Mutation in the Gene Responsible for Cystic Fibrosis and Predisposition to Chronic Rhinosinusitis in the General Population

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Context  Chronic rhinosinusitis (CRS) is a common condition in the US general population, yet little is known about its underlying molecular cause. Chronic rhinosinusitis is a consistent feature of the autosomal recessive disorder cystic fibrosis (CF).

Objective  To determine whether mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which is responsible for CF, predispose to CRS.

Design  Case-control study conducted from 1996 to 1999 in which the DNA of CRS patients and controls was typed for 16 mutations that account for 85% of CF alleles in the general population. Chronic rhinosinusitis patients with 1 CF mutation were evaluated for a CF diagnosis by sweat chloride testing, nasal potential difference measurement, and DNA analysis for additional mutations.

Setting  Otolaryngology–head and neck clinic of a US teaching hospital.

Participants  One hundred forty-seven consecutive adult white patients who met stringent diagnostic criteria for CRS and 123 CRS-free white control volunteers of similar age range, geographic region, and socioeconomic status.

Main Outcome Measures  Presence of CF mutations by DNA analysis among CRS patients vs controls.

Results  Eleven CRS patients were found to have a CF mutation (ΔF508, n=9; G542X, n=1; and N1303K, n=1). Diagnostic testing excluded CF in 10 of these patients and led to CF diagnosis in 1. Excluding this patient from the analyses, the proportion of CRS patients who were found to have a CF mutation (7%) was significantly higher than in the control group (n=2 [2%]; P=.04, both having ΔF508 mutations). Furthermore, 9 of the 10 CF carriers had the polymorphism M470V, and M470V homozygotes were overrepresented in the remaining 136 CRS patients (P=.03).

Conclusion  These data indicate that mutations in the gene responsible for CF may be associated with the development of CRS in the general population.

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eral population, we screened CRS patients and disease-free controls enrolled by the Otolaryngology–Head and Neck Surgery Clinic of the Johns Hopkins Outpatient Center for common CF mutations.

METHODS

Patient and Control Subjects

Adult (age range, 22-75 years) white patients with nasal or sinus symptoms for more than 8 weeks2 at the time of presentation at the Johns Hopkins Otolaryngology–Head and Neck Surgery Clinic, or subjects with a history of at least 4 episodes (each >3 weeks in duration) of recurrent symptoms in the prior 12 months,2,4 were recruited. Each patient entered into the study had evidence of thickened mucosa in the nose and/or sinuses, either by computed tomographic scan or nasal endoscopy. A family history was taken from each patient with specific reference to sinusitis, pulmonary disease, and CF.

White individuals (age range, 23-72 years) drawn from the same geographic region (Maryland and surrounding states) who had less than 10 days of signs or symptoms of rhinosinusitis per year by history or examination were enrolled as controls by the same clinic. The 123 controls included 31 patients with other conditions (eg, head and neck cancer) recruited from the same clinic as was caring for the CRS patients, 35 nonpatient volunteers from the same clinic, and 57 persons who were hospital staff. Blood relatives were identified by last name and excluded. The questionnaire used for the CRS patients was used to determine that the controls did not have CRS. Patients and controls were of similar race, age range, geographic region, and socioeconomic status (most were classified as middle class). Participation rate of controls was 98.4%.

All protocols were approved by the institutional review boards of the Johns Hopkins School of Medicine and the Johns Hopkins Bayview Medical Center, and informed consent was obtained from all subjects. As per the consent form, test results were provided to subjects on request and counseling was given on request following testing.

Analysis of CFTR Genes

Genomic DNA samples extracted from the blood of participants were screened for 16 mutations (R117H, 621+1G→T, R334W, R347P, A455E, ΔI507, ΔF508, 1717-1 G→A, G542X, S549N, G551D, R553X, R560T, 3849+10 Kb C→T, W1282X, and N1303K) that account for 85% of CF alleles in the white populations using the multiplex reverse dot hybridization system (Roche Molecular Systems, Alameda, Calif).16,17 This test also identified the 5T, 7T, and 9T variants of the splice acceptor site in intron 8 and F508C, 1507V, and 1506V (exon 10) polymorphisms of the CFTR gene.

To identify mutations other than the 16 common CF mutations, CFTR gene exons and flanking introns were analyzed using denaturing gradient gel electrophoresis (DGGE) described by Mack et al18 with the following modifications of polymerase chain reaction primers, annealing temperatures, DGGE gel gradients, and running times: exon 8, 5′-primer (GC)n TAAAGTAGATGTATAAATGC, 3′-primer ATTTTATTTCGCAATAGATAT; annealing temperature, 50°C; gradient range, 0% to 50%; running time, 15 hours; exon 9, 5′-primer TGAAATACTGTGAGAAACTC, 3′-primer (GC)n CCTTCCAGCACTAAACTA; annealing temperature, 50°C; gradient, 0% to 50%; running time, 8 hours; and exon 23, 5′-primer (GC)n CTGTCTGTGATATTATGGT, 3′-primer GTTATCAAAGTTACACACTA; annealing temperature, 51°C; gradient, 20% to 70%; running time, 7.5 hours.

A total of 115 DNA samples containing different mutations16 distributed over the entire coding region of CFTR were used to assess the sensitivity of the DGGE method in this study. Patient samples with an abnormal migration pattern were sequenced (ABI model 377, Applied Biosystems Inc, Foster City, Calif) at the Johns Hopkins DNA Analysis Facility. For the M470V locus, each allele was determined by restriction enzyme analysis by HphI digestion.18

Sweat Test and Nasal Potential Difference Measurement

Sweat chloride concentration was determined by quantitative pilocarpine iontophoresis by the Johns Hopkins Cystic Fibrosis Clinic as described.19,20 Nasal potential difference (NPD) measurements were performed at the Johns Hopkins Pediatric Clinical Research Unit Outpatient Center according to a published protocol21 with a few modifications.22 All perfusion solutions were at room temperature. After establishing the location of the most negative potential difference (PD) baseline during perfusion with a Ringer’s solution at 0.5 mL/min, amiloride hydrochloride (10−4 mol/L) (spectrum AM 122-02) in Ringer’s (pH 7.4) was applied at 5 mL/min for 2 minutes or until a stable plateau was attained. The perfusion was changed to gluconate-substituted chloride-free Ringer’s solution with amiloride hydrochloride (10−4 mol/L) at 5 mL/min over the next 2 minutes or until a plateau was achieved to assess unstimulated chloride transport. This solution was then replaced with chloride-free Ringer’s solution containing isoproterenol hydrochloride (10−4 mol/L) and amiloride hydrochloride (10−4 mol/L) at 5 mL/min for 3 minutes to assess cyclic adenosine monophosphate–augmented chloride transport. For data analysis, PD values are reported as those obtained at the stable plateau after perfusion of each solution.

Statistical Analysis

Comparisons of CF mutation frequencies between patient and control groups were evaluated using the Fisher exact 2-tailed test. The M470V distributions in patient and control groups were analyzed using χ² analysis. The odds ratios (ORs) were calculated using the classic case-control 2×2 table. The 95% confidence intervals (CIs) were calculated using the exact method. Nasal potential difference values were compared using the unpaired Student t test. A P value of .05 or less was considered significant. Sta-
CFTR GENE AND CHRONIC RHINOSINUSITIS

Table 1. Cystic Fibrosis (CF) Mutations and Cystic Fibrosis Transmembrane Regulator (CFTR) Variants in Chronic Rhinosinusitis (CRS) Patients and Controls*

<table>
<thead>
<tr>
<th>CF mutations</th>
<th>CRS, No. (%)</th>
<th>Non CRS, No. (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>∆F508/+</td>
<td>(n = 147)</td>
<td>(n = 123)</td>
<td></td>
</tr>
<tr>
<td>∆F508/M470V</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>G542X/M470V, L967S</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>N1303K/+; M470V/M, R75Q/+†</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(ΔF508/2789+5G→A)‡</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Frequency of CF carriers</td>
<td>10 (7)</td>
<td>2 (2)</td>
<td>.04§</td>
</tr>
</tbody>
</table>

| CFTR variants | ST Variant in non-CF carriers|| 5T/+ |
|---------------|-------------------------------|-------|
| 5T/±          | (n = 136)                    | (n = 121) |
| Codon 470 genotypes among non-CF carriers||                  |
| M/M           | 21 (16)                      | 23 (19) | .25§ |
| M/V           | 55 (40)                      | 64 (53) |         |
| V/V           | 60 (44)                      | 34 (28) |         |

*Plus (+) indicates wildtype. †Whether the 3 CFTR alterations occur together on the same chromosome (phase) is not known. ‡Fisher exact test. ¶Indispensals having CF mutations were excluded from these analyses to avoid confounding. §χ² Test.

RESULTS

Screening CRS Patients for CF Mutations

Blood samples were collected from 147 white patients recruited in a sequential fashion into a prospective treatment study of CRS. Samples from a control group of 123 disease-free whites of similar age range and geographic region were collected concurrently. Eleven CRS patients were found to have CF mutations (TABLE 1); 9 had the common mutation, ∆F508, 1 had G542X, and 1 had N1303K. Two ∆F508 carriers were identified in the control group. The OR of CRS in CF allele carriers (n = 13) identified in the study was 4.9 (95% CI, 1.0-46) in comparison with noncarriers (n = 257). The 5T variant of the polypyrimidine tract in intron 8 reduces splicing efficiency of CFTR and has been associated with CBAVD.6,23 However, there was no statistically significant difference in the frequency of the 5T variant between patients and controls, and none of the CF mutation carriers had the 5T variant. Among 147 CRS patients and 123 controls, 1 patient reported a family history of CF (first cousin). This patient did not have an identified CF mutation.

To determine whether any patients had mutations other than the 16 common CF mutations, the DNA of patients having 1 CF mutation or the 5T variant was analyzed by DGGE and sequencing. Using this technique, we previously demonstrated that at least 96% of mutations in the exons and flanking intron regions of the CFTR gene can be detected.16 A 97% sensitivity was achieved using 115 samples with previously identified mutations16 in this study. Sequence analysis of samples with an abnormal DGGE pattern identified an R75Q mutation in the N1303K carrier and an L967S mutation in the G542X carrier. Neither R75Q or L967S is a CF-causing mutation (Cystic Fibrosis Mutation Data Base, http://www.genet.sickkids.on.ca/cftr/). A second CF-causing mutation (2789+5G→A) was found in patient 1624 (Table 1).

Nine of the 10 CRS patients (patient 1624 was excluded because of having been diagnosed as having CF) having a CF mutation also had valine at codon 470 rather than methionine (M470V) (Table 1). The M470V variant does not cause CF, but CFTR with valine functions differently from wild-type CFTR with methionine at codon 470.18,24 In 8 cases, pedigree analysis or DNA sequencing confirmed that the M470V variant was in the other CFTR gene (Table 1). Seven of the 8 patients had ∆F508 and M470V, which both occur on exon 10; thus, sequencing from each direction can identify whether they occur together. Patient 1386 had changes in exon 10 (M470V), exon 15 (L967S), and exon 11 (G542X), and pedigree analysis was used to show that the M470V variant and the L967S variant segregated independently of G542X. Neither of the ∆F508 carriers in the control group carried the M470V allele. To further explore the possible role of this variant in rhinosinusitis, we analyzed the entire remaining (those not having a CF mutation) patient group and control group (Table 1). The frequency of the M470V allele was higher in the CRS group (61%) than the control group (53%) and higher than the published frequency of this allele in the general population (50%).25 The distribution of M470V genotypes differed significantly between the 2 groups (P = .03) due to an excess of M470V homozygotes (Table 1). The OR of CRS in M470V homozygotes was 2.0 (95% CI, 1.2-3.3).

In Vivo Evaluation of CFTR Function in CF Mutation Carriers

Cystic fibrosis transmembrane regulator function in the sweat gland of patients having CF mutations was evaluated by determination of sweat chloride concentration and in nasal epithelia via measurement of NPD. One patient (No. 1600) decided not to continue the study and did not have sweat testing or NPD analysis. The patient having 2 CF mutations (No. 1624) had an elevated sweat chloride concentration (102 mmol/L) (normal level, <60 mmol/L).20 The N1303K carrier patient (No. 1344) had 2 borderline and 1 elevated sweat chloride concentration measurements (55, 58, and 78 mmol/L), while the re-
main patients had values in the normal range (Table 2).

The baseline PD in nasal epithelia reflects ongoing sodium reabsorption and is elevated 2-fold in CF. Patient 1624 had an elevated baseline value (−29 mV), which was in the range of that of previously studied CF patients22 (Table 3). The remaining CRS patients had values well below those in the CF range (Table 3). Inhibition of sodium reabsorption by amiloride hydrochloride lowers the PD, although the change is typically greater in CF patients than in persons not having CF.21 While the amiloride hydrochloride response of patient 1624 (change in PD [ΔPD] = 21.1 mV) was in the range of that of the CF patients, the remaining CRS patients had a mean ΔPD that was similar to that of persons not having CF (Table 3). The most discriminating feature of the NPD for CF is the ΔPD in response to perfusion with a chloride-free solution containing amiloride hydrochloride and isoproterenol hydrochloride.21,26 The mean (SD) ΔPD for persons not having CF was −15.8 (6) mV, whereas CF patients and CRS patient 1624 had no such response to this maneuver (ΔPD = 2.4 mV). The mean (SD) ΔPD for the remaining CRS patients with 1 CF mutation (−11[7.1] mV) was significantly different from the mean ΔPD of the CF patients (P <.001).

**COMMENT**

Chronic rhinosinusitis is a common disorder that is likely to have a number of different causes.2 The prominence of sinus disease in certain inherited conditions suggests that genetic factors may play a role in CRS.27 To pursue this concept, we screened patients with CRS for mutations in the CFTR gene. The CFTR gene is a reasonable candidate since sinusitis is a consistent feature of CF.28 We initiated our CF mutation analysis in white patients because they accounted for most CRS patients visiting our clinic and the frequencies of CF alleles have been extensively studied in whites.29 Screening for the common CF mutations identified 11 mutation carriers among 147 CRS patients. Since this number of carriers was higher than expected, we had to exclude the possibility that the excess was due to patients with mild CF presenting with sinus disease.

Newly updated guidelines for the diagnosis of CF require 1 clinical manifestation characteristic of CF and evi-

### Table 2. Cystic Fibrosis Transmembrane Regulator (CFTR) Genotypes and Clinical Features of Chronic Rhinosinusitis Patients Having a Cystic Fibrosis Mutation*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>CFTR Genotype</th>
<th>Age of Onset, y</th>
<th>Sinus Surgery, No. of Times</th>
<th>Polyposis</th>
<th>Pulmonary Status</th>
<th>Gastrointestinal Status</th>
<th>Sweat Chloride Concentration, mmol/L†</th>
<th>Other Pertinent Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1344</td>
<td>F</td>
<td>N1323K+/+; M470V/M; R75Q/+‡</td>
<td>&lt;10</td>
<td>3</td>
<td>–</td>
<td>Recurrent bronchitis</td>
<td>Lactose intolerant</td>
<td>55, 58, 78</td>
<td></td>
</tr>
<tr>
<td>1386</td>
<td>M</td>
<td>G542X/M470V, L967S</td>
<td>16</td>
<td>14</td>
<td>+</td>
<td>Asthma, Pseudomonas aeruginosa pneumonia§</td>
<td>Normal</td>
<td>&lt;15</td>
<td>Fathered 3 children, genetically confirmed</td>
</tr>
<tr>
<td>1379</td>
<td>F</td>
<td>ΔF508/M470V</td>
<td>&lt;10</td>
<td>8</td>
<td>–</td>
<td>Normal</td>
<td>Normal</td>
<td>31</td>
<td>Problem conceiving</td>
</tr>
<tr>
<td>1380</td>
<td>M</td>
<td>ΔF508/M470V</td>
<td>59</td>
<td>2</td>
<td>+</td>
<td>Asthma</td>
<td>Normal</td>
<td>39</td>
<td>Fathered 2 children</td>
</tr>
<tr>
<td>1468</td>
<td>F</td>
<td>ΔF508/M470V</td>
<td>20</td>
<td>2</td>
<td>–</td>
<td>Normal</td>
<td>Normal</td>
<td>29</td>
<td>Problem conceiving</td>
</tr>
<tr>
<td>1509</td>
<td>F</td>
<td>ΔF508/M470V</td>
<td>26</td>
<td>None</td>
<td>–</td>
<td>Asthma</td>
<td>Normal</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>1493</td>
<td>F</td>
<td>ΔF508/+</td>
<td>38</td>
<td>1</td>
<td>+</td>
<td>Asthma</td>
<td>Normal</td>
<td>21</td>
<td>Graves disease</td>
</tr>
<tr>
<td>1523</td>
<td>F</td>
<td>ΔF508/+</td>
<td>20</td>
<td>1</td>
<td>−</td>
<td>Normal</td>
<td>Normal</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>1534</td>
<td>F</td>
<td>ΔF508/M470V</td>
<td>38</td>
<td>None</td>
<td>−</td>
<td>Normal</td>
<td>Normal</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td>F</td>
<td>ΔF508/M470V</td>
<td>15</td>
<td>1</td>
<td>+</td>
<td>Normal</td>
<td>Normal</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>1624</td>
<td>F</td>
<td>ΔF508/2789+5G→A</td>
<td>45</td>
<td>2</td>
<td>+</td>
<td>Pulmonary disease since childhood</td>
<td>Pancreatitis, fat maldigestion</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>1633</td>
<td>M</td>
<td>ΔF508/M470V</td>
<td>24</td>
<td>2</td>
<td>0</td>
<td>Asthma</td>
<td>Normal</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

*Patient ages ranged from 43 to 67 years.
†Normal level of sweat chloride concentration is less than 60 mmol/L.20 ND indicates not determined.
‡Whether the 3 CFTR alterations occur together on the same chromosome (phase) is not known.
§Three years ago the patient had a ventilatory defect (forced expiratory volume in 1 second/forced vital capacity 82% predicted).
¶Identical twin of patient 1523, included here for comparison of genotype and phenotype, not part of the primary patient study group.

### Table 3. Potential Difference (PD) Measurements of Nasal Epithelia in Chronic Rhinosinusitis (CRS) Patients Having Cystic Fibrosis (CF) Mutations*

<table>
<thead>
<tr>
<th>Patients†</th>
<th>Baseline PD, mV</th>
<th>Amiloride ΔPD, mV</th>
<th>ΔPD of Chloride-Free Solution Containing Amiloride and Isoproterenol, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRS patients</td>
<td>−14.4 (3.9)</td>
<td>7.6 (4.1)</td>
<td>−11 (7.1)</td>
</tr>
<tr>
<td>Persons without CF‡</td>
<td>−18 (7.9)</td>
<td>8.3 (5.9)</td>
<td>−15.8 (6)</td>
</tr>
<tr>
<td>CF patients‡</td>
<td>−36.3 (5.2)</td>
<td>20.2 (5.4)</td>
<td>5.2 (2.0)</td>
</tr>
</tbody>
</table>

*Values are expressed as mean (SD). ΔPD indicates change in PD.
†Total number of patients tested = 9 (patient 1624 is not represented in these values because of being diagnosed as having CF, and patient 1600 declined sweat or nasal PD testing).
‡Data are derived from 10 volunteers not having CF and 10 CF patients previously studied at Johns Hopkins General Clinical Research Unit.25
dence of CFTR dysfunction in the sweat gland or nasal epithelia or molecular evidence at the DNA level. 30 All 11 patients had radiographic evidence of sinus disease, one of the phenotypic features consistent with CF. Only 1 patient with CRS (No. 1624) had consistent evidence of CFTR dysfunction in vivo (elevated sweat chloride concentration [102 mmol/L], and abnormal NPD); this patient had 2 CF-causing mutations (ΔF508 and 2789+5G→A) and was diagnosed as having CF at 46 years of age. After excluding the single CF patient, the CRS group had 10 CF mutation carriers, a significant excess compared with the control group (P = .04). Based on a CF carrier rate of 1 in 28 (0.036) in the general population and a test sensitivity of 85%, we expected 3 or 4 carriers in the control group. However, only 2 CF carriers were identified among 123 controls. Because the number of expected carriers was small, it is not clear whether CF carrier frequency in the disease-free controls differs from that of the general population. However, if the OR of CRS in CF carriers is about 5-fold higher than in the general population, it is possible that the sinus disease-free population could have a lower CF carrier frequency.

Most of the CF carriers with CRS had variants in their other CFTR gene. An analogous situation occurs in patients with CBAVD. A substantial fraction of patients with CBAVD have 2 mutations; one that is observed in CF patients and a second that is a variant not associated with CF. 31 The interpretation at the biochemical level is that the second mutation permits CFTR to function sufficiently to escape the CF phenotype, but some epithelial tissue dysfunction does occur. Indeed, in addition to CRS, patient 1344 had subtle sweat gland dysfunction and a history of bronchitis while patient 1386 had sputum infected with Pseudomonas aeruginosa, an organism common in CF patients but unusual in the general population. 7 Both patients were found to carry mutations that are not associated with CF (R75Q and L967S). Furthermore, the M470V variant was found in 9 of the 10 CRS patients with a CF mutation, and homozygotes for M470V were in significant excess in the remaining CRS patients. It is interesting to note that patients having a CF mutation that eliminates function and the M470V variant are similar at the cellular level to M470V homozygotes; only CFTR with the M470V variant is functional in their cells. 26 Since the chloride channel activity of CFTR with valine at codon 470 is reduced compared with CFTR with methionine at codon 470, it is tempting to speculate that CFTR dysfunction due to M470V predisposes certain individuals to CRS.

We screened for 16 CFTR mutations (of the >900 mutations reported on the Cystic Fibrosis Mutation Data Base [http://www.genet.sickkids.on.ca/cftr/]), accounting for 85% of CF alleles in whites. An underestimate would be 15% at most; thus, 1 or 2 CRS patients in our study may have rare CF alleles undetected by the standard panel. To identify them, the CFTR genes of all patients would have to be analyzed, a substantial amount of work infeasible in a clinical setting. If certain rare CF alleles occurred only in CRS patients, they should have been found in combination with common CF alleles in the 22 CRS patients (11 CF allele carriers and 11 ST carriers) for whom the entire CFTR gene was screened.

How might CFTR dysfunction contribute to development of CRS? The importance of CFTR to the normal function of the sinus epithelium is illustrated by CF. Severe reduction in CFTR function observed in CF patients leads to chronic sinus disease that usually begins early in childhood. Therefore, it seems reasonable that less severe reductions in CFTR function may be associated with CRS in the absence of CF. The inability of NPD measurements to identify reduced CFTR function in the CRS patients with CF mutations is not surprising. This test has been developed and refined to distinguish CF from other disorders and does not discriminate a CF carrier from a person not having CF mutations. 32 It is possible that consequences of altered CFTR function may be manifested only under stress, such as an acute upper respiratory infection. Altered viscosity and abnormal electrolyte composition of sinus secretions due to CFTR dysfunction may increase the chance that rhinosinusitis will progress to a chronic phase. The presence of other genetic factors and/or specific environmental exposures may also increase the likelihood that persons with reduced CFTR function eventually develop CRS. While only a minor fraction of CRS patients in this study had CF mutations, our observation provides fresh molecular insight into a common chronic disorder and suggests new avenues of investigation.

REFERENCES

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As the day’s problems accumulate, I have three piles of work in front of me: first you tackle the one about which you can make immediate decisions, get it done and over with; then after appraising the second pile, containing insufficient data, arrange for the collection of the required missing information; finally there is the third pile of imponderables which should be filed or thrown into the basket; above all, don’t waste any time on them.
—Walter W. Palmer (1882-1950)