Fletcher Factor Deficiency: Family Study and Detection

By Charles F. Abildgaard and Janet Harrison

Eight of 11 children of a known Fletcher factor-deficient individual were found to have normal activated partial thromboplastin times, normal levels of factors VIII, IX, XI, and XII, and a mean Fletcher factor level of 53% (range 40%-72%), suggesting a heterozygous state for the gene-controlling Fletcher factor production. All partial thromboplastin time reagents containing celite or kaolin were sensitive to Fletcher factor deficiency, while one reagent containing ellagic acid did not detect this abnormality. The finding of an abnormal partial thromboplastin time that is corrected by a 10-min incubation period is presumptive evidence for Fletcher factor deficiency.

Since the initial description by Hathaway et al. of a previously unrecognized coagulation factor in four siblings, only three additional examples of "Fletcher factor" deficiency have been reported, and two other cases have been recognized. Study of other members of the original Fletcher family revealed normal coagulation screening tests in both parents and six additional siblings. Fletcher factor levels were not measured. Except for a normal Fletcher factor level in the plasma of the mother of one additional patient, no other family studies have been reported. Recent study of eight of 11 children of one of the previously described Fletcher factor-deficient patients (see reference 2, case 3) revealed entirely normal screening and quantitative assays, except for Fletcher factor levels which ranged from 40% to 72% (mean 53%). The details of these studies are reported below.

MATERIAL AND METHODS

Blood samples were obtained from the previously identified Fletcher factor-deficient patient, a 50-yr-old black male, and from eight of his 11 children, ages 14-29 yr. Blood was collected in plastic syringes and added to polycarbonate centrifuge tubes containing citrate-citric acid anticoagulant (1 ml anticoagulant per 9 ml blood). The samples were centrifuged for 15 min at 12,000 rpm at 4°C and plasma was removed using plastic droppers. The plasma was aliquoted into plastic tubes and was either tested immediately or quick-frozen in alcohol and dry ice and stored at -40°C until tested. Coagulation screening tests and coagulation factor assays were done by methods previously reported from this laboratory. The Fletcher factor assays were done by a one-stage partial thromboplastin time method with celite-whetherin, using the father’s deficient plasma as substrate. The procedure was modified by using a 2-min incubation period and testing serial dilutions of plasma from 1/80 to 1/640. Attempts to perform the assay without incubation resulted in prolonged clotting times at all dilutions; using incubation times greater

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Table 1. Partial Thromboplastin Reagents Used in Study

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Source</th>
<th>Activator</th>
<th>Partial Thromboplastin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celite-inosithin</td>
<td>Authors' laboratory</td>
<td>Celite</td>
<td>Inosithin</td>
</tr>
<tr>
<td>Platein plus activator</td>
<td>Warner Chilcott</td>
<td>Celite</td>
<td>Cephalin</td>
</tr>
<tr>
<td>Fibrolet</td>
<td>Bioquest</td>
<td>Celite</td>
<td>Cephalin</td>
</tr>
<tr>
<td>Platein-kaolin</td>
<td>Warner Chilcott</td>
<td>Kaolin</td>
<td>Cephalin</td>
</tr>
<tr>
<td>Partial thromboplastin</td>
<td>Hyland</td>
<td>Kaolin</td>
<td>Cephalin</td>
</tr>
<tr>
<td>Activated cephaloplastin</td>
<td>Dade</td>
<td>Ellagic acid</td>
<td>Cephalin</td>
</tr>
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</table>

than 2 min, the clotting times were uniformly shortened, resulting in an inadequate slope or an actual plateau. Comparative partial thromboplastin time (PTT) studies were done with the reagents listed in Table 1.

RESULTS

The relative sensitivity of various partial thromboplastin time reagents to the defect in Fletcher-deficient plasma was determined at different incubation times with each PTT reagent, and the results are presented in Table 2. All of the tests done with reagents utilizing cellite or kaolin for activation resulted in prolonged clotting times at the incubation time recommended for the particular reagent.

Table 2. Results of Partial Thromboplastin Screening Tests on Fletcher Factor-Deficient Plasma

<table>
<thead>
<tr>
<th>Incubation Time (min)</th>
<th>Celite Inosithin</th>
<th>Platein Plus Activator</th>
<th>Fibrolet</th>
<th>Platein-Kaolin</th>
<th>Hyland Partial Thromboplastin</th>
<th>Activated Cephaloplastin</th>
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<tr>
<td>2</td>
<td>101</td>
<td>134</td>
<td>111*</td>
<td>142</td>
<td>134</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>105</td>
<td>118</td>
<td>66</td>
<td>97*</td>
<td>127</td>
<td>42</td>
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<tr>
<td>4</td>
<td>109*</td>
<td>96</td>
<td>64</td>
<td>128</td>
<td>120</td>
<td>31*</td>
</tr>
<tr>
<td>5</td>
<td>89</td>
<td>78*</td>
<td>42</td>
<td>92</td>
<td>93*</td>
<td>28</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>35</td>
<td>27</td>
<td>49</td>
<td>36</td>
<td>25</td>
</tr>
</tbody>
</table>

* Result at recommended incubation time for each reagent when used with fibrometer.
† The normal range is reported for the incubation time recommended for each reagent.
All tests were done using the fibrometer and are reported as the mean (sec) of two determinations.

Table 3. Coagulation Factor Assay Results on Children of Fletcher Factor-deficient Patient

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Factor VIII</th>
<th>Factor IX</th>
<th>Factor XI</th>
<th>Factor XII</th>
<th>Fletcher Factor</th>
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<tr>
<td>14</td>
<td>M</td>
<td>198</td>
<td>85</td>
<td>61</td>
<td>84</td>
<td>60</td>
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<tr>
<td>14</td>
<td>F</td>
<td>159</td>
<td>58</td>
<td>102</td>
<td>79</td>
<td>47</td>
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<tr>
<td>15</td>
<td>M</td>
<td>133</td>
<td>58</td>
<td>58</td>
<td>62</td>
<td>72</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>113</td>
<td>76</td>
<td>66</td>
<td>41</td>
<td>50</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>129</td>
<td>88</td>
<td>70</td>
<td>61</td>
<td>48</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>156</td>
<td>146</td>
<td>128</td>
<td>49</td>
<td>48</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>131</td>
<td>90</td>
<td>106</td>
<td>69</td>
<td>61</td>
</tr>
<tr>
<td>29</td>
<td>F</td>
<td>172</td>
<td>100</td>
<td>112</td>
<td>112</td>
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<tr>
<td>Mean</td>
<td></td>
<td>149</td>
<td>88</td>
<td>88</td>
<td>70</td>
<td>53</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>113-198</td>
<td>58-146</td>
<td>61-128</td>
<td>41-112</td>
<td>40-72</td>
</tr>
</tbody>
</table>

* Fifty normal adults studied in authors' laboratory.
In contrast, the reagent which utilizes the effect of ellagic acid for activation produced a normal clotting time at the recommended incubation time (4 min), and only moderate prolongation of the clotting time was observed at shorter incubation times. Prolongation of the incubation time to 10 min resulted in normal (or near normal) clotting times with all reagents tested. Partial thromboplastin times were performed with all of the reagents and incubation times used in the above study on plasma from each of eight children of the Fletcher factor-deficient donor (three additional children were unavailable for study). All of the results were normal. Assays for factor VIII, factor IX, factor XI, factor XII, and Fletcher factor were done on plasma of the same individuals, and the results are presented in Table 3. The mean value for Fletcher factor was 53° (range 40° - 72°), while the mean values for the other coagulation factors were all within the usual expected normal range.

DISCUSSION

Eight years have elapsed since the original description of Fletcher factor deficiency, yet only five additional cases have been recognized. The fact that Hattersley detected three unrelated individuals during a 2-yr period, as a result of performing the activated coagulation times as a routine preoperative screening procedure, suggests that the defect is not rare. It is of interest that two of Hattersley’s patients are black, the father of Hathaway’s original patients is “part black” and the two unreported individuals are black.

Although the PTT has been used with increasing frequency as a routine preoperative screening procedure during recent years, individual methods vary as to reagents and incubation times used. As illustrated in Table 2, at least one commercial PTT reagent (Activated Cephaloplastin-Dade) is insensitive to Fletcher factor deficiency. This reagent contains ellagic acid which activates factor XII directly and probably accounts for its insensitivity to deficiency of Fletcher factor. Hathaway has previously demonstrated that ellagic acid shortens the prolonged recalcification of Fletcher-deficient plasma. The correction of the prolonged PTT in Fletcher-deficient plasma by increased incubation time also may account for the defect being missed in some laboratories, since there is great variation in PTT methodology.

This unique property of Fletcher-deficient plasma provides a simple presumptive screening test for the deficiency, since prolonging the incubation time of the PTT does not result in correction of the abnormal clotting time in deficiencies of factors VIII, IX, XI, or XII. The recent observations that Fletcher-deficient plasma lacks prekallikrein have stimulated great interest in further study of such plasma. Screening programs to detect such individuals must be done with PTT reagents and incubation times that are sensitive to the defect. For preoperative screening programs, it is not essential to detect Fletcher factor-deficient individuals, since they do not manifest any bleeding tendencies.

The results of Fletcher factor assays in the eight offspring of one deficient individual suggest that they are heterozygous for the gene controlling Fletcher factor production. It is of interest that all of these individuals had entirely normal partial thromboplastin times, which suggests that the heterozygous
state for Fletcher deficiency does not account for the phenomenon of “slow
activation” (individuals with slightly prolonged partial thromboplastin times
which correct to normal with 10-min incubation). Additional family studies
will be required to document the hereditary pattern of Fletcher deficiency.

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