53* mutations [26]. PCR primers were labeled

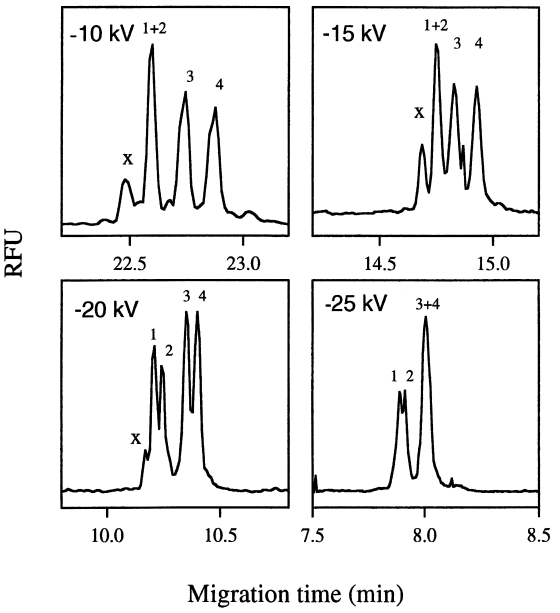

Fig. 4. Separation of ssDNA components of a heterozygous sample at various field strengths. The peaks denoted ×, 1 and 4 derived from the CC genotype, and 2 and 3 from the TT genotype. Electrophoretic conditions: buffer, 1×TBE; sieving medium, 6% PA; temperature, 25°C; applied voltage, −10 to −25 kV. RFU, relative fluorescence units. Reprinted from Ref. [46].

with two different color fluorescent dyes, FAM and JOE. SSCP analysis was carried out on an ABI 310 CE instrument (Perkin-Elmer) equipped with a multiple-channel LIF detector using 3% GeneScan polymer (commercial name) containing 10% glycerol. Five single-point mutations in exons 7 and 8 of the p53 gene were identified. Furthermore, they performed a blind trial with 10 samples, in which six were correctly identified as containing one of the reference mutations, two corresponded to wild type, and two were correctly identified non-reference mutations [24].

SSCP analysis was also applied for detecting the mutations of the ras oncogenes [22,25]. Arakawa et al. designed a multiple sheath-flow capillary electrophoresis system with LIF and applied it to the SSCP analysis of 'the *k-ras* gene' [22]. The 71-bp DNA fragments covering seven single-point mutations of the *k-ras* gene were amplified with Texas Red labeled primers. The He–Cd laser was used as an excitation light. They conducted the SSCP analysis of eight samples simultaneously using an in-house built multiple capillary array system. This high-throughput system, in fact, has a potential to analyze 100–200 samples simultaneously and will become a promising tool to screen a large number of samples in clinical diagnosis.

3.2. CBS gene and MTHFR gene

Homocysteine is metabolized by one of two pathways: remethylation and trans-sulfuration, which

Table 1
Selected applications of SSCP analysis by capillary electrophoresis

Gene	Location of mutation	DNA purification	Electrophoretic conditions	Detection mode	Ref.
p53	Codon 12 973–13 344	Ethanol precipitation	4% PA, TBE buffer, 25°C	UV	[19]
p53	Exon 7	No	2% PA+5% glycerol, TBE buffer, 25°C	Fluorescence	[21]
p53	Exons 7–8	No	3% GeneScan polymer+10% glycerol, TBE buffer, 25°C	LIF	[26]
ras	Codon 12	Ethanol precipitation	9% PA, TBE buffer, room temperature	LIF	[22]
CBS	Exons 7–10	Spin column	6% PA, TBE buffer, 20°C	LIF	[63]
MTHFR	Codons 677, 1298	Spin column	6% PA, TBE buffer, 20°C	LIF	[46,63]
Factor V	Codon 1691	Spin column	6% PA, TBE buffer, 20°C	LIF	[23,63]
Factor IX	Exons 7–8	(Unreported)	3% GeneScan polymer+10% glycerol, TBE buffer, 30°C		
LDL receptor	Exons 1–18	No	GeneScam polymer+10% glycerol, TBE buffer, 30°C	LIF	[57]
HFE	Codons 187, 845	(Unreported)	2.8–5% GeneScan polymer+10% glycerol, TBE buffer, 30°C	LIF	[56,65]
KVLQT1	Exon 6	No	4% POP polymer+10% glycerol, BE buffer, 14–45°C	LIF	[68]

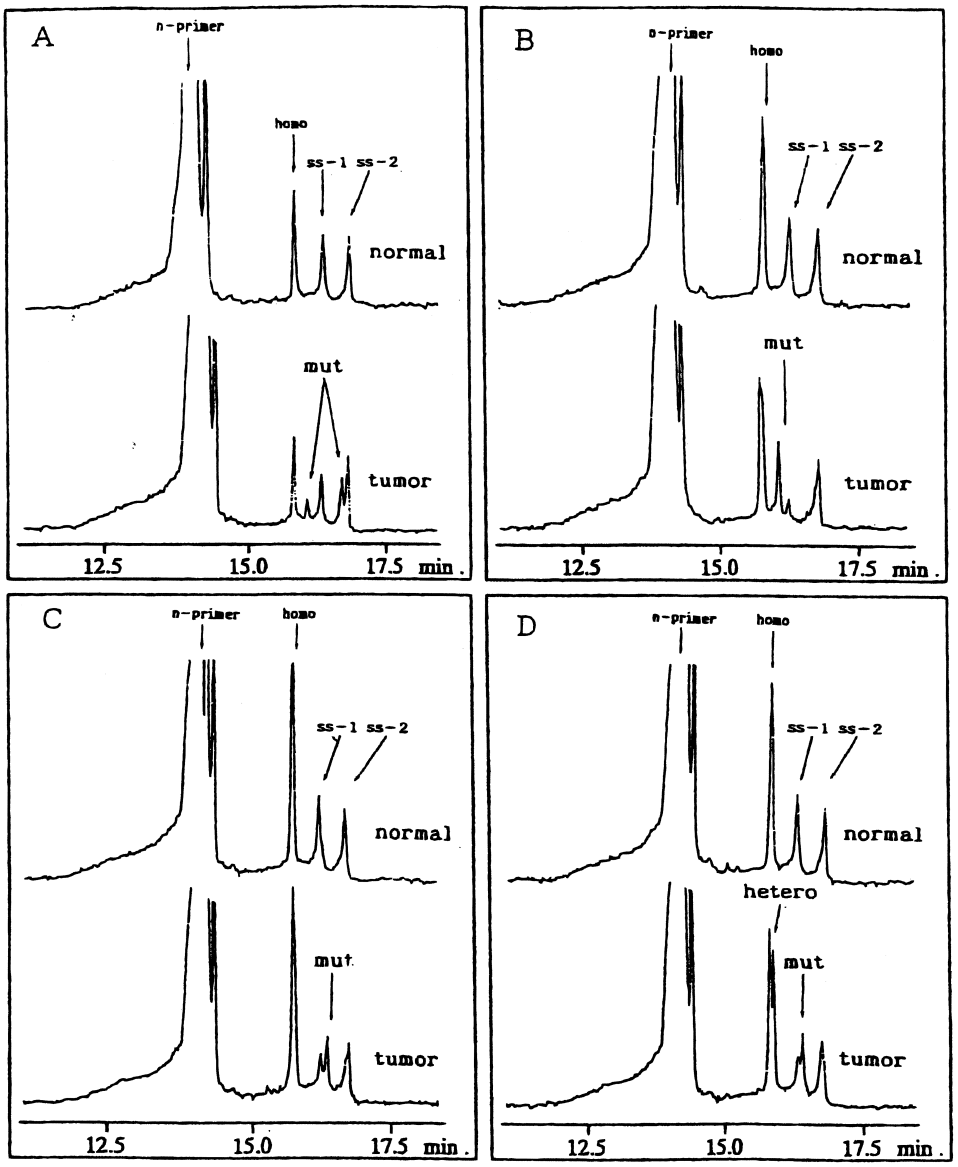

Fig. 5. CE-FSSCP analysis using a 2% polyacrylamide polymer system. DNA samples examined were from cancer tissue (tumor) and normal tissue from patients 104, 106, 137, and 232. For patients 104 (A), 2106 (B), and 232 (C). Cancer tissue samples yielded a DNA peak in addition to the peak produced by normal tissue samples, but this new peak was not detected for patient 137 (D). Homo, ss-1 and ss-2 indicate positions of homoduplex and single-stranded DNA molecules. The peaks of mutated alleles are indicated by mut. Reprinted from Ref. [21].

involve two enzymes, the *CBS* and *MTHFR*. A deficiency of the *CBS* activity is the most frequent cause of homocystinuria, an autosomal recessive disease. The major of clinical features includes optic lens dislocation, mental retardation, skeletal abnormalities and premature thrombotic events. To date, 92 mutations have been identified in the *CBS* gene, about two-thirds of which are missense mutations [70,71]. A thermolabile variant of the *MTHFR* is associated with reduced enzyme activity and in-

creased plasma homocysteine level, which has been widely studied as an independent risk factor for atherosclerosis [72–74].

We developed SSCP analyses of mutations in *CBS* and *MTHFR* genes using a CE-LIF system. DNA fragments were obtained by PCR amplification using fluorescein-labeled primers using genomic DNA from whole blood as templates [62,63]. SSCP analyses were performed on commercial capillary electrophoresis instruments with an in-house built LIF detector with a sheath flow cuvette and a commercial LIF detector (P/ACE). The rapid separation of ssDNA fragments with high resolution were obtained by the use of 6% short-chain PA as sieving medium. Five of six single-point mutations in exons 7–10 of the *CBS* gene, C785T, T833C, G919A, T959C and C1105T from a homocystinuria patient samples, were identified by SSCP analysis. The C667T and A1298G common mutations of the *MTHFR* gene also were clearly differentiated in these separation conditions. Furthermore, we developed a novel SSCP analysis method with a multiple injection mode. Three injections with 1.2-min intervals were performed in one run. Analysis of three samples was completed in 14 min, i.e., a mean analysis time per sample of <5 min [46].

3.3. Factor V gene and factor IX gene

The G1691A (Leiden) mutation in the *factor V* gene is probably the most common genetic predisposition to venous thrombosis, conferring a 7-fold increased risk in heterozygous individuals, and has recently been identified as a strong risk factor for myocardial infarction in young women [75,76]. We detected the G1691A mutation of the factor V gene by SSCP analysis in combination with CE-LIF. SSCP analyses were performed in coated and uncoated capillaries, respectively [23,63]. Three genotypes of the G1691A, G1691G and A1691A were unambiguously distinguished. This data indicates that SSCP based on CE is not only used for screening of unknown mutations, but also for identification and genotyping of known mutations.

Hemophilia B is a X-linked recessive disorder of the homeostasis and mostly due to mutations in the *factor IX* gene affecting either its transcription, mRNA maturation, mRNA translation or the fine structure of the *factor IX* [77]. Inazuka et al. described an efficient mutation screening for the *factor IX* gene using SSCP analysis [36]. Using genomic DNA from hemophilia B patients as templates, DNA fragments were amplified by PCR and post-labeled with two fluorescent dyes, R110 and R6G. Eleven mutations in exons 7 and 8 of the factor IX gene were identified by SSCP analysis.

3.4. Low-density lipoprotein (LDL) receptor gene

The LDL receptor family consists of cell-surface receptors that recognize extracellular ligands and internalize them for degradation by lysosomes. They play an important role in cholesterol homeostasis. Mutations in the *LDL receptor* gene cause familial hypercholesterolemia (FH) and premature coronary artery disease. Furthermore, the *LDL receptor* protein also plays a center role in the clearance of plasma-activated α_2-macroglobulin and apolipoprotein E-enriched lipoproteins, which is associated with Alzheimer's disease. To date, more than 350 different mutations of the *LDL receptor* gene have been reported [78,79]. Geisel et al. systematically analyzed the mutations of the *LDL receptor* gene by SSCP in combination with a CE-LIF system [57]. Twenty fragments from the promoter and all 18 exons were amplified by PCR using the fluorescent primers labeled with FAM and HEX. Fifty-nine of 61 genetic varieties distributed in 16 exons were correctly identified by SSCP analysis using 5% GeneScan polymer with 10% glycerol. The results demonstrated that SSCP analysis based on CE was a robust method for the detection of mutations in the LDL receptor gene.

3.5. Hemochromatosis (HFE) gene

Hereditary hemochromatosis is an autosomal recessive disease in which increased iron absorption causes iron overload and irreversible tissue damage [80]. Two common mutations, the G845C (C282Y) and C187G (H63D) in the *HFE* gene, are known to be the cause of the hereditary disease. The simultaneous detection of the G845C and C187G mutations was developed by Wenz et al. [65]. The multiplex PCR was applied to amplify two DNA fragments containing codons 187 or 845 using two pairs of

primers labeled with different fluorophors (FAM and TET). SSCP analyses were performed on an ABI 310 CE instrument in 2.8% GeneScan polymer in the presence of 10% glycerol using an uncoated capillary. A blind trial showed that the genotypes of 20 samples were in concordance with those obtained by RFLP method. Another successful SSCP analysis of the *HFE* gene was reported by Bosserhoff et al. [56]. Two *HFE* gene fragments spanning codons 187 and 845 were amplified by duplex PCR using genomic DNA from peripheral blood or tissue sections of paraffin-embedded liver biopsies and the fluorescent primers labeled with FAM and TAMRA. SSCP analyses were carried out on an ABI 310 CE instrument. Eighty-five samples were successfully analyzed. The error genotypes of three samples from RFLP analysis, which was due to incomplete restriction digestion in combination with poor PCR efficiency, were corrected by SSCP analysis.

3.6. KVLQT1, HERG and MYH7 genes

Long QT syndrome (LQTS) is an inherited cardiac disorder that causes syncope and sudden death from arrhythmias. Characteristic electrocardiograms frequently show a prolonged QT interval, T wave alternations, and notched T waves. The main cause of LQTS may be attributed to mutations in the *KVLQT1*, *HERG*, *SCN5A* and *KCNE1* genes encoding cardiac ion channels [81]. Mutations in the cardiac β-myosin heavy chain gene (*MYH7*), and other genes (such as *MYBPC3*) encoding cardiac sarcomere proteins may cause familial hypertrophic cardiomyopathy (F-HCM), an autosomal dominant disease characterized by myocardial hypertrophy [82]. Larsen at al. developed a high-throughput SSCP analysis method for detection of mutations in the *KVLQT1*, *HERG* and *MYH7* genes [27]. The DNA fragments, ranging in size from 166 to 1223 bp, were amplified by PCR using fluorescent primers. Thirty-four point mutations in 17 different sequence contexts associated with the inherited cardiac disorder LQTS and F-HCM were analyzed. A sensitivity of 100% was obtained under three different running temperatures using 4% POP polymer with 10% glycerol (the partial results are shown in Table 2). In order to enhance the efficiency of CE analysis, they developed multiplex SSCP analysis to simultaneously detect the mutations of four DNA fragments in exons 2, 5, 6 and 7 of the *KVLQT1* gene amplified by multiplex PCR. The results obtained from the multiplex SSCP analyses were in line with that from analyses of individual exons, except the differences in DNA concentration which was due to unequal competition between the amplimers in multiplex PCR reaction. Surprisingly, 24 single-point mutations in DNA fragments of 838 and 1233 bp in length were clearly differentiated by SSCP analysis. The method was successfully applied to the mutation screening of patient samples with LQTS and two novel mutations were found, which were identified by DNA sequencing.

3.7. Other genes

It has been reported that CE was applied for SSCP analysis of the *human β-globin* gene [83].

SSCP analysis was also used for detection of the mutations from other organism besides human. Mutations of the *divE* genes from *E. coli* were detected by SSCP analysis based on CE-UV detection [22]. SSCP analyses of mutations in the *lacI* gene and *IGF1-BP3* (insulin-like growth factor 1-binding protein 3) gene were successfully performed on an in-house built CE system with two-dye laser-induced fluorescence [20]. Twenty-three single-point mutations in the *B. subtilis gyra* gene were identified by SSCP analysis using post PCR product labeling with two different fluorescent dyes [36]. SSCP analysis based on CE was applied for the detection of the mutations in human hepatitis B virus genome, and the *16S rRNA* gene from *Psudomonas aeruginosa* and Gram-negative bacilli isolated from patients with cystic fibrosis [84].

4. Conclusions

The successful applications described above demonstrate that SSCP analysis based on CE is a robust method for screening of unknown mutations and identification of known mutations. This new method can be automated and is characterized by simplicity, high speed, high sensitivity and good reproducibility compared to conventional SSCP analysis based on

Table 2
Detection of mutations in genomic DNA samples (Reprinted from Ref. [27])

(No.) Mutation/amino acid change (if any)[a]	Product length (bp)	Position of mutation (from 5′-end)	Electrophoresis temperature (°C)						
			14[b]	20	25	30	35	40	45
KVLQT1									
(1) c470 T>G/F157C	186	115	+	+	+	+	+	−	−
(2) c470 G>A	194	138	+	+	+	−	+	+	+
(3) c1096 C>T/R366W	166	88	+	+	+	+	+	+	+
(4) c1638 G>A	213	137	+	+	+	+	+	+	+
(5) c1986 C>T	232	49	+	+	+	−	−	−	−
HERG									
(6) C1467 C>T	265	143	+	+	+	+	−	−	+
(7) c1692 A>G	260	176	+	+	+	−	−	−	−
(8) c1914 G>C	260	198	+	+	−	−	+	+	+
(9) c1886 A>G/N629S	285	141	+	+	+	−	+	−	−
(10) c1956 T>C[c]	227	127	−	−	−	−	+	+	−
MYH7									
(11) g5908 C>T[c]	276	216	−	+	−	−	+	+	+
(12) g5908 T>C[c]	276	216	−	+	−	−	−	−	−
(13) g7648 A>G[c]	204	125	+	+	+	+	+	+	+
(14) g7864 T>C[c]	235	130	−	−	+	+	+	−	−
(15) g8867 C>T[c]	193	112	+	−	−	−	−	−	−
(16) g9600 C>T[c]	231	105	+	+	+	+	−	+	+
(17) g9633 G>A[c]	231	91	+	+	+	+	+	+	+
(18) g9633 A>G[c]	231	91	+	+	+	+	+	+	+
(19) g12245 A>G[c]	330	79	−	+	+	+	+	+	−
Sensitivity (detected/total) (%)			74	84	74	53	68	58	47

+, mutation detected; −, mutation not detected.
[a] Numbering refers to Genbank accession numbers AF000571 (*KVLQT1*), U04270 (*HERG*) and X52889 (*MYH7*).
[b] The lowest possible electrophoresis temperature for the assay was 14°C.
[c] Single nucleotide polymorphism not previously reported.

slab gel electrophoresis. Furthermore, capillary array electrophoresis instruments are now available commercially, which will overcome the drawbacks of most current CE instruments that analyze only one sample per run. SSCP analysis in combination with capillary array electrophoresis, multiple color fluorescent labeling and multiplex PCR technique will become a high-throughput screening method for mutations and be widely used for analysis of large numbers of samples in clinical diagnosis.

Finally, It should be noted that some new techniques such as MALDITOF-MS [84], denaturing HPLC [85] and microchip techniques [86,87] have been successfully applied for analysis of DNA fragments and have shown great potential to become powerful tools for analyses of genetic mutations and polymorphisms.

5. List of abbreviations

CBS	Cystathionine β-synthase
CE	Capillary electrophoresis
dsDNA	Double-stranded DNA
FAM	5-Carbonfluorescein
FH	Familial hypercholesterolemia
HEC	Hydroxyethylcellulose
HEPES	*N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid
HMC	Hydroxymethylcellulose
HEF	Hemochromatosis
HPMC	Hydroxypropylmethylcellulose
HPLC	High-performance liquid chromatography
JOE	2′,7′-Dimethyl-4′5′-dichloro-6-carboxyfluorescein

LDL	Low-density lipoprotein
F-HCM	Familial hypertrophic cardiomyopathy
LIF	Laser-induced fluorescence
LQTS	Long QT syndrome
MALDI-TOF-MS	Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry
MES	2-(*N*-Morpholino)ethanesulfonic acid
MTHFR	Methylenetetrahydrofolate reductase
MYH7	β-Myosin heavy chain
PA	Polyacrylamide
PCR	Polymerase chain reaction
PDMA	Polydimethylacrylamide
PEO	Poly(ethylene oxide)
R110	6-Carboxyrhodamine
R6G	*N*,*N*′-Diethyl-2′,7′-dimethyl-6-carboxyrhodamine
RFLP	Restriction fragment length polymorphism
ROX	6-Carboxy-X-rhodamine
SSCP	Single-strand conformation polymorphism
ssDNA	Single-stranded DNA
TAMRA	*N*,*N*,*N*′,*N*′-Tetramethyl-6-carboxyrhodamine
TAPS	*N*-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid
TBE	Tris–boric acid–EDTA

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