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# Use of Constant Denaturant Capillary Electrophoresis of Pooled Blood Samples to Identify Single-Nucleotide Polymorphisms in the Genes (*Scnn1a* and *Scnn1b*) Encoding the α and β Subunits of the Epithelial Sodium Channel

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***Background:*** **The epithelial sodium channel (ENaC) is composed of three homologous subunits: α, β, and γ. Mutations in the *Scnn1b* and *Scnn1g* genes, which encode the β and the γ subunits of ENaC, cause a severe form of hypertension (Liddle syndrome). The contribution of genetic variants within the *Scnn1a* gene, which codes for the α subunit, has not been investigated.**

***Methods:*** **We screened for mutations in the COOH termini of the α and β subunits of ENaC. Blood from 184 individuals from 31 families participating in a study on the genetics of hypertension were analyzed. Exons 13 of *Scnn1a* and *Scnn1b*, which encode the second transmembrane segment and the COOH termini of α- and β-ENaC, respectively, were amplified from pooled DNA samples of members of each family by PCR. Constant denaturant capillary electrophoresis (CDCE) was used to detect mutations in PCR products of the pooled DNA samples.**

***Results:*** **The detection limit of CDCE for ENaC variants was 1%, indicating that all members of any family or up to 100 individuals can be analyzed in one CDCE run. CDCE profiles of the COOH terminus of α-ENaC in pooled family members showed that the 31 families belonged to four groups and identified families with genetic variants. Using this approach, we analyzed 31 rather than 184 samples. Individual CDCE analysis of members from families with different pooled CDCE profiles revealed five genotypes containing 1853G→T and 1987A→G polymorphisms. The presence of the mutations was confirmed by DNA sequencing. For the COOH terminus of β-ENaC, only one family showed a different CDCE profile. Two members of this family (n = 5) were heterozygous at 1781C→T (T594M).**

***Conclusion:*** **CDCE rapidly detects point mutations in these candidate disease genes.**



Variations in genome sequence are likely to be one of the underlying factors in interindividual susceptibility to complex diseases such as hypertension or diabetes. The identification of single-nucleotide polymorphisms (SNPs)[5] in relation to multigenic diseases is critically dependent on rapid and efficient screening methods. Several technologies to detect mutations, including direct DNA sequencing, single-strand conformation polymorphism analysis, and heteroduplex analyses are available

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[5] Nonstandard abbreviations: SNP, single-nucleotide polymorphism; CDCE, constant denaturant capillary electrophoresis; ENaC, epithelial sodium channel; and nt, nucleotide(s).

[reviewed in *(1)*]. Recently, novel methods suitable for the high-throughput detection of SNPs have been developed *(2, 3)*. These innovations include the use of DNA chips *(4)*, microchip electrophoresis *(5)*, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry genotyping *(6)*, and automated DNA sequence analysis or real-time pyrophosphate DNA sequencing *(7)*.

One strategy to increase the efficiency of mutation detection in genetic studies is to pool DNA samples prior to analysis *(8, 9)*. Highly sensitive methods allow the detection of SNPs in pools containing a large number of DNA samples. The use of constant denaturant capillary electrophoresis (CDCE) *(10)* to detect mutations is well suited for the analysis of pooled samples *(11)*. CDCE has been demonstrated to be an efficient method for the detection of mutations in tumors *(12, 13)*. A slightly modified method for CDCE analysis that allows automation on a commercially available capillary electrophoresis apparatus has been reported recently *(14)*. Theoretical considerations on the use of CDCE to identify genes underlying different diseases are presented in the report by Tomita-Mitchell et al. *(15)*. CDCE is based on the differential mobility of partially melted DNA fragments in gel matrixes *(16)*. Separation of DNA fragments containing a difference in a single base pair can be achieved by electrophoresis of the DNA at defined temperatures. Partial denaturation of the DNA fragments at defined temperatures leads to different mobilities of standard and mutant DNA fragments, as either homoduplexes or heteroduplex sequences, in a gel.

Essential hypertension arises from the interplay between different sequence variants in one or several as yet undefined genes and environmental factors. The candidate gene approach to understand the genetics of human essential hypertension identified two important genes contributing in the regulation of blood pressure, the genes that encode for angiotensinogen *(17)* and the epithelial sodium channel (ENaC). The ENaC plays a critical role in the distal part of the nephron, where the amount of sodium excreted in the urine is controlled. The ENaC is composed of three homologous subunits: $\alpha$, $\beta$, and $\gamma$ ENaC. Mutations in the COOH termini of $\beta$- and $\gamma$-ENaC cause a rare monogenic form of severe hypertension (Liddle syndrome) as a result of excessive sodium reabsorption in the distal nephron of the kidney *(18, 19)*. These mutations are mainly base substitutions, leading to stop codons and truncation of proteins, or frameshift mutations. Among the mutations identified, ~88% and 12% of the mutations were found in the $\beta$ and $\gamma$ subunits, respectively *(20)*.

Because the mutations linked to Liddle syndrome were observed in the COOH termini of the $\beta$ and $\gamma$ subunits of ENaC *(18)*, we determined the presence of mutations in exon 13 (COOH terminus) of the *Scnn1a* gene to gain some insight into the role of $\alpha$-ENaC in the pathogenesis of more common forms of hypertension. In this report, we apply CDCE to screen for those variations in pooled DNA samples and show that families can be categorized by their CDCE profiles. For comparison, we also analyzed the COOH terminus of the $\beta$ subunit in that population. The presence of genetic changes in the *Scnn1a* and *Scnn1b* genes of families with different profiles was confirmed by DNA sequencing.

## Materials and Methods

### SAMPLES

Genomic DNA was extracted from peripheral blood from 184 inhabitants of the Seychelles Islands by standard procedures. These individuals are members of 31 families participating in a large study on the genetics of hypertension. The University of Lausanne Human Studies Review Board approved this study.

### PCR

Using the pairs of primers shown in Table 1, we amplified exon 13 of the *Scnn1a* gene as two fragments: 13a, corresponding to the 5′ end of exon 13, and 13b, corresponding to the 3′ end of the exon. Fragment 13a contained the first 193 bases of exon 13 [nucleotides (nt) 1620–1813; codons 541–605]. Fragment 13b contained bases 142–390 of exon 13 (nt 1765 to *31; codons 589 to the stop codon 670 and 31 bases in the 3′ untranslated region).

Exon 13 of the *Scnn1b* gene was also amplified as two fragments, B13a and B13b, using the pairs of primers shown (Table 1). Fragment B13a contained 14 bases of the flanking intron and the first 219 bases of exon 13 (codons 516–594). Fragment B13b contained bases 219–416 of exon 13 (codons 579 to the stop codon 641 and 31 bases in the 3′ untranslated region).

One of the primers had a GC-clamp added for optimal resolution of mutant fragments and/or a fluorescein group to permit detection by CDCE. PCR was carried out using *Taq* polymerase (Amersham) in 20-$\mu$L reaction volumes containing 50 ng of genomic DNA, 10 pmol of each primer, and 400 pmol of deoxynucleotide triphosphates in the buffer provided. When pooled DNA samples were analyzed, equivalent amounts of DNA from the individuals were mixed before amplification. Thermal cycling conditions were as follows: initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 50s, 65 °C for 45s, and 72 °C for 50s; with a final extension period of 7 min at 72 °C.

### CDCE

The CDCE apparatus was as described by Khrapko et al. *(10)*. Briefly, 30 cm × 50 $\mu$m (i.d.) fused-silica capillaries (Polymicro Technologies) coated with linear polyacrylamide chains were used. A portion of the capillary was maintained at the temperature optimal for separation of the fragments (described below) by a water jacket connected to a circulating water bath. A window made in the external coating of the capillary was aligned to a low-power 488 nm argon laser to detect the fluorescein-labeled products. The capillary was filled with 5% linear poly-

**Table 1. PCR primers used in amplification of exons 13 of the *Scnn1a* and *Scnn1b* genes.**

| Fragment | Sense primer | Antisense primer | Fragment size, bp |
|---|---|---|---|
| 13a | 5′-agaaccctctgtcccatcgtc-3′ | 5′-gggcgggggcggcgcgggacgggcgcgggcggcgcgggcgggaggatgccaggggtggaggc-3′ F1 | 249 |
| 13b | 5′-cgccgcgcgcccgcgccgtccgcgccccgcccccgaagcgatactggtctccag-3′ F1 | 5′-gccttggtgtgagaaacctctc-3′ | 315 |
| B13a | 5′-gggcgggggcggcgggacgggcgcgggcggcgcgggcggtggcctgagctcacccccagctc-3′ F1 | 5′-cgtgtcaggctggaagccaaag-3′ | 233 |
| B13b | 5′-gggcgggggcggcgggacgggcgcgggcggcgcgggcggtggaggcccacac-3′ F1 | 5′-acagtcttggctgctcagtgag-3′ | 197 |

acrylamide, which was replaced prior to each run *(21)*. PCR products were diluted in water (1 $\mu$L of sample in 20 $\mu$L of water) and loaded on the CDCE by electrokinetic injection for 1 min without further purification. The fluorescein-labeled PCR products were electrophoresed at 18 $\mu$A.

Data analysis was performed with the statistical package S-plus, Ver. 4.5 (Math Soft). The WinMelt program (MedProbe AS) was used to determine the melting temperatures reflecting the temperatures suitable for the separation of exon 13 fragments containing single base differences. DNA samples from different individuals of a family were pooled, amplified, and analyzed by CDCE, or the DNA from each individual was amplified and analyzed separately on the CDCE.

### DNA sequencing

The DNA sequences of the PCR fragments were determined by cycle sequencing using the Taq DNA Sequence reagent set according to the supplier's instructions (Roche) or by automatic sequencing performed by Microsynth.

### Statistical analysis

The ANOVA model used in the analysis of retention times of the peaks was:

$$y_{ij} = \mu + \alpha_i + \beta_j + \text{error}_{ij}$$

with $i = 1, \ldots, 5$ and $j = 1, \ldots, 4$ being peak and days, respectively. The model was fitted with the program S-plus.

## Results

### Optimized conditions for resolution of sequence variants in exon 13 of *Scnn1a*

The theoretical melting properties of fragments 13a and 13b were first obtained using the WinMelt program. The results for fragment 13b are shown in Fig. 1 as an example. Fig. 1A shows the melting profile of fragment 13b in the presence of a GC-clamp at the 5′ end of the sequence. A high-melting domain created by the GC-clamp transformed bases 142–390 of exon 13 (nt 1765 to *31) to a low-melting region with a theoretical melting temperature of 79.3 °C. Different melting profiles were observed for fragments containing an A→G mutation in nt 1987, double mutations (G→T transversion in nt 1853 and A→G transition in nt 1987), and the standard sequence in the database (standard sequence) indicating that mutant standard sequences should be resolved (i.e., should have different mobilities in a gel matrix).

The optimal temperature for the resolution of fragment 13b was obtained by performing CDCE runs at different temperatures. The temperatures used were 79.3 ± 1 °C with 0.1 °C increments. Using DNA samples whose sequence corresponded to the *Scnn1a* standard sequence,

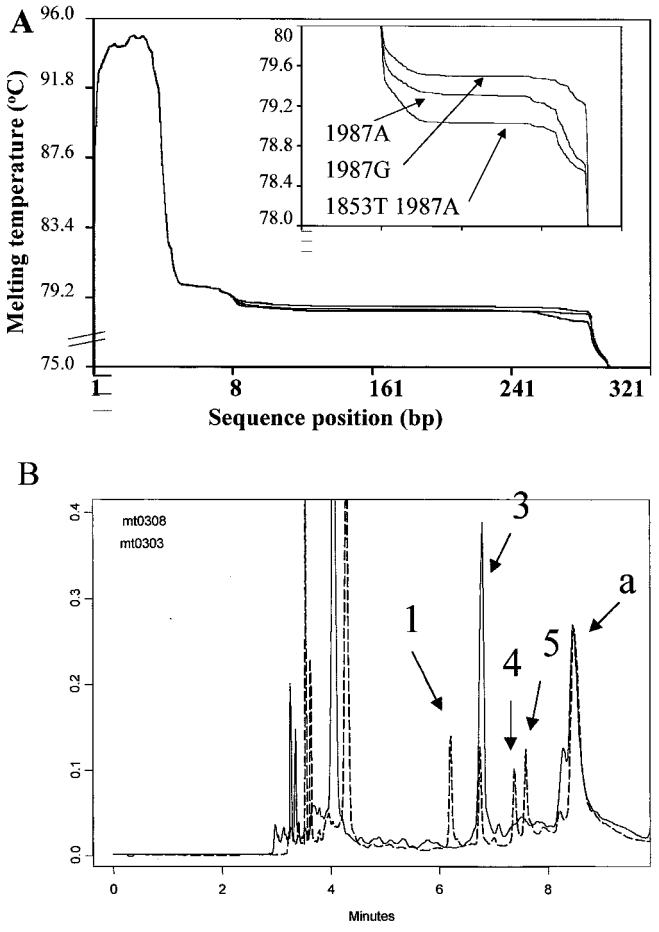



Fig. 1. Optimization of conditions for resolution of mutant and standard fragments by CDCE.

(*A*), melting profiles of fragment 13b encompassing nt 1765 to *31 (codon 589) to nt 2039 (stop at codon 670 and 31 bases in the 3′ untranslated region) of the *Scnn1a* (α-ENaC) gene and fragment 13b containing an A→G mutation in nt 1987 (codon 663) or fragment 13b with double mutations, G→T transversion in nt 1853 (codon 618) and A→G transition in nt 1987 (codon 663). The *inset* shows the melting profiles between 78 and 80 °C. (*B*), CDCE at 79.2 °C was performed using our standard sequence (——) and a 1987 A→G heterozygous sequence (– – –). The *arrows* indicate the positions of the homoduplexes (*peaks 1* and *3*) and the heteroduplexes (*peaks 4* and *5*). The peaks at ~4 min are the primers. *Peak a* denotes the position tentatively assigned to the single strand.

the 1987A homozygote (GenBank accession no. NM 001038, Z92981), or a DNA sample from a 1987A→G heterozygote, we found that the standard and heterozygous samples showed different profiles at 79.2 °C (Fig. 1B). Two major peaks (peaks 3 and a in Fig. 1B) were present in the CDCE profile (79.2 °C) of the standard sequence. The second major peak (peak a) increased with higher temperatures and was therefore assigned as the position of single-stranded DNA. Peak 3 was assigned to the position of the homoduplex DNA. In the 1987A→G heterozygote, four peaks were observed in addition to peak a. Because only one peak was present at temperatures lower than the theoretical melting temperature (data not shown), the presence of the additional peaks indicated that the mutant and standard peaks were resolved at 79.2 °C. The four peaks represented the two homoduplex peaks and the two heteroduplex peaks. Because one of the peaks (peak 3) comigrated with the standard peak, it was assigned to the homoduplex A fragment.

The detection limit of CDCE under the conditions used for the resolution of fragment 13b was next determined using DNA samples amplified from a homozygous (1987G) individual and a 1987A→G heterozygote. Fragment 13b from samples containing different ratios of DNA from homozygotes and heterozygotes was also amplified and analyzed by CDCE. Fig. 2 shows the CDCE plots of DNA from a homozygote (0% heterozygote), DNA from a homozygote containing 1% or 5% DNA from a heterozygote, and DNA from a heterozygote. In the homozygous sample (Fig. 2A), only one peak (peak 1) at ~6 min was observed. Because the DNA sample was from an individual who was homozygous G at nt 1987, peak 1 was assigned to the G:C homoduplex fragment. In the sample containing 1% DNA from a heterozygote (Fig. 2B), in addition to peak 1, peaks 3, 4, and 5 were also visible. Peak 3 migrated in the same position as the A:T homoduplex peak of the standard sequence (Fig. 1B). Peaks 4 and 5 were not observed in DNA samples from the homozygous individuals and were therefore presumed to be the G:T or A:C heteroduplexes. Peaks 3, 4, and 5, which were present in increasing amounts in the samples containing 5% or 100% DNA from heterozygous individuals, supported the assignments of the peaks. Because peaks 3, 4, and 5 were already observed in the presence of 1% DNA from heterozygous individuals, we concluded that a mutant present at ≥1% of the sample alleles would be detected under the conditions used.

To evaluate the reproducibility of CDCE, we analyzed a sample containing a mixture of three mutants (50% 1853G→T heterozygotes, 16.7% 1987A→G heterozygotes, and 33.3% 1853G→T:1987A→G compound heterozygotes) several times. Fig. 3 shows five overlapping analyses of the same sample performed on a single day. The profiles observed in the different analyses were similar, with each showing the presence of five peaks. The retention times of the primer and the five peaks measured in eight analyses (the five shown in Fig. 3 and three others each measured on a separate day) are shown in Table 2. An ANOVA model with factors day (four levels) and peak (five levels) fitted the data well with a multiple $R^2$ of 99%. The ANOVA results are shown in Table 3. The error variance was estimated as 14, i.e., a SD of the relative retention times of the peaks of ~3.7 s.

### MUTATION SCREENING IN POOLED SAMPLES

In the first screen for α-ENaC variants, exon 13 of the *Scnn1a* gene was amplified from pooled genomic DNA from all available members (n = 2–12) of any one of the 31 families and analyzed by CDCE. Analysis of the CDCE profiles of fragment 13a in the 31 families showed that the profiles for fragment 13a were similar to the standard

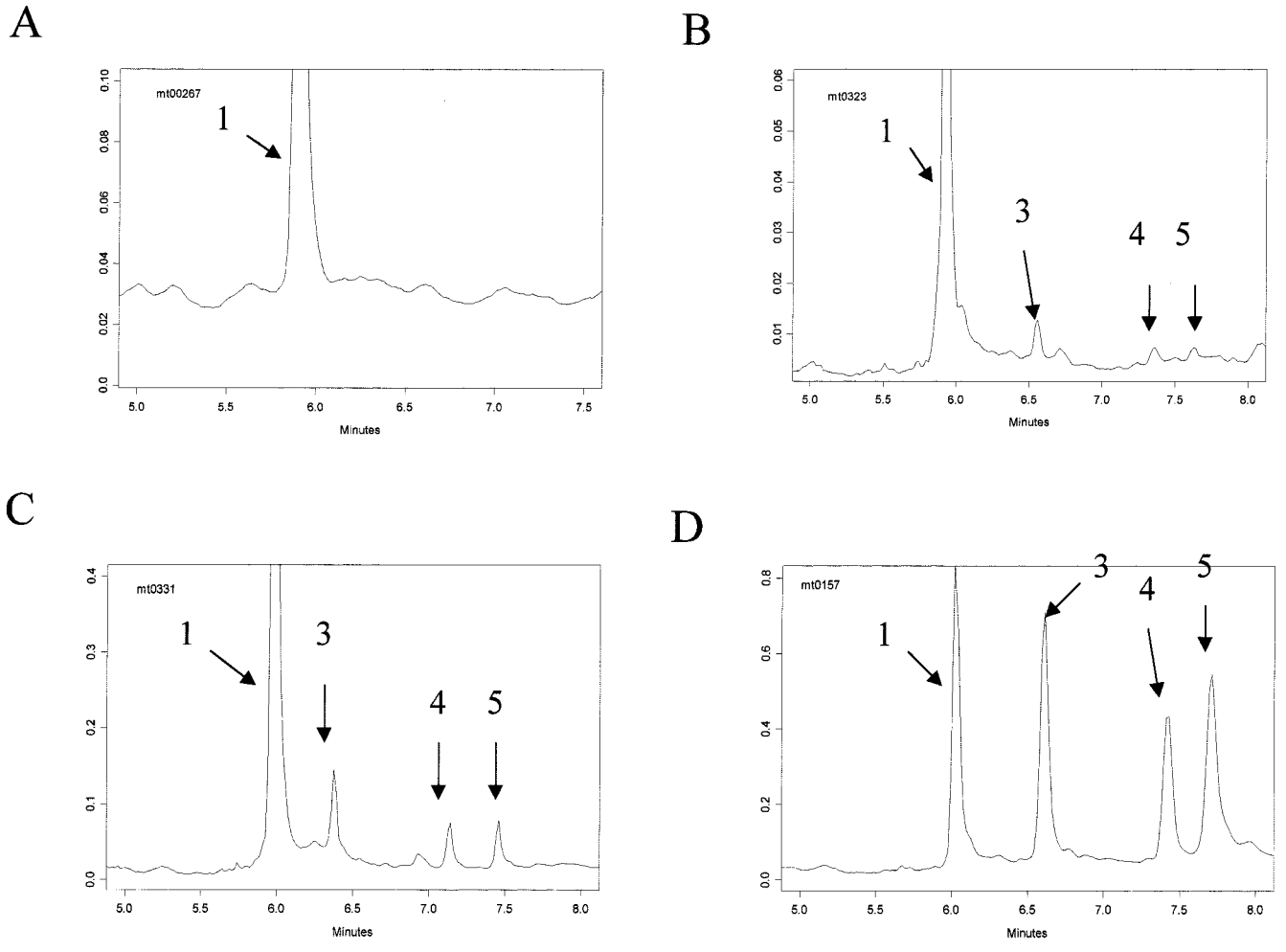



Fig. 2. Detection limit of CDCE.

Reconstruction CDCE analyses were performed with samples containing DNA from an individual homozygous G at nt 1987 and samples with different amounts of DNA from a 1987 G→A heterozygote. Shown are CDCE plots for samples from a 1987G homozygote (*A*), a 1987G homozygote containing 1% DNA from a 1987G→A heterozygote (*B*), a 1987G homozygote containing 5% DNA from a 1987G→A heterozygote (*C*), and a 1987G→A heterozygote (*D*). *Peaks 1* and *3* are the 1987 G:C and the 1987 A:T homoduplexes, respectively. *Peaks 4* and *5* represent the G:A or A:G heteroduplexes.

*Scnn1a* sequence. This result indicated the absence of variants in fragment 13a. Analysis of their DNA sequence (data not shown) confirmed their similarity.

Regarding fragmented 13b, CDCE analysis of the 31 families showed that they could be categorized according to the observed peaks (CDCE profiles) into four groups. Each group was predicted to have defined genotypes. The CDCE profiles of 18 families showed the presence of only one homoduplex peak. One family showed a CDCE profile that comigrated with our standard DNA (data not shown). The peaks in the remaining 17 families were found to be in the same positions and were assigned to group 1. Fig. 4A shows the CDCE profiles from two representative families. The presence of only one homoduplex peak (peak 1) in their CDCE profiles suggested that they were homozygotes and that the genotypes of all the members of those families were identical. The CDCE profiles obtained from the families, however, could not be superimposed with that of the standard sequence. Peak 1 migrated faster than peak 3 of the standard sequence (Fig. 4A). Therefore, the families were predicted to have sequences that were different from the standard sequence used as control.

The other 13 families clearly differed in their CDCE profiles from the standard sequence, indicating the presence of *Scnn1a* exon 13 variants. Examples of these whole-family CDCE profiles are shown in Fig. 4, B–D.

Two families (families 17 and 19) had CDCE profiles containing three homoduplex peaks (peaks 1–3; Fig. 4B), and they were assigned to group 2. Group 3 was characterized by CDCE profiles (Fig. 4C) that contained peaks 1 and 2. Five families (9, 11, 28, 37, and 40) had CDCE profiles of group 3. The presence of peaks 1 and 3 was a signature of the CDCE profiles of group 4 (Fig. 3D). Using this criterion, we assigned six families (18, 20, 34, 46, 53, and 56) to group 4.

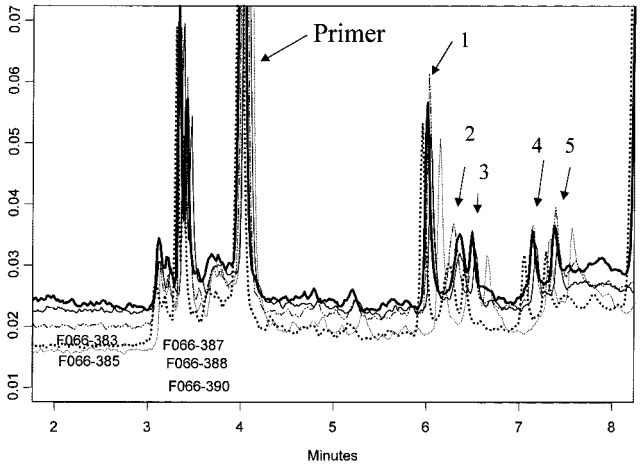



Fig. 3. Reproducibility of CDCE.

CDCE analysis of a sample containing a mixture of mutants (50% 1853 G→T heterozygotes, 16.7% 1987 G→A heterozygotes, and 33.3% 1853G→T: 1987A→G compound heterozygotes) was repeated five times. Each different electropherogram represents a different run.

### ANALYSES OF INDIVIDUAL FAMILY MEMBERS

To interpret the various peaks observed in the CDCE profiles, we characterized the individuals of one family (family 17) from group 2 by CDCE and DNA sequence analysis. The pedigree of the family and the CDCE analyses of fragment 13b are shown in Fig. 5. The CDCE profile obtained from pooled DNA of members of family 17 was found to contain three homoduplex peaks (Fig. 5B, peaks 1–3). Analysis of individual CDCE profiles showed the partitioning of the peaks among the family members (Fig. 5C). The CDCE profile of the mother contained peaks 1 and 2. Peaks 1 and 3 were observed in the CDCE profile of the father. Two of the children had peaks 2 and 3, similar to those of their mother and their father, respectively. The CDCE profiles of two other children (twins) contained peak 1 from the father and peak 2 from the mother. From the CDCE and DNA sequence analysis, we could confirm the identity of peak 1 as the 1853G:1987G homoduplex and peak 3 as the 1853G:1987A homoduplex. Peak 2 was assigned to the 1853T:1987G homoduplex.

Among the members of family 17, a slightly higher blood pressure (139/93 mmHg) was observed in the father, who was heterozygous at nt 1987. In the population under study, only 15 individuals showed that genotype. Analysis involving a larger number of individuals is required to determine whether that genetic variant is linked to increased blood pressure.

**Table 2. Retention times of sample peaks[a] relative to primer peaks.**

| | Retention time, s | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Peak | Day 1 | Day 2 | Day 3 | Day 4a | Day 4b | Day 4c | Day 4d | Day 4e |
| 1 | 125.2 | 116.85 | 128.5 | 119.3 | 121.0 | 120.3 | 119.1 | 124.6 |
| 2 | 144.2 | 137.7 | 145.4 | 138 | 143.1 | 137.1 | 136.6 | 144.4 |
| 3 | 157.8 | 146.6 | 160.3 | 147.9 | 150.3 | 148.3 | 146.2 | 154.6 |
| 4 | 203.7 | 188.3 | 206.9 | 187.2 | 190.4 | 187.7 | 184.7 | 194.9 |
| 5 | 220.0 | 202.6 | 223.1 | 201.6 | 204.6 | 203.2 | 199.1 | 210.2 |

[a] Peak numbers refer to those indicated in Fig. 3.

**Table 3. ANOVA results for the relative retention times of sample peaks.[a]**

| | Degrees of freedom | Sum of squares | Mean square | *F* | Pr(*F*) |
|---|---|---|---|---|---|
| Peak | 4 | 41960.92 | 10490.23 | 753.25 | 0.00 |
| Day | 3 | 993.48 | 331.16 | 23.78 | $2.78 \times 10^{-8}$ |
| Residuals | 32 | 445.65 | 13.93 | | |

[a] Data shown in Table 2.

### CHARACTERIZATION OF MUTATIONS

The DNA from all members of families showing CDCE profiles consistent with the presence of ENaC variants were analyzed individually. CDCE analysis and DNA sequence determination were performed to correlate the different CDCE patterns with variations in the DNA sequence. Five CDCE profiles were obtained when the family members were analyzed separately. Different mutations could be assigned to the five CDCE profiles obtained after analysis of the 31 families (Table 4). The CDCE profiles were 100% concordant with the nucleotide sequences in all (n = 40) individuals who were analyzed.

### ANALYSIS OF GENETIC VARIANTS IN EXON 13 OF *Scnn1b*

CDCE was used to screen for mutations in the COOH terminus of the β subunit of ENaC (*Scnn1b*). Conditions for the separation of mutations in fragments B13a and B13b were optimized as described for the α subunit. The optimal temperatures for resolution of fragment B13a and B13b were 81 and 79.6 °C, respectively (data not shown). Among the 31 families who were analyzed, 30 families showed one homoduplex peak in their fragment B13a CDCE profiles (Fig. 6A). Only one family was found to show a different CDCE profile (Fig. 6B). Subsequent analysis of the members of this family by CDCE and DNA sequencing showed that two of the five members were heterozygous (C/T) at base pair 1781. All the families exhibited similar CDCE profiles in their fragment B13b, indicating the lack of genetic variation in this sequence (data not shown).

## Discussion

In this report, we show the application of CDCE for mutation detection in families participating in a study on the genetics of hypertension. We evaluated the presence of genetic variants in the COOH termini of *Scnn1a* and *Scnn1b* in 184 individuals from 31 families. CDCE was used to screen for mutations in pooled family samples containing DNA from 2–12 individuals. Exon 13 of *Scnn1a* was amplified from the genomic DNA in two overlapping fragments, fragment 13a, which contained the 5′ region of the exon, and fragment 13b, which contained the 3′ region. Reconstruction experiments indicated that muta-

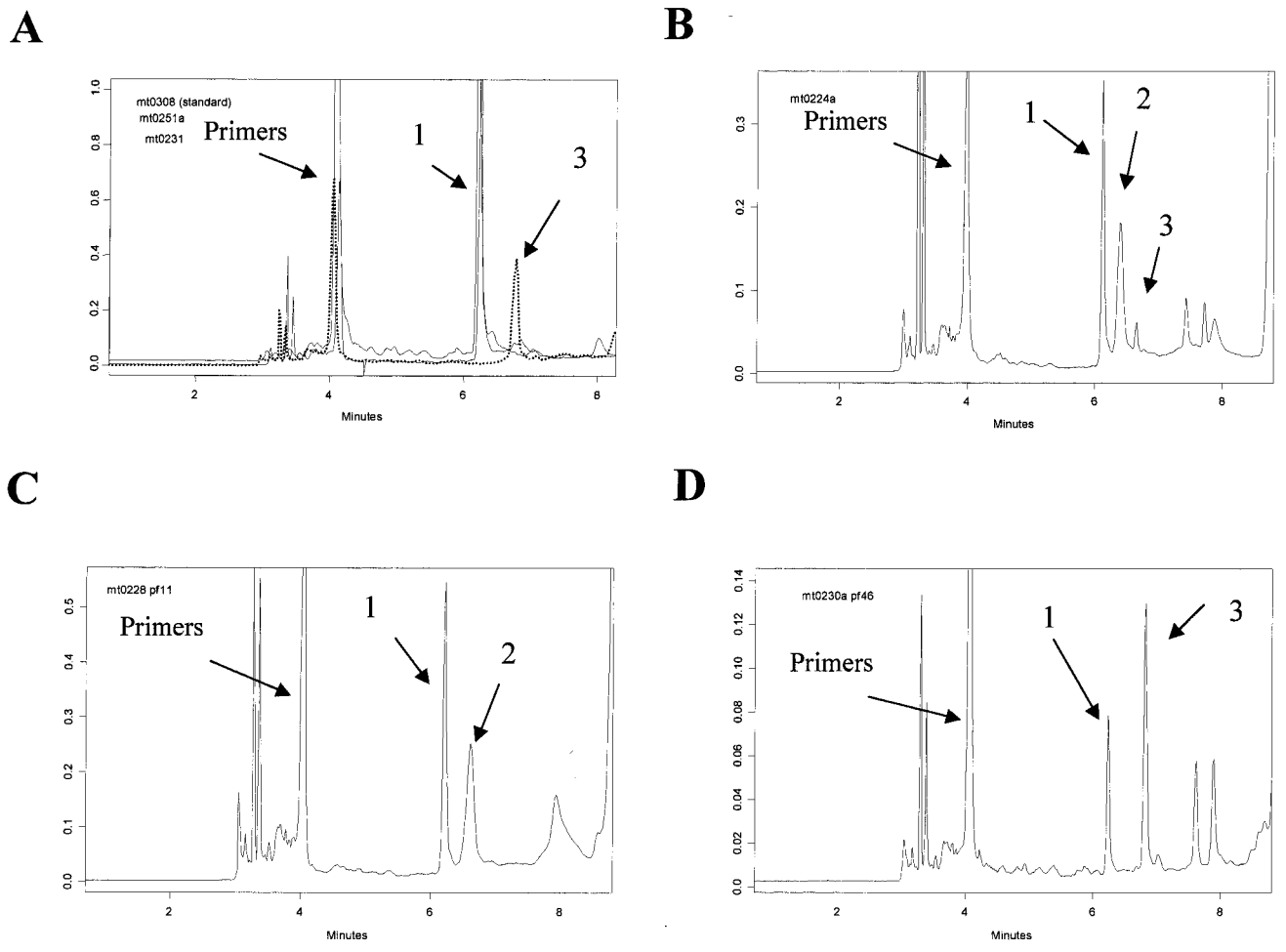



Fig. 4. CDCE analysis using pooled DNA samples.

(*A*), CDCE profiles of two representative families of group 1. The CDCE profile of our standard sequence (*dotted line*) is included for comparison. (*B*), CDCE profile of family 17 (n = 6), representative of group 2. (*C*), CDCE profile of family 11 (n = 4), representative of group 3. (*D*), CDCE profile of family 46 (n = 4), representative of group 4. Fragment 13B was amplified using genomic DNA from pooled DNA samples as described in the text. After amplification, the DNA samples were subjected to analysis on the CDCE at 79.2 °C. The *numbered peaks* were used to discriminate among different sequences in the fragment. The positions of the homoduplexes only are noted.

tions present in fragment 13b were resolved at 79.2 °C and that a limit of detection of 1% was achieved. With this approach, 30 of the 31 families analyzed had CDCE profiles that were different from our standard sequence. The families could be separated into four groups according to the peaks observed in the CDCE profiles from pooled DNA of family members. Detailed analysis of one of those families (family 17) showed the segregation of the CDCE peaks observed in the pooled sample among the individuals.

Individual analysis of the members (n = 184) of the families (n = 31) yielded five different CDCE profiles that corresponded to five genotypes (Table 4). Both previously reported SNPs in exon 13 of $\alpha$-ENaC *(22, 23)* were detected in our study. The 1853 G→T (C618F) polymorphism has been reported to occur at a frequency of 8% in Africans *(22)*. Of the 184 individuals who were analyzed, 82.3% were homozygous 1853G and 17.7% were heterozygous 1853G→T. No homozygous 1853T individuals were observed in the families studied. Because these 184 individuals were from 31 families, the different allele frequencies were estimated from the expected parental genotypes. The 1853G and 1853T allele frequencies were 0.9 and 0.1, respectively. A previously reported 1987A→G (T663A) polymorphism was found in 80% (homozygous G), 4.4% (homozygous A), and 15.5% (heterozygous) of the population under study. The allele frequencies in the parents were 18.2% 1987A and 83.3% 1987G. In previously reported studies, 54% and 31% of the individuals were homozygous 1987G and 1987A, respectively *(23, 24)*. Of the individuals in the present study, 2.9% were compound 1853G→T:1987A→G heterozygotes.

The DNA sequences corresponded to the CDCE profiles in all cases tested (40 of 40), showing that the CDCE profile accurately reflected the mutations that were present. To confirm that the presence of only one other peak in addition to peak a in pooled family samples was indicative of their homogeneity, members of the families

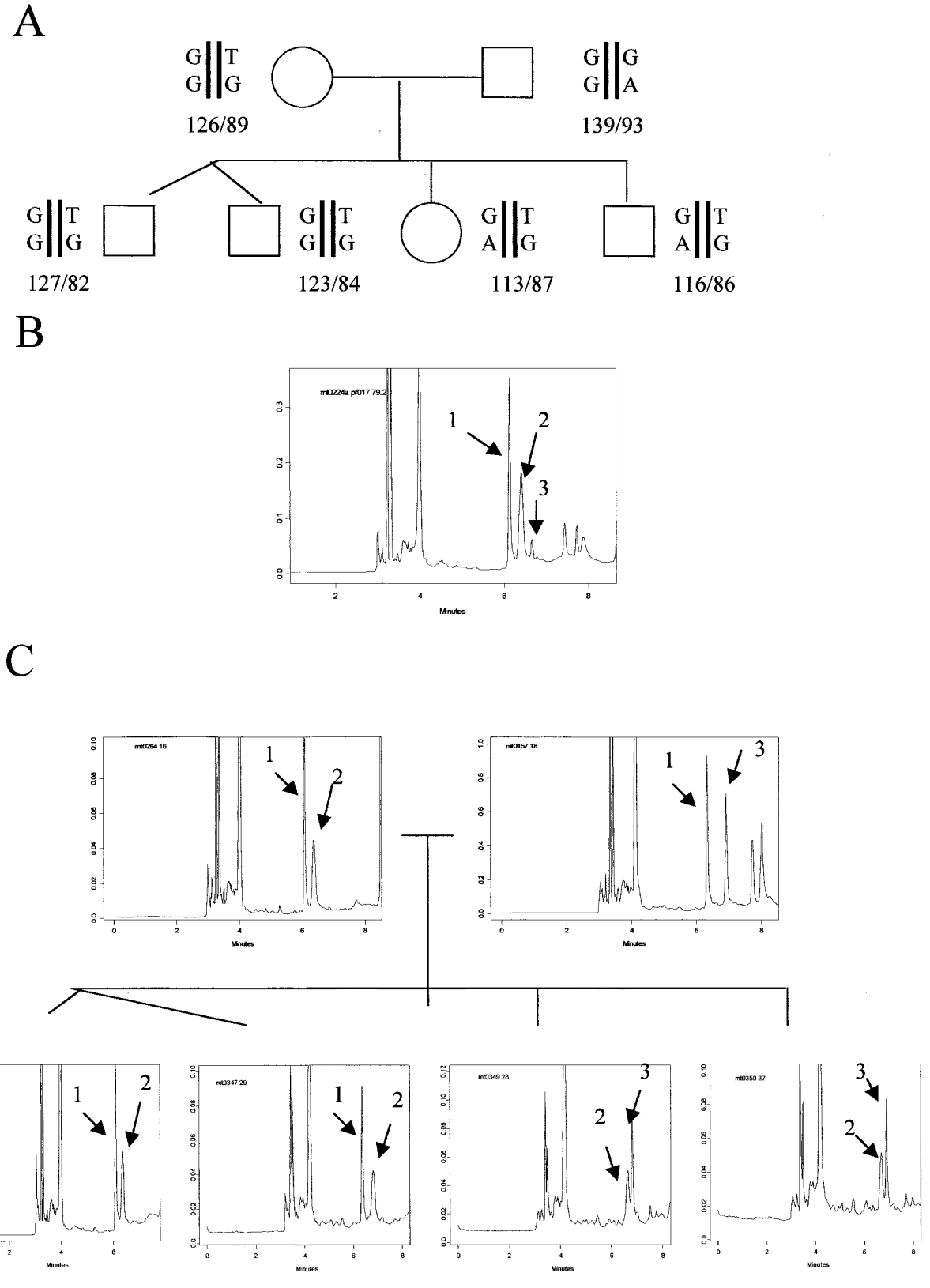
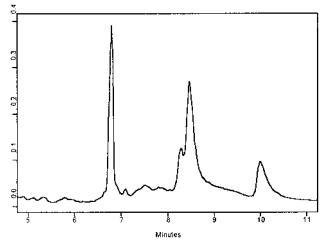
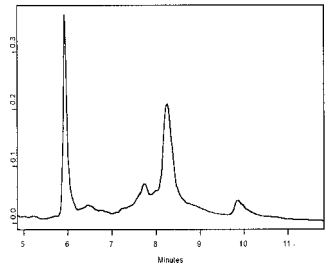
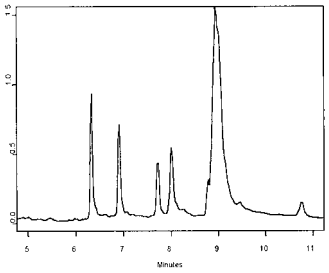
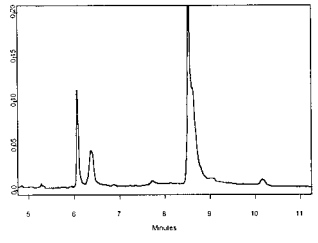
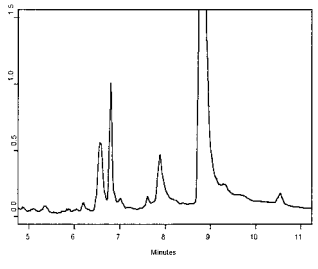



Fig. 5. Pedigree and CDCE analysis of fragment 13b of *Scnn1a* (α-ENaC) gene in family 17.

(*A*), pedigree of family 17. The blood pressures (systolic/diastolic, in mmHg) of the individuals are noted *below* their genotypes. (*B*), CDCE analysis of pooled family members. (*C*), CDCE analysis of family members. Fragment 13b was amplified using genomic DNA from pooled family members or each individual as described in the text. After amplification, the DNA samples were subjected to analysis on the CDCE at 79.2 °C. The peaks appearing at ~4 min are the primers. The *numbered peaks* are used to discriminate among different sequences in the fragment. The positions of the homoduplexes only are noted.

**Table 4. Different CDCE profiles and the DNA sequences present.**

| CDCE | nt 1853[a] (codon 618) | nt 1987[a] (codon 663) | n[b] | n[c] |
|---|---|---|---|---|
|  Profile 1 | G/G | A/A | 8 | 2/2 |
|  Profile 2 | G/G | G/G | 118 | 13/13 |
|  Profile 3 | G/G | A/G | 15 | 10/10 |
|  Profile 4 | G/T | G/G | 27 | 12/12 |
|  Profile 5 | G/T | A/G | 5 | 3/3 |

[a] Bases present in the two alleles.
[b] Number of individuals showing this CDCE profile.
[c] Number sequenced that confirmed genotype of CDCE profile.

showing such CDCE profiles (group 1) were also analyzed separately by either CDCE or DNA sequence determination. Individual analysis of 78 family members of group 1 showed that the genotypes of all the individuals analyzed were identical. This indicated that DNA sequencing in samples where no mutations were detected by CDCE found no additional mutations.

Ten different SNPs have been detected in the COOH

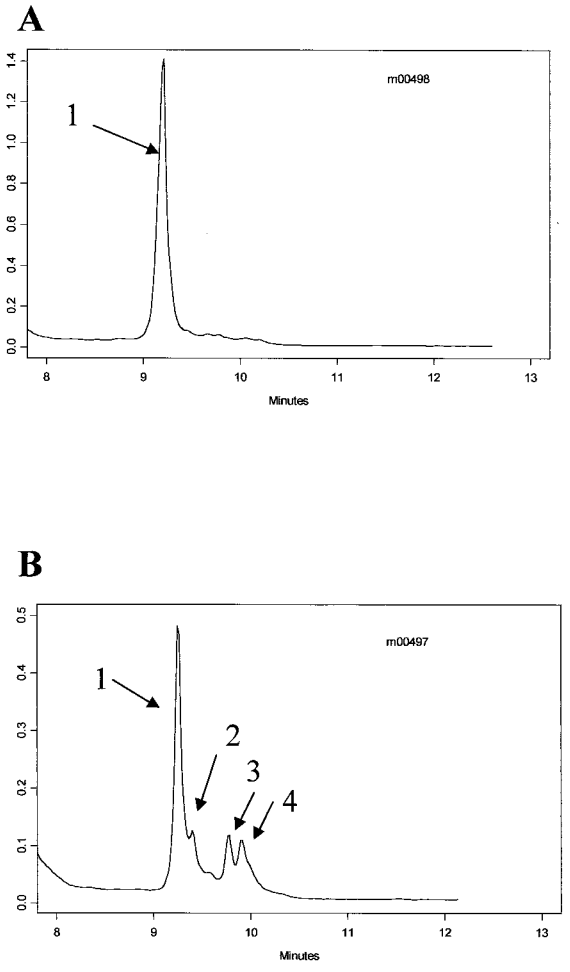



Fig. 6. CDCE analysis of fragment B13a using pooled DNA samples.

(*A*), CDCE profile of one representative family in which only one homoduplex peak was observed. (*B*), CDCE of the family in which four peaks were observed. Fragment B13a was amplified using genomic DNA from pooled DNA samples as described in the text. After amplification, the DNA samples were subjected to analysis on the CDCE at 81 °C.

terminus of the β subunit of ENaC *(25)*. The population we are studying, however, showed less genetic variations in this region. Only one family had a different CDCE profile. Two members of that family were heterozygous for the T594M mutation of the β subunit. Therefore, the most commonly identified mutation (T594M) of the sodium channel, which has been associated with hypertension in black people resident in London *(26)*, did not appear to be frequent in the Seychelles population.

Several advantages are obtained by an initial analysis of pooled DNA samples. The ability of CDCE to detect mutations in mixed populations *(10, 11)* makes this method ideal for the analysis of pooled DNA samples. When CDCE is used in a protocol similar to the one in the present study (based on the 1% detection limit achieved under the conditions that were used), all members from any one family or up to 100 individuals can be examined in a single CDCE run that takes ~15 min. With this strategy, the presence of mutations in an entire gene can theoretically be scanned in a family within a relatively short time. Because the CDCE profiles accurately reflect the heterogeneity of the sample, a screen of pooled DNA samples from each family locates families with mutations. Only those families that show different profiles from the control sequence need to be characterized in detail. Even larger pooled samples up to 100 000 individuals can be screened using optimized protocols already demonstrated for mitochondrial and nuclear mutations *(27, 28)*. The genotypes of individual members of those families can be determined and lead to the identification of mutations. Members of the families can also be grouped according to their profiles before further analyses. A representative number of each CDCE profile needs to be sequenced because the genotypes are accurately reflected in the CDCE profiles.

In our study, genetic changes were found in all the families that were predicted to contain mutations from the CDCE profile. We would have sequenced 62 individuals if only members of families that were identified in the CDCE prescreen had been analyzed. This translates to a reduction of 60% of sequencing reactions. In addition, individual CDCE analysis and grouping of CDCE profiles can be carried out before sequencing. Five different CDCE profiles were obtained after the analysis of individual samples. The nucleotide determination of two representatives from each CDCE profile would have reduced the number of samples to be sequenced from 184 to 10.

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