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

Glenn E. Palomaki, PhD[1], Edward M. Kloza, MS[1], Geralyn M. Lambert-Messerlian, PhD[1], James E. Haddow, MD[1], Louis M. Neveux, BA[1], Mathias Ehrich, MD[2], Dirk van den Boom, PhD[2], Allan T. Bombard, MD, MBA[2,3,4], Cosmin Deciu, MSc[3], Wayne W. Grody, MD, PhD[5], Stanley F. Nelson, MD[6], and Jacob A. Canick, PhD[1]

Purpose: Prenatal screening for Down syndrome has improved, but the number of resulting invasive diagnostic procedures remains problem- atic. 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 sequenc- ing 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), the false-positive rate was 0.20% (3/1471), and the testing failed in 13 pregnancies (0.8%); all were euploid. Before unblinding, the primary testing laboratory also reported multiple alter- native 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 mater- nal 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 evi- dence supports introducing this testing on a clinical basis. ***Genet Med* 2011:XX(XX):000–000.**

Key Words: *Down syndrome, prenatal screening, massively parallel shotgun sequencing, fetal DNA, clinical validation, detection rate, false-positive rate*

From the [1]Division of Medical Screening and Special Testing, Department of Pathology and Laboratory Medicine, Women & Infants Hospital, Alpert Medical School of Brown University, Providence, Rhode Island; [2]Sequenom Inc., and [3]Sequenom Center for Molecular Medicine, San Diego, California; [4]Department of Reproductive Medicine, University of California at San Diego, San Diego, California; and [5]Departments of Pathology and Labora- tory Medicine, Pediatrics, and Human Genetics and [6]Departments of Human Genetics, Pathology and Laboratory Medicine, and Psychiatry and Biobe- havioral Sciences, David Geffen School of Medicine, University of Califor- nia, Los Angeles, California.

Glenn E. Palomaki, PhD, Division of Medical Screening and Special Test- ing, Department of Pathology and Laboratory Medicine, Women & Infants Hospital, 70 Elm Street, 2nd Floor, Providence, Rhode Island 02903. E-mail: gpalomaki@ipmms.org.

Disclosure: Palomaki and Canick (Co-Principal Investigators) were mem- bers of the Sequenom Clinical Advisory Board for 6 months and resigned when the study was funded. Van den Boom, Ehrich, Bombard, and Deciu are employees and shareholders of Sequenom, Inc.

Role of the Sponsor: Sequenom Center for Molecular Medicine (SCMM) was responsible for developing an internally validated laboratory developed test (LDT) for detecting Down syndrome in maternal plasma using MPSS and for providing clinical interpretation of the test results. SCMM also identified, equipped, and trained an independent laboratory to test a subset of samples through a separate contract with UCLA. The sponsor did not control study design, identify, or communicate with Enrollment Sites, thaw or test samples prior to the formal testing period, have access to patient information prior to all testing being completed, analyze study results, prepare drafts of the manuscript, or have final decisions on manuscript content.

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Currently, the most effective prenatal screening tests for Down syndrome combine maternal age with information from sonographic measurement of nuchal translucency in the first trimester and measurements of several maternal serum screening markers obtained in the first and second trimesters.[1,2] This approach detects up to 90% of all cases at a false-positive rate of 2%. Given the prevalence of Down syndrome, 1 of every 16 screen positive women offered invasive diagnostic testing (amniocentesis or chorionic villus sampling) will have an af- fected pregnancy and 15 will not. As many as 1 in 200 such invasive procedures are associated with fetal loss, a major adverse consequence of prenatal diagnosis.[3,4] This has led to adjusting screening cutoffs to minimize the false-positive rate.
In practice, false-positive rates of 5% are common.

The 1997 discovery that 3–6% of cell-free DNA in maternal blood was of fetal origin prompted studies to determine whether Down syndrome could be detected noninvasively.[5] In 2008, two groups identified fetal Down syndrome, using massively paral- lel shotgun sequencing (MPSS).[6,7] This technique sequences the first 36 bases of millions of DNA fragments to determine their specific chromosomal origin. If the fetus has a third chromo- some 21, the percentage of chromosome 21 fragments is slightly higher than expected. Subsequent reports have extended these observations and suggest that a detection rate of at least 98% can be achieved at a false-positive rate of 2% or lower.[8–10]
Although promising, these studies were relatively small (range 13–86 Down syndrome cases and 34–410 euploid control samples), DNA sequencing was not performed in CLIA-certi- fied laboratories, and throughput and turnaround times did not simulate clinical practice. The current independent, collabora- tive study addresses these and other shortcomings.

MATERIALS AND METHODS

See "Expanded Methods," Appendix A, Supplemental Digital Content 1, http://links.lww.com/GIM/A213, for complete details.

Overall study design

Our study (clinicaltrials.gov NCT00877292) involved pa- tients enrolled at 27 prenatal diagnostic centers worldwide (Enrollment Sites). Women at high risk for Down syndrome based on maternal age, family history or a positive serum and/or sonographic screening test provided consent, plasma samples, and demographic and pregnancy-related information. Institu-

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

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

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,[9] 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 confidential 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.

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