

samples. We included a daily in-house quality control with DBS prepared with CAL2 in EDTA-blood from a patient without detectable AAT (nominal values, 0.10–3.00 g/L). Eluted DBS samples for the nephelometric assay were not diluted (AAT reading range, 0.003–0.028 g/L) or were diluted 1 to 6 (AAT reading range, 0.028–0.167 g/L) to be in the working range of the assay.

We evaluated the effect of different matrices on the recovery of AAT, defined as the percentage of AAT in the DBS compared with the corresponding serum or plasma concentration (see Fig. 1 in the Data Supplement that accompanies the online version of this letter at <http://www.clinchem.org/content/vol52/issue5>). The recovery of AAT in the eluate from DBS at a 1:21.8 dilution varied according to the complexity of the matrix, which acts as a molecular sieve, and the concentration of the protein itself. Thus, we used 2 regression curves to evaluate all serum/plasma samples vs the corresponding DBS (Fig. 1). The choice of curve was based on the nephelometric reading of the eluate. If the DBS reading, increased 21.8-fold, was <0.22 g/L, curve A (range, 0.16–0.53 g/L) was used, whereas if the DBS reading was  $\geq$ 0.22 g/L, curve B (range, 0.54–2.93 g/L) was used. The correlation coefficients were 0.9889 and 0.9098 for samples with low and medium-to-high concentrations of AAT, respectively.

We evaluated the precision, recovery, and sensitivity of the AAT assay for the concentration range read by our instrument (0.03–0.17 g/L) by adding calibrator to the DBS, prepared with CAL2 (successive additions of 0.03 g/L) in EDTA-blood from a patient without detectable AAT. The results are presented in Table 1 of the online Data Supplement. Intra- and interday imprecision (CV) was 0.6%–3.2% and 0.3%–3%, respectively (see Table 1 in the online Data Supplement).

We studied 114 samples submitted to our center for a laboratory diagnosis of AATD. These samples had been genotyped and sequenced

(5, 6). Fifty-one samples came from PI\*MM individuals (healthy), and 63 came from patients with AATD. The AATD was either severe or intermediate: 8 were PI\*MR (where R indicates rare deficient variants) (6), 8 were PI\*MS, 25 were PI\*MZ, 4 were PI\*SZ, 14 were PI\*ZZ, and 4 were PI\*ZR. In this group, albeit small, an AAT concentration of 1.13 g/L represented the best cutoff to differentiate AATD patients from healthy individuals (sensitivity, 0.92; specificity, 0.90).

In conclusion, this method allowed AAT to be assayed from a 6-mm disk and provided the same quantitative results on both conventional and DBS samples.

We gratefully acknowledge the skillful technical assistance of Carla Viliani and Laura Pirolini. This research was supported by a Ricerca Corrente from the Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) San Matteo, Pavia; a grant from Istituto Superiore di Sanità Progetto "Malattie Rare"; and an educational grant from Bayer.

#### References

- Rodriguez F, Jordi R, Costa X, Cotrina M, Galimany R, Vidal R, et al. Rapid screening for  $\alpha_1$ -antitrypsin deficiency in patients with chronic obstructive pulmonary disease using dried blood specimens. *Am J Respir Crit Care Med* 2002; 166:814–7.
- Wencker M, Marx A, Konietzko N, Schaefer B, Campbell EJ. Screening for  $\alpha_1$ -Pi deficiency in patients with lung disease. *Eur Respir J* 2002; 20:319–24.
- Spence WC, Morris JE, Pass K, Murphy PD. Molecular confirmation of  $\alpha_1$ -antitrypsin genotypes in newborn dried blood specimens. *Biochem Med Metab Biol* 1993;50:233–40.
- Costa X, Jordi R, Rodriguez F, Miravittles M, Cotrina M, Gonzalez C, et al. Simple method for  $\alpha_1$ -antitrypsin deficiency screening by use of dried blood spot specimens. *Eur Respir J* 2000; 15:1111–5.
- Ferrarotti I, Zorzetto M, Scabini R, Mazzola P, Campo I, Luisetti M. A novel method for rapid genotypic identification of  $\alpha_1$ -antitrypsin variants. *Diagn Mol Pathol* 2004;13:160–3.
- Ferrarotti I, Baccheschi J, Zorzetto M, Tinelli C, Corda L, Balbi B, et al. Prevalence and phenotype of subjects carrying rare variants in the Italian Registry for  $\alpha_1$ -antitrypsin deficiency. *J Med Genet* 2005;42:282–7.

Marina Gorrini<sup>1</sup>  
 Ilaria Ferrarotti<sup>1</sup>  
 Anna Lupi<sup>2</sup>  
 Tiziana Bosoni<sup>3</sup>  
 Paola Mazzola<sup>1</sup>  
 Roberta Scabini<sup>1</sup>  
 Ilaria Campo<sup>1</sup>  
 Michele Zorzetto<sup>1</sup>  
 Francesco Novazi<sup>3</sup>  
 Maurizio Luisetti<sup>1\*</sup>

<sup>1</sup> Centro Diagnosi Deficit Ereditario AAT  
 Laboratorio di Biochimica e Genetica  
 Clinica Malattie Apparato Respiratorio  
 and

<sup>3</sup> Servizio Analisi Chimico Cliniche  
 Istituto di Ricovero e Cura a Carattere Scientifico  
 Policlinico San Matteo  
 Pavia, Italy

<sup>2</sup> Dipartimento di Biochimica A. Castellani  
 Università di Pavia  
 Pavia, Italy

\* Address correspondence to this author at: Clinica Malattie Apparato Respiratorio, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Policlinico San Matteo, Piazza Golgi, 27100 Pavia, Italy. Fax 39-0382-502269; e-mail [m.luisetti@smatteo.pv.it](mailto:m.luisetti@smatteo.pv.it).

DOI: 10.1373/clinchem.2005.062059

#### Prenatal Diagnosis of the WAS R86H Sequence Variation in Heterozygous Twins

To the Editor:

A healthy 28-year-old woman was heterozygous for the Wiskott–Aldrich syndrome gene (WAS) sequence variation, and the syndrome-causing variant was inherited by her first-born son (Fig. 1). The 2.5-year-old hemizygous boy developed characteristic features of the WAS, including eczema, thrombocytopenia, and recurrent infections of the lower respiratory tract from early infancy. The mother presented recently with a new pregnancy, and the abdominal

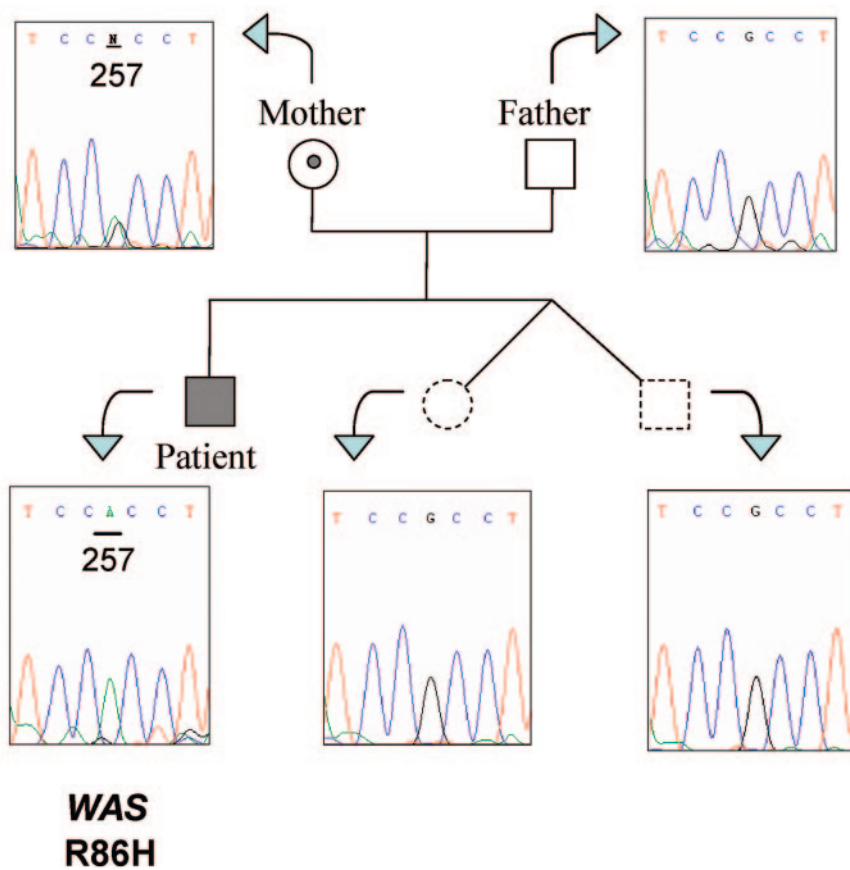


Fig. 1. Pedigree and sequences of the WAS gene in the affected family.

The WAS sequence variant in the hemizygous patient was inherited from the healthy mother, who was heterozygous for the R86H variation. The *dashed symbols* represent the fetuses in the twin pregnancy.

ultrasound scan obtained at 7 weeks of gestation revealed that she had conceived twins. We counseled the mother on the complexity and risks of invasive prenatal diagnostic procedures and made it clear that the potential risk of disease in a male fetus is 50%; nevertheless, she decided to continue her pregnancy and to undergo invasive prenatal evaluation. At 11 weeks of gestation, chorionic villus sampling by a transabdominal approach was performed with no complications. Rapid karyotyping revealed a male and a female fetus. We then isolated genomic DNA from the chorionic villus samples and performed bidirectional DNA sequencing of the WAS gene. Neither of the fetuses carried the R86H WAS sequence variant found in the mother and her son (Fig. 1).

The WAS is a rare X-linked immu-

nodeficiency disorder characterized by eczema, thrombocytopenia with small platelets, recurrent pyogenic infections, and high incidences of autoimmune diseases and malignancies (1). The genetic basis of the disease is a sequence variant in the WAS gene encoding a 502-amino acid protein that is expressed in the cytosol of hematopoietic cells (2, 3). Sequence variations occur throughout the WAS gene and include missense, nonsense, and splice-site variants; insertions; and deletions (4). In a recent study, 141 unique sequence variations were identified in 265 patients affected with WAS or X-linked thrombocytopenia, including 4 R86H missense variants (4). The predominant WAS sequence variations were missense variants, which were typically located in exons 1 to 4.

Genomic medicine provided a

powerful means to detect WAS sequence variations in this early pregnancy and to assist the mother in her decision-making on the fate of her fetuses. This report is the first to describe a case of DNA analysis in a twin pregnancy at risk for WAS. As in singleton pregnancies, chorionic villus sampling is also used as an invasive tool for early sex determination and DNA assays in multiple pregnancies (5). We decided to use chorionic villus sampling because it has considerable advantages over amniocentesis by offering rapid karyotyping and DNA studies at an early stage of pregnancy. In multifetal pregnancies, early diagnosis by chorionic villus sampling also facilitates selective termination of an affected fetus. Remarkably, fetal termination performed earlier in pregnancy may be associated with a higher survival rate of the unaffected twin (6). It is important to emphasize that only experienced centers should perform these procedures because of the technical challenges and the expertise needed.

#### References

1. Snapper SB, Rosen FS. A family of WASPs. *N Engl J Med* 2003;348:350–1.
2. Derry JM, Ochs HD, Francke U. Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. *Cell* 1994;78:635–44.
3. Ochs HD, Notarangelo LD. Structure and function of the Wiskott-Aldrich syndrome protein. *Curr Opin Hematol* 2005;12:284–91.
4. Jin Y, Mazza C, Christie JR, Giliani S, Fiorini M, Mella P, et al. Mutations of the Wiskott-Aldrich syndrome protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation. *Blood* 2004;104:4010–9.
5. Weisz B, Rodeck CH. Invasive diagnostic procedures in twin pregnancies. *Prenat Diagn* 2005;25:751–8.
6. Evans MI, Goldberg JD, Horenstein J, Wapner RJ, Ayoub MA, Stone J, et al. Selective termination for structural, chromosomal and Mendelian anomalies: international experience. *Am J Obstet Gynecol* 1999;181:893–7.

Krisztina Alapi<sup>1</sup>  
Melinda Erdős<sup>1</sup>  
Olga Török<sup>2</sup>  
László Maródi<sup>1\*</sup>

Departments of

<sup>1</sup> Infectious and Pediatric Immunology  
and

<sup>2</sup> Obstetrics and Gynecology

University of Debrecen

Medical and Health Science Center

Debrecen, Hungary

\* Address correspondence to this author at: Department of Infectious and Pediatric Immunology, University of Debrecen, Medical and Health Science Center, H-4012 Debrecen, Hungary. Fax 36-52-430-323; e-mail lmarodi@jaguar.dote.hu.

DOI: 10.1373/clinchem.2005.064816

### Comparison of 3 Third-Generation Assays for Bio-intact Parathyroid Hormone

To the Editor:

Current second-generation parathyroid hormone (PTH) assays suffer

from interference by circulating PTH fragments. Newer assays that measure bio-intact PTH, i.e., the biologically active whole PTH molecule (1–84 PTH), have overcome this problem (1, 2). Our study was designed to evaluate and compare 3 commercial third-generation assays based on a chemiluminescence immunoassay, ELISA, and IRMA for bio-intact PTH and to compare these with a second-generation PTH assay.

EDTA-plasma samples were collected from 40 healthy blood donors (age range, 18–67 years; mean age, 39 years) and from 26 patients with

predialysis chronic kidney disease (age range, 28–86 years; mean age, 65 years). Bio-intact PTH was measured by (a) a chemiluminescence immunoassay on the Nichols Advantage<sup>®</sup> platform (Nichols Institute Diagnostics) (2); (b) an IRMA using the Duo PTH reagents (Scantibodies Laboratory) (1); and (c) an ELISA using the Human BioActive Intact PTH reagents (Immutopics), which is a 2-site assay in which 2 goat polyclonal antibodies recognize epitopes within the midregion/carboxy terminus and at the initial amino terminus. For comparison, we performed

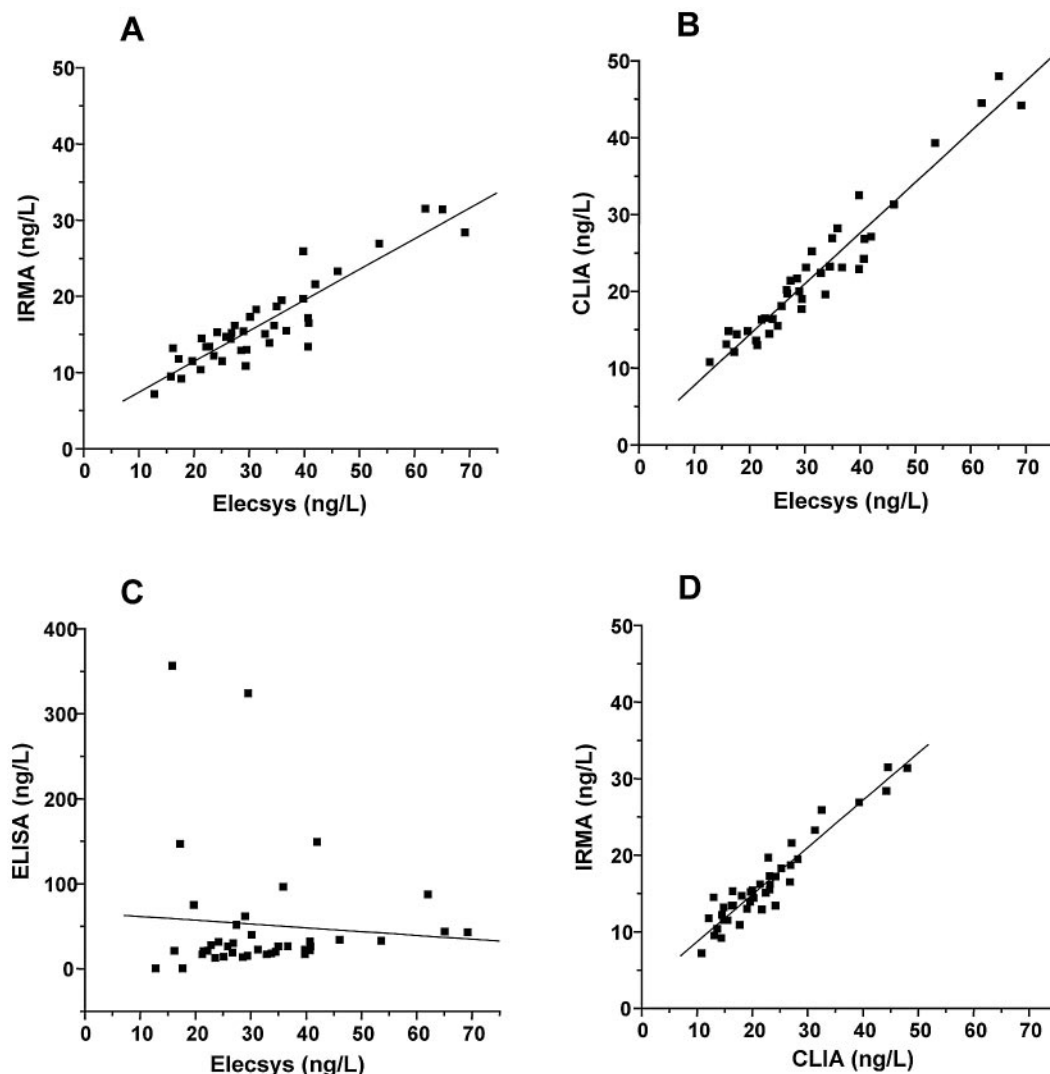


Fig. 1. Regression analysis for comparison of the 3 bio-intact PTH assays [IRMA (A), chemiluminescence immunoassay (CLIA; B), and ELISA (C)] vs the second-generation Elecsys assay, and for the comparison between the IRMA and the chemiluminescence immunoassay (D).

The data are for the healthy individuals ( $n = 40$ ). The regression analysis equations are as follows: (A),  $y = 0.40x + 3.4$  ng/L ( $r = 0.90$ ;  $P < 0.001$ ); (B),  $y = 0.66x + 1.1$  ng/L ( $r = 0.96$ ;  $P < 0.001$ ); (C),  $y = -0.44x + 66$  ng/L ( $r = -0.10$ ;  $P$ , not significant); (D),  $y = 0.62x + 2.5$  ng/L ( $r = 0.95$ ;  $P < 0.001$ ).