DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study

Glenn E. Palomaki, PhD1,2, Edward M. Kloza, MS3, Geralyn M. Lambert-Messerlian, PhD4, James E. Haddow, MD1, Louis M. Neveux, BA1, Mathias Ehrich, MD2, Dirk van den Boom, PhD2, Allan T. Bombard, MD, MBA2,3,4, Cosmin Deciu, MSc3, Wayne W. Grody, MD, PhD5, Stanley F. Nelson, MD6, and Jacob A. Canick, PhD1

Purpose: Prenatal screening for Down syndrome has improved, but the number of resulting invasive diagnostic procedures remains problematic. Measurement of circulating cell-free DNA in maternal plasma might offer improvement. Methods: A blinded, nested case-control study was designed within a cohort of 4664 pregnancies at high risk for Down syndrome. Fetal karyotyping was compared with an internally validated, laboratory-developed test based on next-generation sequencing in 212 Down syndrome and 1484 matched euploid pregnancies. None had been previously tested. Primary testing occurred at a CLIA-certified commercial laboratory, with cross validation by a CLIA-certified university laboratory. Results: Down syndrome detection rate was 98.6% (209/212), and the false-positive rate was 0.20% (3/1471), and the false rate failed in 13 pregnancies (0.98%); all were euploid. Before unblinding, the primary testing laboratory also reported multiple alternative interpretations. Adjusting chromosome 21 counts for guanine cytosine base content had the largest impact on improving performance. Conclusion: When applied to high-risk pregnancies, measuring maternal plasma DNA detects nearly all cases of Down syndrome at a very low false-positive rate. This method can substantially reduce the need for invasive diagnostic procedures and attendant procedure-related fetal losses. Although implementation issues need to be addressed, the evidence supports introducing this testing on a clinical basis. DOI: 10.1097/GIM.0b013e3182368a0e

Copyright © American College of Medical Genetics. Unauthorized reproduction of this article is prohibited.
fetal outcome). A strong negative association of fetal fraction with maternal weight was observed in case and control women (eFig. B8, Appendix B, Supplemental Digital Content 1, http://links.lww.com/GIM/A213), with weights of 100, 150, and 250 pounds associated with predicted fetal fractions of 17.8%, 13.2%, and 7.3%, respectively. No association was found for gestational age, maternal race, or indication for testing. Other associations were small and usually nonsignificant.

Massively parallel shotgun sequencing testing for Down syndrome

Testing was performed over 9 weeks (January to March, 2011) by 30 scientists, molecular technicians/technologists with training on the assay protocols, and related instrumentation. Historical reference ranges were to be used for interpretation, with real-time review of new data a requirement. Review of the first few flow cells by the Laboratory Director (before sign out) revealed that adjustments to the reference data were necessary (Expanded Methods, Appendix A and eFigs. B17–B19, Appendix B, Supplemental Digital Content 1, http://links.lww.com/GIM/A213). After data from six flow cells were generated, results were assessed by the Oversight Committee according to the interim criteria, and the conditional decision was made to allow the testing to continue. At the conclusion of testing, but before unblinding, SCMM requested a second aliquot for 85 of the 90 test failures among the 1696 enrollees (5.3%; 95% CI, 4.3–6.5) (eFig. B36, Appendix B, Supplemental Digital Content 1, http://links.lww.com/GIM/A213). The second result was used for final interpretation. Genetics in Medicine • Volume XX, Number XX, XX2011

<table>
<thead>
<tr>
<th>Enrollment site</th>
<th>Location</th>
<th>Clinical investigator</th>
<th>Singleton pregnancy</th>
<th>Normal karyotype</th>
<th>Other</th>
<th>Patients enrolled</th>
</tr>
</thead>
<tbody>
<tr>
<td>North York General Hospital</td>
<td>Toronto, Canada</td>
<td>Wendy S. Meschino, MD</td>
<td>41</td>
<td>651</td>
<td>86</td>
<td>778</td>
</tr>
<tr>
<td>Istituto G. Gaslini</td>
<td>Genoa, Italy</td>
<td>Pierangela De Biasio, MD</td>
<td>27</td>
<td>492</td>
<td>35</td>
<td>554</td>
</tr>
<tr>
<td>Hospital Clinic Barcelona</td>
<td>Barcelona, Spain</td>
<td>Antoni Borrell, MD, PhD</td>
<td>24</td>
<td>291</td>
<td>44</td>
<td>359</td>
</tr>
<tr>
<td>Centrum Lekarske Genetiky</td>
<td>Ceske Budejovice, Czech Republic</td>
<td>David Cutka, MD</td>
<td>14</td>
<td>362</td>
<td>19</td>
<td>395</td>
</tr>
<tr>
<td>Hospital Italiano</td>
<td>Buenos Aires, Argentina</td>
<td>Lucas Otaño, MD, PhD</td>
<td>13</td>
<td>68</td>
<td>14</td>
<td>95</td>
</tr>
<tr>
<td>Dalhousie University</td>
<td>Halifax, Canada</td>
<td>Michiel Van den Hof, MD</td>
<td>12</td>
<td>115</td>
<td>18</td>
<td>145</td>
</tr>
<tr>
<td>Rotunda University</td>
<td>Dublin, Ireland</td>
<td>Fergal Malone, MD</td>
<td>12</td>
<td>70</td>
<td>12</td>
<td>94</td>
</tr>
<tr>
<td>Semmelweis University</td>
<td>Budapest, Hungary</td>
<td>Csaba Papp, MD, PhD</td>
<td>10</td>
<td>64</td>
<td>9</td>
<td>83</td>
</tr>
<tr>
<td>IMALAB s.r.o. Medical Laboratories</td>
<td>Zlin, Czech Republic</td>
<td>Jaroslav Louchy, RNDr</td>
<td>9</td>
<td>238</td>
<td>8</td>
<td>255</td>
</tr>
<tr>
<td>CEMIC</td>
<td>Buenos Aires, Argentina</td>
<td>Maria Laura Igarzabal, MD</td>
<td>8</td>
<td>224</td>
<td>49</td>
<td>281</td>
</tr>
<tr>
<td>University of Iowa</td>
<td>Iowa City, IA</td>
<td>Kristi Borowski, MD</td>
<td>8</td>
<td>135</td>
<td>30</td>
<td>173</td>
</tr>
<tr>
<td>Women &amp; Infants Hospital</td>
<td>Providence, RI</td>
<td>Barbara O’Brien, MD</td>
<td>6</td>
<td>99</td>
<td>21</td>
<td>126</td>
</tr>
<tr>
<td>University of Pécs</td>
<td>Pécs, Hungary</td>
<td>Béla Veszprémi, MD, PhD</td>
<td>4</td>
<td>172</td>
<td>31</td>
<td>207</td>
</tr>
<tr>
<td>University of Alabama at Birmingham</td>
<td>Birmingham, AL</td>
<td>Joseph Biggio, MD</td>
<td>4</td>
<td>169</td>
<td>20</td>
<td>193</td>
</tr>
<tr>
<td>Rambam Medical Center</td>
<td>Haifa, Israel</td>
<td>Zeev Weiner, MD</td>
<td>4</td>
<td>133</td>
<td>10</td>
<td>147</td>
</tr>
<tr>
<td>Cedars Sinai PDC</td>
<td>Los Angeles, CA</td>
<td>John Williams, MD</td>
<td>3</td>
<td>192</td>
<td>28</td>
<td>223</td>
</tr>
<tr>
<td>Northwestern University</td>
<td>Chicago, IL</td>
<td>Jeffrey Dungan, MD</td>
<td>3</td>
<td>88</td>
<td>11</td>
<td>102</td>
</tr>
<tr>
<td>Henry Ford Hospital</td>
<td>Detroit, MI</td>
<td>Jacquelyn Roberson, MD</td>
<td>3</td>
<td>74</td>
<td>14</td>
<td>91</td>
</tr>
<tr>
<td>University of Virginia</td>
<td>Charlottesville, VA</td>
<td>Devereux N. Saller, Jr, MD</td>
<td>3</td>
<td>21</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>University of British Columbia</td>
<td>Vancouver, Canada</td>
<td>Sylvie Langlois, MD</td>
<td>2</td>
<td>67</td>
<td>14</td>
<td>83</td>
</tr>
<tr>
<td>Intermountain Healthcare</td>
<td>Salt Lake City, UT</td>
<td>Nancy Rose, MD</td>
<td>2</td>
<td>67</td>
<td>9</td>
<td>78</td>
</tr>
<tr>
<td>Brigham and Women’s Hospital</td>
<td>Boston, MA</td>
<td>Louise Wilkins-Haug, MD</td>
<td>2</td>
<td>21</td>
<td>8</td>
<td>31</td>
</tr>
<tr>
<td>Baylor College of Medicine</td>
<td>Houston, TX</td>
<td>Anthony Johnson, DO</td>
<td>2</td>
<td>20</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Yale University</td>
<td>New Haven, CT</td>
<td>Maurice J. Mahoney, MD, JD</td>
<td>1</td>
<td>31</td>
<td>9</td>
<td>41</td>
</tr>
<tr>
<td>New Beginnings Perinatal Consultants</td>
<td>Providence, RI</td>
<td>Marshall Carpenter, MD</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>University of Calgary</td>
<td>Calgary, Canada</td>
<td>Jo-Ann Johnson, MD</td>
<td>0</td>
<td>52</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td>Royal North Shore Hospital</td>
<td>Sydney, Australia</td>
<td>Vitomir Tasevski, PhD</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td></td>
<td>218</td>
<td>3,930</td>
<td>516</td>
<td>4,664</td>
</tr>
</tbody>
</table>

Table 1 Clinical sites enrolled in the study, along with related enrollment and outcome information