Application of molecular techniques in population genetic studies of cystic fibrosis in the Netherlands

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Chapter 2

Validation of determination of ΔF508 mutations of the cystic fibrosis gene in over 11,000 mouthwashes.

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Abstract

Mouthwashes can be used as DNA resource for mutation detection. Because collection and DNA isolation is simple and cheap, it could especially be used for large numbers of samples. To determine the failure rate (the proportion of mouthsamples in which no PCR product was obtained) and the specificity of buccal epithelial cell mutation detection in large numbers of samples, we collected mouthwashes and blood samples from 11,413 blood donors and tested the mouthwashes for the ΔF508 mutation, which has an estimated frequency of 75% among cystic fibrosis chromosomes in The Netherlands. Blood samples were tested for the ΔF508 mutations only if the mutation was identified in the mouthwash or in case of a failure to obtain PCR products. The sensitivity of the test was determined in mouthwashes of 75 ΔF508 carriers known from earlier family studies. These samples were offered blindly between the mouthwashes of the blood donors. Both specificity and sensitivity of the mouthwash procedure were 100%. The overall failure rate was 5.6 %. This large figure was caused mainly by insufficient rinsing of the mouth in one particular blood bank. Exclusion of the results of this blood bank
bank reduced the failure rate to 1.8%. Our results confirm that also for large number of samples the mouthwash procedure is suitable for mutation detection and with proper instructions can be used in community screening.

Introduction

White blood cells are the main source of DNA used for gene analysis (Miller et al.1980). Blood sampling and DNA isolation from great numbers of such samples, however, have a few disadvantages: medical supervision is necessary for blood collection, people may shrink from venopuncture, there is a risk of exposure to blood pathogens for the donor as well as for the investigator, and costs of DNA isolation are high. An alternative is sampling of mouthwashes followed by DNA isolation from buccal cells (Lench et al.1988). This procedure is much simpler and cheaper than existing methods, especially with large numbers of samples. There is no need for medical supervision of sample collection, and the risk of infections is eliminated.

To determine the failure rate (the proportion of mouthwash samples in which no PCR product was obtained), the specificity and the sensitivity of the mouthwash procedure, we studied the presence of the ΔF508 mutation of the cystic fibrosis (CF) gene in a large number of mouthwash samples with known or unknown carrier status.

Materials and Methods

Recruitment and sampling

11,413 blood donors from 15 blood banks were approached in a study to determine the prevalence of carriers of the ΔF508 mutation in the CFTR gene in this country and the failure rate and specificity of the mouthwash procedure. The design was approved by the ethical committee of the Groningen University Hospital. Blood bank officials were instructed how to recruit volunteers among the blood donors. After informed consent, the blood donors were asked to rinse their mouth with 15
ml of 0.9% sterile saline for 10 seconds. The samples were collected in sputum containers (Emergo, Landsmeer, The Netherlands). Matched blood samples of the blood donors were collected in 10 ml heparin tubes. Samples were stored at 4°C until shipment to either one of the two participating laboratories. Here all mouthwash samples were analyzed. In order to determine the proportion of false positives mutations detected in mouthwash DNA were checked in the matched blood samples. Sensitivity was determined in mouthwash samples of 75 known ΔF508 carriers detected in earlier studies of CF families. The samples of these carriers were offered blindly to the investigator among the mouthwashes of the blood donors.

**DNA isolation and (mutation) analysis**

DNA isolation out of buccal samples was performed according to a procedure modified from Lench et al.(1988). Buccal cells were pelleted by centrifugation at 1500 g for 10 minutes. The cell pellet was resuspended in 100 µl 50 mM sodiumhydroxide and boiled for 10 minutes. Samples were neutralised with 14 µl 1 M TRIS (pH 7.5) and 5 µl was taken for PCR analysis. Genomic DNA was extracted from heparin blood according to Miller et al.(1980).

The ΔF508 mutation was determined in the following way. PCR was performed with primers according to Scheffer et al.(1989) in a Pharmacia LKB-Gene ATAQ controller with denaturation temperatures decreasing from 94°C to 88°C in three steps of 10 cycles. The ΔF508 mutation was detected directly on a 13% polyacrylamide gel (50 samples per gel). Mutations detected in mouthwashes were checked in the matched blood samples.

**Results**

Out of 11,413 mouthwashes we were able to analyse PCR products in 10,772 samples. A total number of 254 ΔF508 carriers were detected in this group. All 254 positive results could be confirmed by DNA analysis in matched blood samples. The specificity of the mouthwash procedure was 10501/10501 or 100% (95% CI= 99.96-100%, assuming a sensitivity of 100%, see below).
DNA isolation failures occurred in 641 cases, due to insufficient mouthwashings, leaking containers and food rest contamination. The overall failure rate of mouthwashes therefore was 5.6%. In the matched blood samples of these cases an additional 17 ΔF508 carriers were detected. The difference in the carrier frequencies detected in mouthwashes and blood samples was not statistically significant.

The samples (492) collected from one particular blood bank yielded few successful DNA isolations, with a failure rate of 90 percent. This probably has been caused by insufficient rinsing of the mouth. Exclusion of these data resulted in a total yield of 98.2 % successful DNA analyses (failure rate 1.8%).

Of the failures remaining after excluding those samples of the above mentioned blood bank, almost 40% was caused by containers leaking during transport of the mouthwash samples to the laboratories. Another 40% was caused by insufficient rinsing and 20% by foodrest contamination.

More then 1,000 samples were sent from the blood banks to the laboratories after storage for 14 days at 4°C. DNA isolation from these mouthwashes has the same success rate of over 98%.

We were able to obtain PCR products from 74 out of 75 mouthwash samples of known ΔF508 carriers. In all 74 samples we confirmed carriership of this mutation. This gives a sensitivity of 100% (95 % CI= 95.1-100%). In one case we were unable to isolate DNA due to contamination with food remains. The percentage of successfully analysed mouthwashes of known carriers, therefore, was 98.2% (failure rate 1.8%).

Discussion

We detected a total of 271 ΔF508 carriers out of 11,413 unrelated individuals. Since ΔF508 represents 75% of the CF mutations in The Netherlands (Scheffer et al. submitted), this is in keeping with a prevalence of the CF carrierstatus (all mutations) of about 1 in 30 in The Netherlands (Ten Kate 1977). The specificity and sensitivity of the mouthwash procedure were both 100%.

The validation of DNA isolation out of buccal cells (mouthwashes/mouthbrushes) has been described before (Richard et al. 1993;
Gilfillan et al. 1994). These studies applied multiplex PCR amplifications to detect the most frequent CFTR mutations. The success rates of these studies were comparable with our results. The present study is the first to present data on the sensitivity of the mouthwash procedure as well as effects of storage and transport. Determination of the failure rate and the specificity of the procedure are based upon a substantially larger number of samples than in the previous studies.

Our analysis of 11,413 mouthwashes showed a PCR success rate of 94.4%. After exclusion of the samples from one particular blood bank this percentage increased to 98.2%. Because mutation detection was comparable among the other 14 blood banks, the failures to obtain PCR-products in the samples of one blood bank seem to represent an isolated case.

We did not collect mouthwashes under optimum conditions, because blood donors generally are advised to eat before donating blood. Excluding the one particular blood bank, this resulted in 30 PCR failures out of 10,921 (0.27%).

For the application of the mouthwash procedure one should be aware of the above mentioned problems. The need for giving good instructions should not be underestimated, even with simple collection procedures. If this condition is fulfilled, mouthwashes can easily be used for single mutation detections with a large number of samples, like in carrier screening programmes.

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