Genetics inMedicine | GENETIC LEGACY

Reproducing Genetics

Joe Leigh Simpson, MD[1]

From a very early stage, reproduction was the most interesting aspect of medicine for me and genetics the apotheosis of the sciences. Why study anything other than genetics in obstetrics and gynecology? Such advice was rare during my sentinel years. In the 1960s and 1970s genetics was considered arcane, lacking in tools and having little practical application. Those who expressed interest were politely labeled quixotic. Yet we have watched our dreams evolve quickly and dramatically.

DISCOVERING GENETICS AS A MEDICAL STUDENT

My first human genetics opportunity arose following my first year at Duke University School of Medicine: a summer fellowship with new Ob/Gyn faculty member, Arthur C. Christakos, MD. He had just returned from a fellowship at Columbia University with O.J. Miller, MD, another "atypical" obstetrician-gynecologist, a true doyen of cytogenetics. I was afforded an unparalleled opportunity—to learn how to create a research and service cytogenetics laboratory. One project involved collating every published article on human cytogenetics. They all fit comfortably in a 3-inch binder, an apt characterization of knowledge at the time. Another impressionable event involved a consanguineous family with three 46,XX siblings with ovarian dysgenesis. At that time, ovarian failure in these women was presumed to be the result of undetected 45,X mosaicism. My interest in autosomal genes causing ovarian failure was ignited and has continued to the present.[1]

During medical school, I also worked in the laboratory of James German, MD, at Cornell Medical College. The goal was to exploit autoradiography, given that before chromosomal banding (1971) differential DNA replication was the only way to identify otherwise morphologically indistinguishable chromosomes. We attempted to correlate chromosomal polymorphisms (e.g., prominent acrocentric satellites) with haptoglobin type. This led to my first American Society of Human Genetics presentation in 1967, where several hundred participants crammed themselves into Toronto's Royal York Hotel. I vividly recall participants suggesting that pursuit of a human genetics career could be hazardous, for example, being labeled a eugenicist and leading humanity down the path to ruin.

After medical school I returned to Cornell as a Pediatrics intern. The strategy was to become more familiar with normal childhood development and morphology in order to appreciate the abnormal development that typified many genetic disorders. A highlight was genetic rounds, conducted by James German and his postdoctoral fellow Eberhard Passarge, who opened up for me a panoply of dysmorphic syndromes. Usually no diagnosis was evident, but occasionally we settled on an eponym based on photos in the first edition of David Smith's *Recognizable Patterns of Human Malformations*, then a mere 368 pages.[2]

After Eberhard returned to Essen, I performed genetic consultations myself, even while an ob/gyn resident (newborns seemed not to mind 2 AM consults). These led to identifying the Simpson-Golabi-Behmel syndrome. Such extracurricular genetic pursuits were encouraged by Fritz Fuchs, my chairman and a pioneer who, in Denmark in 1956, had performed the first genetic amniocentesis to assess sex chromatin in amniotic fluid cells for couples at risk for offspring with X-linked recessive disorders.[3] I am indebted to my coresidents, who indulged me at the time, including my focus on sex differentiation. Especially valuable was collaboration with Maria New. She, German, and I described several disorders, including one involving John Opitz in what later proved to be 5α-reductase deficiency.[4]

Additional transformative experiences included the annual American Society of Human Genetics meetings and the March of Dimes birth defects conferences hosted by Victor McKusick at Johns Hopkins University. The latter were frequented by walking eponyms (McKusick, Opitz, Gorlin) and exciting young investigators (Rimoin, Schimke, Hall). After finishing residency in 1973, I spent 2 years as chief of the Obstetrics Service at Brooke Army Medical Center, during which I held a clinical appointment at the new University of Texas Health Services Center at San Antonio and had time to write my first book.[5]

PRENATAL GENETIC DIAGNOSIS

My first academic position was at Northwestern University, heading the Section of Human Genetics in the Department of Obstetrics and Gynecology. My charges included elucidating the genetics of fetal losses and ovarian failure and advancing prenatal genetic diagnoses, respecting that Henry Nadler and Albert Gerbie at the same university had recently demonstrated that genetic amniocentesis during ongoing pregnancies was

[1]March of Dimes Foundation, White Plains, New York, USA. Correspondence: Joe Leigh Simpson (jsimpson@marchofdimes.org)

Submitted 4 February 2015; accepted 16 February 2015.

safe.[6] They welcomed Alice Martin and me setting up a genetics program at the Prentice Women's Hospital, the vision of my chairman, John Sciarra.

Despite Cassandras fearful of fetal damage and eugenics fiascos, amniocentesis scared no obstetrician because the procedure was performed routinely to manage Rh incompatibility. Even as a medical student, culturing amniotic fluid cells for fetal genetic diagnosis had seemed obvious and hence prenatal genetic diagnosis was "just around the corner." Indeed, in 1966 Steele and Breg[7] successfully cultured amniotic fluid cells. Clinical application rapidly followed. At Northwestern, Sherman Elias and I dreamed (as I still do) of definitively testing *every* pregnancy. Anticipating universal testing for pregnant women of any age, Alice Martin led our efforts to generate automated karyotyping, modifying equipment initially developed at the Jet Propulsion Laboratory by Ken Castleman: The goal was a karyotype in every pregnancy.[8]

In 1986 Sherman and I moved to the Department of Obstetrics and Gynecology, University of Tennessee, Memphis, where I became chair and Sherman the director of Reproductive Genetics. The safety and efficacy of chorionic villus sampling were assessed in National Institute of Child Health and Human Development collaborative trials,[9] with Lee Shulman now joining us. Newly minted molecular technologies were evolving. Thus the concept of intact fetal cells in maternal blood for prenatal genetic diagnosis was reopened, following up the 1979 report of flow sorting of Y-chromatin cells from a pregnancy carrying a male fetus by Hertzenberg et al.[10]

In the late 1980s chromosome-specific fluorescent in situ hybridization became a practical option to interrogate fetal cells recovered from maternal blood. In collaboration with Kathy Klinger (Genzyme, Cambridge, MA), we used fluorescence-activated cell sorting to recover nucleated fetal red blood cells from maternal blood. In 1991 our team was the first to detect fetal trisomy (chromosome 18) in cells recovered from maternal blood.[11] The following year we and two other laboratories reported trisomy 21 in maternal blood; the flood gates were opened.[12] In 1994 I moved to Baylor College of Medicine, where Sherman and I cajoled Farideh Bischoff into the pursuit of fetal cells. A National Institute of Child Health and Human Development collaborative study began to optimize recovery of intact fetal cells for the detection of aneuploidy by fluorescent in situ hybridization. This project generated many insights and achieved a trisomy 21 detection rate of 74%.[13] Only later did cell-free fetal DNA become the best *single* marker for prenatal aneuploidy.

A RETURN TO SEX DIFFERENTIATION

During years of emphasizing prenatal diagnosis, my focus unavoidably moved away from sex differentiation, although not for lack of interest. I contributed to every edition of Emery and Rimoin's *Principles and Practice of Medical Genetics*, and I remained active in the Gender Verification Working Group for the Medical Commission of the International Olympics Committee. We advocated for abolishing laboratory tests for gender verification, which at the time (1980s) was unfairly preventing elite athletes with some disorders of sex differentiation from participating in the Olympics. Obligatory genetic testing for sex was finally removed for the Olympics in Seoul, Korea, in 1992.[14]

When sequencing finally became practical, a surfeit of molecular reproductive studies was possible. In 2004, while at Baylor, Aleksander Rajkovic and I began collaboration with Zi-Jiang Chen to interrogate genes potentially causing ovarian failure. We discovered causative perturbations in *NOBOX*[15] and *FIGLA*,[16] as well as many others since then. Although initially disappointed by the failure of our genome-wide association study[17] to detect any significant associations with protein-coding genes (it detected only modest significance for a "gene desert" region (8q22.3)), we later realized these results were typical for complex disorders, especially those affecting fitness. Nevertheless, I predict that 8q22.3 will prove to have regulatory significance.

The lesson is that old pursuits become resurrected once new technologies evolve. Elucidating the genetics of primary ovarian failure, albeit now for me tangential compared with those active in laboratories, seems a pleasing coda to my medical school case report of the consanguineous family with three affected siblings. What has truly changed is that more than academic interest is possible. Oocytes from women who have a mutation that will result in primary ovarian failure should be retrieved, cryopreserved, and later thawed for use in assisted reproductive technologies. In turn, this further fueled my intrigue with preimplantation genetic diagnosis, not only for its complement to traditional prenatal genetic diagnosis but also because of its therapeutic applications. Preimplantation genetic diagnosis also allows the transfer of euploid pregnancies only, reducing spontaneous abortions and increasing the success of assisted reproductive technologies.

CONCLUSION

Medical genetics has been an exciting journey and it has been a privilege for me to be involved. How can any of us not be exhilarated by the developments we have witnessed? Daily there is media attention for a given disorder said to show "newly discovered" genetic tendencies. We had long predicted this, and we smile. Public and professional acceptance of medical genetics has clearly arrived. I personally look forward to everyone having their genome sequenced and their results applied to identify and treat incipient disorders and to prevent pharmacogenetic idiosyncrasies. Conditions now diagnosed during newborn screening could be detected in utero: Why wait for the neonatal manifestations of inborn errors of metabolism? Personalized medicine will lead to better health care and significant cost savings. Medical genetics has made exciting progress, but the pace of change today will seem glacial to the next generation.

DISCLOSURE

The author declares no conflict of interest.

REFERENCES

1. Christakos AC, Simpson JL, Younger JB, Christian CD. Gonadal dysgenesis as an autosomal recessive condition. *Am J Obstet Gynecol* 1969;104: 1027–1030.
2. Smith DW. *Recognizable Patterns of Human Malformation*. WB Saunders, Philadelphia 1970.
3. Fuchs F, Riis P. Antenatal sex determination. *Nature* 1956;177:330.
4. Opitz JM, Simpson JL, Sarto GE, Summitt RL, New M, German J. Pseudovaginal perineoscrotal hypospadias. *Clin Genet* 1972;3:1–26.
5. Simpson JL. Disorders of sexual differentiation. *Etiology and Clinical Delineation*. Academic Press, New York 1976.
6. Nadler HL, Gerbie AB. Role of amniocentesis in the intrauterine detection of genetic disorders. *N Engl J Med* 1970;282:596–599.
7. Steele MW, Breg WR Jr. Chromosome analysis of human amniotic-fluid cells. *Lancet* 1966;1:383–385.
8. Martin AO, Shaunessy M, Sabrin H, et al. Evaluation and development of a system for automated preparation of blood specimens for cytogenic analysis. In: C Lundesteen, J Piper (eds.). *Automation of Cytogenics: Advances in System and Technology*. Springer-Verlag: Berlin. pp 149–173, 1989.
9. Rhoads GG, Jackson LG, Schlesselman SE, et al. The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. *N Engl J Med* 1989;320:609–617.
10. Herzenberg LA, Bianchi DW, Schröder J, Cann HM, Iverson GM. Fetal cells in the blood of pregnant women: detection and enrichment by fluorescence-activated cell sorting. *Proc Natl Acad Sci USA* 1979;76:1453–1455.
11. Price JO, Elias S, Wachtel SS, et al. Prenatal diagnosis with fetal cells isolated from maternal blood by multiparameter flow cytometry. *Am J Obstet Gynecol* 1991;165(6 Pt 1):1731–1737.
12. Simpson JL, Elias S. Isolating fetal cells from maternal blood. Advances in prenatal diagnosis through molecular technology. *JAMA* 1993;270:2357–2361.
13. Bianchi DW, Simpson JL, Jackson LG, et al. Fetal gender and aneuploidy detection using fetal cells in maternal blood: analysis of NIFTY I data. National Institute of Child Health and Development Fetal Cell Isolation Study. *Prenat Diagn* 2002;22:609–615.
14. Ljungqvist A, Simpson JL. Medical examination for health of all athletes replacing the need for gender verification in international sports. The International Amateur Athletic Federation Plan. *JAMA* 1992;267:850–852.
15. Qin Y, Choi Y, Zhao H, Simpson JL, Chen ZJ, Rajkovic A. NOBOX homeobox mutation causes premature ovarian failure. *Am J Hum Genet* 2007;81:576–581.
16. Zhao H, Chen ZJ, Qin Y, et al. Transcription factor FIGLA is mutated in patients with premature ovarian failure. *Am J Hum Genet* 2008;82:1342–1348.
17. Qin Y, Zhao H, Xu J, et al.; China POF Study Group. Association of 8q22.3 locus in Chinese Han with idiopathic premature ovarian failure (POF). *Hum Mol Genet* 2012;21:430–436.