between the SARS cases and controls, between SARS cases with poor outcomes and controls, between the male SARS patients with poor outcome and the male controls, or between the female SARS patients and female controls (Table 1). The observed genotype distributions for each of the five loci among the female controls did not deviate significantly from those expected from the Hardy–Weinberg equilibrium. The genotype frequencies for each of the five SNP loci were not statistically significantly different between the female SARS patients and the female controls. Because males are hemizygous for ACE2, the genotype frequency is equivalent to the allele frequency.

We therefore conclude that although ACE2 serves functionally as the receptor for entry of the SARS coronavirus into human host cells, the evidence provided by this study does not support an association between its common genetic variants and SARS susceptibility or outcome. Despite its X-chromosome location, poor outcomes in male SARS patients do not appear to be related to genetic variants of ACE2.

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References

Comparison of Cystine Determination in Mixed Leukocytes vs Polymorphonuclear Leukocytes for Diagnosis of Cystinosis and Monitoring of Cysteamine Therapy, Elena Levchenko, Adrian de Graaf-Hess, Martijn Wilmer, Lamberts van den Heuvel, Le Monnens, and Henk Blom

Cystinosis is a rare autosomal recessive disorder caused by mutations of cystinosis gene (CTNS; chromosome 17p13), which encodes the lysosomal cystine carrier. The continuous accumulation of cystine in the lysosomes leads to intracellular crystal formation throughout the body. Patients with the common infantile form of cystinosis develop renal Fanconi syndrome 3–6 months after birth and end-stage renal failure before the age of 10 years. Treatment with the aminothiol cysteamine depletes intralysosomal cystine via a disulfide exchange reaction with formation of cysteine-cysteamine mixed-disulfides and cysteine; these exit the lysosomes via lysosomal carriers for lysine and cysteine, respectively (1). When started at an early age, cysteamine treatment prevents or postpones the deterioration of renal function and the occurrence of extrarenal complications such as hypothyroidism, diabetes mellitus, retinopathy, encephalopathy, and myopathy (1).

Accurate measurement of intracellular cystine content is obligatory for the diagnosis of cystinosis as well as for the monitoring of treatment with cysteamine. Historically, cystine has been measured in mixed leukocyte (ML) preparations, despite the fact that it preferentially accumulates in polymorphonuclear leukocytes (PMN) and monocytes (2). We therefore compared intracellular cystine content in ML preparations and in PMN cells of healthy controls, obligate heterozygotes, and patients at diagnosis and under cysteamine therapy. Because the isolation of PMN may pose practical problems in some laboratories, we also investigated whether preservation of whole blood at room temperature influenced intracellular cystine content. If the cystine concentration remains constant, it would allow the shipping of whole-blood samples.

MLs were isolated exactly as described by de Graaf-Hess et al. (3). All solutions were kept at 4 °C. PMN cells were isolated from 10 mL of blood by addition of 2 mL of dextran solution (50 g/L dextran T500, 15 g/L EDTA, 7
g/L NaCl, pH 7.4) in a 15-mL glass tube. After gentle mixing and ~1 h on ice, the clear upper solution was divided between two 15-mL screw-cap polypropylene tubes, brought up to a total volume of 8 mL with phosphate-buffered saline (PBS), and mixed gently. To the bottom of the tubes we carefully added 7 mL of Ficoll (Ficoll-Paque 1077; Amersham-Pharmacia) via a syringe with a 15-cm long needle; the tubes were then centrifuged at 500 \( \times \) g for 20 min at 4 °C in a swing out rotor with no braking. After centrifugation, the interphase containing the lymphocytes and the two liquid layers were completely removed. The pellet, containing PMN cells and some erythrocytes, was resuspended in 1 mL of PBS, and 3 mL of cold water was added for hypotonic lysis of the erythrocytes. After exactly 1.5 min on ice, 1 mL of 36 g/L NaCl solution was added; the solution was then mixed and centrifuged at 600 \( \times \) g for 10 min at 4 °C. The cells were washed with 5 mL of PBS and centrifuged again. Finally, the pellet was resuspended in 0.5 mL of PBS, transferred to a 1.5-mL screw-cap Eppendorf tube, and centrifuged at 1000 \( \times \) g for 5 min at 4 °C. The pellet was then frozen immediately in liquid \( N_2 \) and stored at −80 °C until the cystine was measured. The differential counts of ML and PMN preparations were determined automatically (Advia; Bayer®).

To determine whether blood samples can be preserved at room temperature, we collected fresh whole blood (2×10 mL per patient) in tubes containing 1.5 mL of acid-citrate-dextrose solution (ACD). PMN cells were isolated immediately and after 24 h of preservation at room temperature.

Cystine was measured by HPLC as described previously (3). ML preparations \( (n = 17) \) contained [mean (SD)] 37 (15)% PMN cells, 39 (11)% lymphocytes, and 5 (7)% monocytes.

The mean (SD) intracellular cystine content (nmol/mg of protein) was lower in ML compared with PMN cells in obligate heterozygotes \( [n = 15; 0.07 (0.03) vs 0.27 (0.17) \text{ nmol/mg of protein}; P < 0.001] \) and in patients treated with cysteamine \( [n = 12; 0.15 (0.08) vs 0.94 (0.58) \text{ nmol/mg of protein}; P < 0.001; Fig. 1] \). In two patients at the time of diagnosis (one with the infantile form and one with the late-onset form), the ML cystine content was within the reference interval, whereas it was increased in PMN cells (0.49 and 1.47 nmol/mg of protein, respectively) and in cultured fibroblasts (1.6 and 1.44 nmol/mg of protein, respectively).

Because of the switch from ML to PMN preparations, the dose of cysteamine had to be increased in 12 of 15 of patients under cysteamine therapy because their cystine concentrations measured in PMN cells were clearly above the desired concentration of 0.5 nmol cystine/mg of protein (90th percentile value of cystine concentration in PMNs of heterozygotes in our laboratory).

The mean (SD) cystine content of ML in obligate heterozygotes \( (n = 15) \) was indistinguishable from that of healthy controls \( (n = 8) \) but was clearly increased in PMN cells compared with the control values \( [0.27 (0.17) vs 0.09 (0.03) \text{ nmol cystine/mg of protein}; P < 0.05; Fig. 1] \).

PMN cells of blood samples stored in ACD tubes for 24 h at room temperature \( (n = 7) \) had increased mean (SD) cystine \( [0.95 (0.50) vs 0.59 (0.46) \text{ nmol/mg of protein}; P < 0.05; see Fig. 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue9/] \).

Detection of increased intracellular cystine is required for the diagnosis of cystinosis in patients presenting with Fanconi syndrome. After the diagnosis of cystinosis, intracellular cystine should be measured regularly to evaluate the cysteamine dose. Estimation of the optimum
leukocyte cystine concentration to be achieved under therapy is conjectural because it is not known whether cystine accumulation in blood cells is representative of the storage in other tissues. Generally, it is recommended to strive for a ML cystine concentration <0.5 nmol/mg of protein (1). This is also the upper limit of cystine seen in heterozygotes, who do not develop nephropathy (4). Because we observed a clear difference between cystine content in ML preparations and PMN cells, we suggest that each laboratory produces its own reference values based on the upper cystine values found in heterozygotes.

In 1970, Schultman et al. (2) showed that cystine accumulation in cystinotic leukocytes is located primarily in phagocytic blood cells rather than in lymphocytes. Cystine measurement in purified PMN preparations improved the sensitivity of the method. Our results were comparable to those of Smolin et al. (5), who used a cystine-binding assay for cystine determination. They also found lower cystine in MLs compared with PMNs in heterozygotes, in one untreated patient, and in patients undergoing cysteamine therapy. In our laboratory, the mean intracellular cystine content of healthy controls did not differ between MLs and PMNs, possibly because the low cystine concentrations in healthy persons are close to the detection limit of the HPLC method.

To our knowledge, no missed diagnosis of cystinosis as a result of low cystine concentrations in ML preparations has been reported previously. In our laboratory, the diagnosis of cystinosis could have been missed in two patients if cystine had been measured only in MLs. In one patient, treatment with cysteamine was delayed for 6 months because the cystine concentration in the MLs remained within the reference interval. Later the diagnosis of cystinosis in both patients was confirmed by clearly increased cystine in fibroblasts and mutational analysis of the CTNS gene.

The possible reasons for falsely low cystine in MLs, especially in young children, could be the overrepresentation of lymphocytes in ML preparations, typical for the first year of life. Furthermore, variations in the differential count of MLs in individual patients can lead to unreliable variations in measured cystine because it is expressed per milligram of protein in the total cell preparation.

As described by Kamoun et al. (6), for storage experiments, we also used ACD tubes for blood collection. In our laboratory, the storage of blood at room temperature for 24 h led to increases in intracellular cystine content. Thus, the shipping of whole-blood samples for cystine determinations is not advisable.

In summary, we recommend measurement of cystine in PMNs and not in ML preparations because the recommended approach increases the sensitivity of cystine detection for the diagnosis of cystinosis and provides a better target concentration during the monitoring of cysteamine treatment.

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References


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Oligonucleotide Microarray-Based Mutation Detection of the K-ras Gene in Colorectal Cancers with Use of Competitive DNA Hybridization, Jae-Hyun Park,† Il-Jin Kim,† Hio Chung Kang, Yong Shin, Hye-Won Park, Sang-Geun Jang, Ja-Lok Ku, Seek-Byung Lim, Seung-Yong Jeong, and Jae-Gaeh Park†. Korean Hereditary Tumor Registry, Laboratory of Cell Biology, Cancer Research Institute and Cancer Research Center, Seoul National University, Seoul, Korea; Department of Surgery, Seoul National University College of Medicine, Seoul, Korea; Research Institute and Hospital, National Cancer Center, Goyang, Gyeonggi, Korea; † these authors contributed equally to this work; † address correspondence to this author at: National Cancer Center, 809 Madu-dong, Ilsan-gu, Goyang, Gyeonggi, 411-764, Korea; fax 82-31-920-1511, e-mail park@ncc.re.kr

In cancer research, gene expression and mutations are increasingly investigated by use of oligonucleotide microarrays, which use immobilized oligonucleotides and sequence-specific DNA probe hybridization to investigate differences between nondiseased and cancer tissues (1). In our previous works, we used oligonucleotide microarray-based mutation analysis to detect germline or somatic mutations (2,3).

Activating mutations of the K-ras gene occur in ~20–50% of colorectal cancers, with ~85% of the mutations restricted to codons 12 and 13 (4). K-ras gene mutations have been widely studied as markers for cancer prognosis, and population-based studies have suggested that mutated K-ras might be associated with some tumor phenotypes (4–6). Studies of associations between K-ras mutations and specific clinical features generally require the analysis of large numbers of samples (5). Thus, researchers need a high-throughput technique for assessing K-ras mutations. Oligonucleotide microarrays may provide a valid option because they allow scientists to accurately and rapidly process large numbers of samples.