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# GROWTH

## Genetics & Hormones

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### DR. ROBERT M. BLIZZARD - A LEGACY

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#### Fima Lifshitz, MD

*For the Editorial Board*

*Growth, Genetics and Hormones (GGH)* has been published without interruption for the past 19 years. This journal was conceived and founded in 1984 by Dr. Robert M. Blizzard; the first issue appeared in March 1985. The goal set by him and the editorial board was to integrate reports of current advances in the fields of growth, genetics, endocrinology, metabolism and nutrition by bringing the most pertinent papers, with erudite editorial comments, to the attention of pediatricians, internists, pediatric endocrinologists, geneticists, nutritionists, nurses, and to others interested in these fields.

As Editor-in-Chief, Dr. Blizzard has worked tirelessly since the inception of the journal. He has been personally responsible for selecting, recruiting and stimulating the editorial board. He has elicited the best from all of us. Initially the editorial board consisted of Drs. David L. Rimoin, Fima Lifshitz and Alan Rogol from the United States, Judith G. Hall from Canada, and Dr. Jürgen R. Bierich as a European representative. Subsequently other distinguished Pediatric Endocrinologists from Europe joined the editorial board, including Drs. Jean-Claude Job and James Tanner. The

current editorial board members, serving *GGH* since 1993, are Drs. William Clarke, William Horton, and Allen Root, plus founding members Judith G. Hall and Fima Lifshitz. Dr. Blizzard has spearheaded all aspects of the publication including the content, quality, and format.

Throughout the last 19 years *GGH* has exceeded his goals and has become a well established resource for all 6,000 of its current readers, many of whom cherish the journal and keep each issue in their reference libraries. As well, Dr. Blizzard has made sure that as the cycle of life continues there would be a positive and productive transition for *GGH*. During the past two years he has fostered a smooth passage to ensure that upon completion of his tenure as Editor-in-Chief the journal will continue to serve the needs of our colleagues and continue to grow. He personally has overseen all transitional aspects and bestowed responsibility for the future of *GGH* to me as Editor-in-Chief.

Dr. Blizzard requested that a short announcement be inserted about his retirement in this his last issue Vol. 19 No. 4. He wished to see that the many readers who have read *GGH* throughout the years were thanked and appreciation was expressed to all those who have contributed to *GGH* by writing lead articles and to those who have been consistent readers. We pass this message along for him, and the editorial board joins him in saying "thank you".

The editorial board, wishing to acknowledge the many years of service and the most important contributions of Dr. Blizzard, has prepared a brief outline of the accomplishments of this founding editor, teacher, pediatric endocrinologist, clinician, scientist, and man described below. This tribute to him is but a token way to bid him farewell and to imprint his legacy, so that future generations of our colleagues also may be inspired by him.

First and foremost, Dr. Blizzard will be remembered and recognized as a teacher and educator. He is an accomplished teacher, and his competence as an educator and preceptor is well known. He was trained (1955-1957) by Lawson Wilkins and he was "trained to

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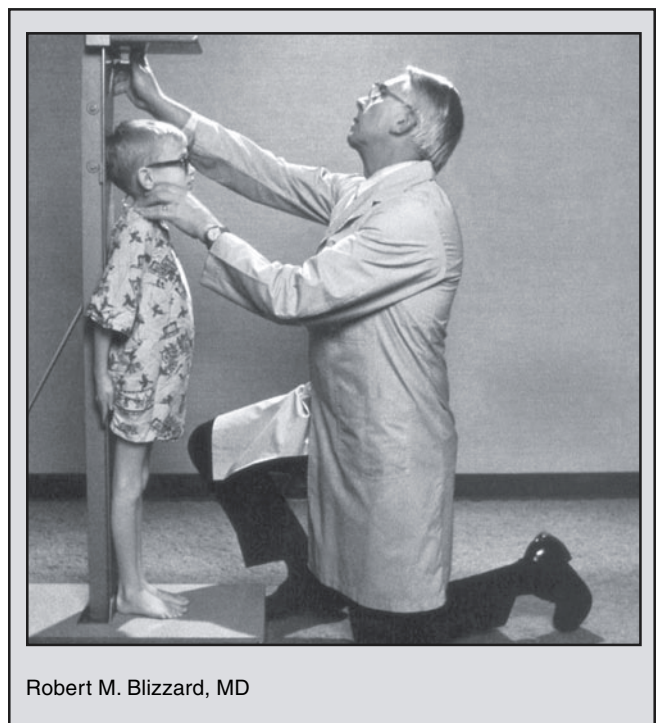
train”, when there were only approximately 20 pediatric endocrinologists in the country. He prides himself in being a pediatric endocrinologist for 48 years (1955-2003) and throughout his career he set the course for his students. Over 50 fellows, including myself and other members of the editorial board, undertook and completed their training with him. Forty-five of these are now in academic positions. Many are full professors including three deans, an associate vice president for health affairs, several chiefs of staff of children’s hospitals, and several pediatric department chairpersons in the U.S. and abroad. He is proud of the fact that most of his fellows have established their own pediatric endocrinology training programs, and thus provided an ongoing transmission of the teaching of Lawson Wilkins and himself to second and third generations of pediatric endocrine fellows. He has received multiple teaching awards including those from the Johns Hopkins Hospital, the University of Virginia, and other prestigious universities. Very possibly the teaching award of which he is most proud is his election to alpha omega alpha in 1970 by the members of the Johns Hopkins Alpha Omega Alpha (AOA) Society. His accomplishments as a student had not qualified him for AOA membership, and, therefore, his election by the student membership was particularly gratifying, since only one faculty member per year was elected to the society.

He also is proud of the opportunity to have served as Acting Chairman of the Department of Pediatrics at the Johns Hopkins University School of Medicine (1972 and 1973) and as Chairman of the Department of Pediatrics at the University of Virginia, School of Medicine (1974-1987). At these institutions he fostered 15 generations of pediatric residents who sought and attained their pediatric training in his departments. Most of them are now in academic and/or clinical practice in the US. A significant number are abroad. For his educational activities he has been honored with other prestigious awards. Among them are the Ayerst (1973) and Williams (1994) distinguished service and leadership awards bestowed by the American Endocrine Society. Recently he has been honored to be elected to the Johns Hopkins Society of Scholars (2002) and honored by the establishment (2002) of the Robert M. Blizzard Annual Lectureship at the annual meetings of the Lawson Wilkins Pediatric Endocrine Society (2002). He also has been honored by invitations to deliver over 150 named lectureships and visiting professorships at many national and international academic institutions. He was elected to the prestigious Hall of Fame of Miami Children’s Hospital in 1997. Those who attended the teachings of Dr. Blizzard have always recognized his talents in teaching, and most have asked for more!

However, his contributions as an educator transcend

the traditional teaching role through which he personally touched so many individuals and imparted his knowledge. Dr. Blizzard made major contributions to continuing medical education by serving on multiple editorial boards of journals, editing and publication of several textbooks, and by his 19 years of editorship of *GGH*. The number of physicians and other scientists whom he reached via this journal through the years cannot be easily counted nor measured, but *GGH* is currently read regularly, as previously stated, by over 6,000 colleagues world-wide. Thus, the impact of Dr. Blizzard as an educator can be summarized as “the teacher par excellence”.

In the field of endocrinology, he is particularly known for his contributions in the areas of growth and in autoimmunity, with over 200 original peer-review papers published in the literature. His picture is a clear testimony to his legacy as a clinician. He has always, in the Wilkins’ style, promoted accurate measurements of children in assessing growth. This is still the gold standard in the evaluation of children with short stature. Dr. Blizzard was a pioneer in this field, publishing his first studies on the action of human growth hormone in 1959, one year after the first publication by Dr. Raben of the use of human growth hormone in growth hormone deficient individuals. His interest continues in this field to this day. Dr. Blizzard, along with Dr. Joanne Brasel and Dr. James Wright in the early 1960s published several important papers that changed the approach to the diagnosis and treatment of growth hormone deficiency. Included were the observations that growth hormone deficiency can be manifested by delayed growth even in the first year of life, previously not thought to be the case, and that the



Robert M. Blizzard, MD

acute metabolic response to human growth hormone did not correlate with its growth promoting effects when growth hormone deficient children were treated. The search for reliable indicators to predict a quantitative response to growth hormone is still ongoing.

Subsequently, Dr. Blizzard authored or co-authored 56 publications in peer-review journals pertaining to growth hormone or growth factors. These studies clarified the role that growth hormone played in producing the adolescent growth spurt, and the phenomenon of growth hormone production and its relationship to steroid production during this stage of life. A series of articles published under his tutorage unequivocally demonstrated that growth hormone increases at the time of adolescence when testosterone is produced in males. These studies showed that growth hormone and testosterone each have separate mechanisms of action in promoting growth, as well as permissive actions in the relationship to the secretion of each other.

In 1971 Dr. Blizzard stimulated his associates to design a pump that would permit a constant withdrawal of blood over a 24-hour period, that would permit the measurement of integrated concentrations of circulating hormones. Dr. Avinoam Kowarski was successful in this endeavor, and he and Dr. Robert Thompson, Dr. Claude Migeon, and Dr. Blizzard first reported the determination of integrated concentrations of human growth hormone and true secretion rates of human growth hormone. The importance of pulsatility and the intricacies of growth hormone production at various stages of life were subsequently delineated using this technique in studies with Dr. Alan Rogol, Dr. Paul Martha, Dr. Nelly Mauras, Dr. Kathleen Link, and others at the University of Virginia.

While being a leader throughout his life and an innovative initiator of investigative protocols, he appropriately was appointed Director of the Clinical Research Center at the University of Virginia (1980-1983), while serving simultaneously as Department Chairman. He collaborated extensively with his colleagues in the Divisions of Endocrinology in Internal Medicine (Dr. Michael Thorner in particular among others). He coauthored 15 papers concerning the effect of growth hormone releasing hormone in humans - both as a diagnostic and therapeutic agent.

Although not as well known, Dr. Blizzard initiated and significantly contributed in elucidating the possible role of decreased growth hormone production during adult life in the aging process. He, his associate Dr. Ann Johanson, and his group initially demonstrated that older males secrete less growth hormone than do young males, and that older males receiving growth hormone retain nitrogen, comparable to that seen in growth hormone deficient young adults. They also reported that

growth hormone administered to older males generated insulin-like growth factor I, comparably to that generated in growth hormone deficient children. He subsequently described the changes in pulsatility of growth hormone secretion in older men and women as compared to younger subjects.

These studies led to the involvement of Dr. Blizzard in the first study to evaluate the effect of chronic growth hormone administration in older males. His research was not only at the intellectual/research level; he was the first of five males in a study which he initiated to receive growth hormone every day over a period of 30 months. He and his colleagues demonstrated that growth hormone had no significant effect upon skin collagen and its amino acid composition. He was obliged to stop the study in 1985 because of the report of possible contamination of native pituitary extracts by the prion producing Creutzfeldt-Jakob disease. However, the results of this project undoubtedly stimulated other investigators to assess the effect of growth hormone in the elderly.

Another major contribution of Dr. Blizzard was the concept that psychosocial dwarfism (also called emotional deprivation, maternal deprivation, the garbage can syndrome, and reversible hyposomatotropism) resulted from transient growth hormone deficiency. He insists that major credit in the concept be accepted by Dr. Dagfinn Aarskog, Dr. Gerald Powell, Dr. Salvatore Raiti, and others. The demonstration of the pathophysiology of such alterations gave great impetus to studying how the hypothalamus and its neurotransmitters are controlled by higher cerebro-cortical centers which has been the subject of countless studies. To date Dr. Blizzard continues to be considered a world authority on psychosocial dwarfism or reversible hyposomatotropism.

Although currently known by young pediatric endocrinologists and academicians more for his work in the field of growth, he contributed significantly in other fields of endocrinology. In 1959 he initiated a study to determine aldosterone excretion in virilizing adrenal hyperplasia. He was the lead author of an article in the *Journal of Clinical Investigation* demonstrating that salt-losing congenital virilizing adrenal hyperplasia was due to decreased aldosterone secretion.

Between 1955 and 1980 Dr. Blizzard was a leading international investigator and authority on autoimmune endocrine diseases. He suggested that many endocrine diseases characterized by glandular atrophy, including adrenal insufficiency, hypoparathyroidism, premature ovarian failure, and insulin dependent diabetes mellitus were of autoimmune origin. He studied this model in his laboratory over the next 25 years and applied his

findings in the clinic setting which led to publications of 27 papers in peer-review journals.

In his laboratory with the assistance of Dr. Robert Chandler, he was one of the first investigators to demonstrate that Addison's disease was frequently of autoimmune origin and the first to elucidate the physical and biochemical characteristics of the antigens involved. In 1966 he reported that hypoparathyroidism was also related to antibody formation. In 1960 he had demonstrated that there was a high incidence of antibodies against thyroid microsomes and thyroglobulin in the serum of mothers of athyreotic cretins. Dr. Blizzard postulated that autoimmune thyroid disease in the pregnant woman might be the etiology of at least some cases of congenital athyreotic cretinism. At the Pediatric Endocrine Research Meetings in May 1987, Dr. Dussault of Canada presented confirmatory evidence of this hypothesis, and acknowledged that the concept and early data had been presented by Dr. Blizzard years previously.

In the early 1960's he proposed that some cases of insulin dependent diabetes mellitus were probably of autoimmune origin with destruction of the beta cells of the pancreas. This observation was based on his earlier papers reporting the associations of diabetes mellitus with Addison's disease and hypoparathyroidism. In 1961, he submitted a grant to the National Institutes of Health (NIH) proposing to study this concept. The grant was rejected stating that the concept was preposterous; subsequently it was demonstrated that indeed many patients with insulin dependent diabetes mellitus had antibodies to beta cells and the role of autoimmunity in insulin dependent diabetes mellitus was firmly established.

Even subsequent to 1979, when Dr. Blizzard was devoting the majority of his investigative time to problems of growth, he published major papers concerning autoimmunity. These papers further elucidated the associations of various types of autoimmune diseases, and particularly clarified the associations of the various types of polyglandular autoimmune adrenal disease with other endocrine disorders. At an international autoimmune conference held in Pisa, Italy, in 1979, Blizzard proposed a classification of polyglandular autoimmune diseases, which was accepted internationally and continues to be used today with only minor modifications.

Blizzard has recorded many other "firsts" in the field of pediatric endocrinology, including, with the collaboration of Dr. Ann Johanson and Dr. Harvey Guyda and other fellows, the elucidation of the intricacies of luteinizing hormone and follicle stimulating hormone secretion during childhood and puberty, and the abnormalities

found in sexual precocity. In his laboratory, along with Dr. Robert Penny, he demonstrated that gonadotropin levels were elevated in hypothyroid children who have associated sexual precocity. He reported with Dr. Johanson that patients with gonadal agenesis or Turner syndrome grew significantly when treated with anabolic agents.

Other firsts included a description and report of the Johanson-Blizzard syndrome of congenital anomalies in congenital hypothyroidism and a description of the syndrome of congenital adrenal cortical-unresponsiveness to ACTH with Dr. Claude Migeon. In addition, Dr. Blizzard actively contributed to and participated in the treatment and research of patients with central sexual precocity utilizing gonadotropin releasing hormone agonists (GHRHa) to block pubertal development. Dr. Blizzard was proud of his capability to work collegially and collaboratively with others to promote multicenter investigation. An example was his collaboration over several years with Dr. Paul Boepple, Dr. William Crowley, and others in Boston in studying the role of GnRH analogues.

In 1961, in association with Dr. Alfred Wilhelmi, Chairman of the Department of Biochemistry at Emory University, the National Pituitary Agency was established. The purpose of this agency was to collect human pituitaries at autopsy examination, extracting their hormones, and to distribute these hormones on a national basis for investigation and therapy. He organized this collection and distribution program under the auspices of the (NIH), and was the Director of the agency until 1967. Dr. Blizzard inspired and led a lay group of individuals to develop an organization of parents and others to assist in the collection of human

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pituitary glands. Their success led to the establishment of the Human Growth Foundation in 1965. The scope of this organization grew and eventually became a support source for families of children with growth disorders with chapters across the country and with an ability to fund research in the area of growth disorders. After the National Pituitary Agency and The Human Growth Foundation were firmly established, Dr. Blizzard was followed by Dr. Salvatore Raiti, one of his former fellows, as director. It was this program that led to, and made possible, all of the investigation pertaining to pituitary hormones that occurred in humans in the subsequent 24 years (1961-1985) before synthetic growth hormone became available.

In 1993 he was asked to establish the Genentech Foundation for Growth and Development, a grant awarding organization separate from Genentech Inc., with an independent board and decision making authority. In the 8 ½ years of its existence under his leadership this foundation provided more than \$18 million dollars in grants to clinical investigators, to basic science investigators, to physicians receiving training in the fields of growth and development, and to support professional and personal education of growth and development in these fields.

Discussing the many contributions of Dr. Blizzard to

pediatrics and to science is an easy and enjoyable endeavor, particularly because he always attempted to recognize the contributions of those with whom he worked professionally. Examples of his appreciation for professional collegiality and recognition are cited in the text above. A major professional colleague of Dr. Blizzard and contributor to the success of Growth, Genetics & Hormones for 19 years is Ms. Juanita Bishop, his trusted and dependable assistant of over 20 years.

Describing the human qualities of Dr. Blizzard also is an easy and enjoyable endeavor. He is an exceptional human being, and it is worth noting the comforting way he talked to his patients and families and his ability to put them at ease despite their difficult problems. He has a special skill to develop closeness with others lasting a lifetime, and to nourish and support his patients, students, fellows, and associates. This is what I and his other associates appreciate the most!

The cycle of life continues, with the publication of this issue Dr. Robert M. Blizzard has officially retired from the editorship of *GGH*. He has had a most prestigious and distinguished career with enough accomplishments for many lifetimes. He now plans to enjoy more time with his family. We anticipate he will continue that which he does best, inspiring and teaching. As he has thanked so many of us, we thank him for all!

#### Abstracts from the Literature

### Screening Newborns for Inborn Errors of Metabolism by Tandem Mass Spectrometry

Newborn screening for inborn errors of metabolism has been in place in many countries for many years. Strong arguments have been made for screening not only for improving care of patients identified through screening, but also for reducing the cost of this care. Indeed, there are numerous examples, PKU most notably, of how early diagnosis and treatment have prevented serious illness or death from these disorders. However, as Wilcken and colleagues point out, formal evidence for the clinical effectiveness of screening is lacking, especially for rarer diseases, such as inborn errors of metabolism. Randomized, controlled trials of screening have been very limited because of the rarity of these disorders and also because of the strong conviction based on clinical experience that there is a benefit from early diagnosis.

Against this backdrop Wilcken et al compared the effectiveness screening for inborn errors of metabolism in all newborns with tandem mass spectrometry from 1998 to 2002 to conventional biochemical screening performed because of clinical suspicion from 1974 to 1998. The study population lived in New South Wales (Australia) and the Australian Capital Territory and totaled six million. Thirty-one disorders were selected for study. PKU and pterin disorders were excluded

because effective screening by other methods had been in place for many years; also excluded were disorders known to be benign or of maternal origin.

The diagnosis rates were reported in four-year brackets, i.e., 1974-1978, 1978-1982 ... 1998-2002, etc. During the six four-year periods preceding the implementation of tandem mass spectrometry screening, 22-34 cases were diagnosed per period giving rates from 6.6 to 9.0 cases per 100,000 births. Diagnoses were made at different ages depending on the age of clinical presentation. There were no trends toward increased overall rates of diagnosis between 1982 and 1998 even though some of the 31 disorders were first recognized during these periods.

Between 1998 and 2002, when all infants were tested between 48 and 72 hours after birth, 57 infants were diagnosed with one of the 31 inborn errors or 15.7 diagnoses per 100,000 births. Of these, 48 infants were diagnosed by screening, while six were diagnosed clinically before or at the same time as the screening result became available, usually within 24 hours of testing. Two patients, siblings with ornithine transcarbamylase deficiency born to a mother with known risk, did not undergo screening. Seven patients

whose diagnoses were made later on clinical grounds had negative results on newborn screening.

Although results showed an increase in the rate of diagnosis following the introduction mass spectrometry screening in newborns, most of the increase could be accounted for by the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency and to a lesser extent by the diagnosis of other disorders of fatty acid oxidation.

The authors calculated the cost of establishing a diagnosis. The incremental cost of the tandem mass spectrometry screening was \$0.70 (USD) per newborn. The cost of confirmatory testing was \$217 and the cost per relevant disorder detected was \$3,939 if PKU was excluded or \$2,519 if it was included. They concluded that their approach provides a rapid and inexpensive way to screen for a wide range of very rare metabolic diseases and that it identifies more cases than are diagnosed clinically. However they caution that it is not yet clear which patients identified through newborn screening would have become symptomatic if screening had not been performed.

Wilcken B et al. *New Eng J Med* 2003;348:2304-2312.

**Editor's Comment:** *This paper brings to the fore the debate over the extent to which tandem mass spectrometry technology should be used to screen for a growing number of inborn errors of metabolism. As*

*noted in a recent article by Marshall,<sup>1</sup> the debate pits parents and often physicians who advocate the application of this technology against ethicists with concerns over costs and public health officials with concerns over how the potentially large amount of genetic data will be managed. The Wilcken study demonstrates the successful implementation of the technology in a public health setting. It documents that the technology leads to an increased rate of diagnosis at low cost, especially for disorders of fatty acid oxidation, although acknowledges the possibility that some patients diagnosed as newborns may not have become symptomatic if screening had not been performed. Readers should note that metabolic screening by tandem mass spectrometry was highlighted by a recent lead article in GGH.<sup>2</sup> This article explains how technology works, provides guidelines for its use and describes its successful application in North Carolina. Together, these articles provide support for advocates of wider use of tandem mass spectrometry for newborn screening.*

## References

1. Marshall E. *Science* 2001;294:2272-2274.
2. Millington D, Koeberl D. *GGH* 2003;19:32-38.

William A. Horton, MD

## The Effect of Clitoral Surgery on Sexual Outcome in Individuals Who Have Intersex Conditions with Ambiguous Genitalia: A Cross-Sectional Study

It is estimated that intersex conditions occur in one per 2,000 live births. In the past, treatment had been based on the assumption that infants were gender neutral at birth, and that assignment of sex of rearing in early years which is reasonably compatible with the appearance of the external genitalia would provide a normal gender identity and partner orientation in adulthood. Subsequently, it has been recognized that there is a complex interaction between prenatal and postnatal factors that lead to the development of gender and sexual identity.

In the United States and in most western European societies, female rearing was most frequently recommended to parents whose infant had ambiguous genitalia. When the decision to raise the child as a female was made, surgery was usually undertaken to remove any ambiguity of the genitalia and to feminize the external appearance. This was done with the hope of a good psychosocial outcome.

Minto et al undertook a study involving individuals with several intersex conditions which included ambiguous genitalia, and who were living as adult females. Individuals were recruited from the Androgen

Insensitivity Syndrome Support Group, the Adrenal Hyperplasia Network and the Intersex Clinic at University College in London Hospital.

Questionnaires were distributed and individuals could respond anonymously or identify themselves, in which case, their records would be examined with their permission. The self-administered questionnaires included the Golombok-Rust inventory of sexual satisfaction (GRISS) for women. Of the 39 patients included in this study, 11 had no clitoral surgery and 28 had had clitoral surgery. Almost all individuals who had undergone gonadectomy were taking hormone replacement therapy. Historical trends were noted in that most individuals seen before 1979 had undergone clitorrectomy, while those operated on since 1980 usually underwent nerve-sparing clitoral reduction surgery. Many individuals also had vaginal reconstructive surgery.

The authors did multiple types of analysis of the data; however, the bottom line is that of the 39 participants, 13 individuals had never been sexually active and the 28 sexually active individuals had below normal scores in terms of sexual function. A low score on sensuality

was evident in the clitoral surgery group when compared to the non-surgical group. Both groups had difficulty with orgasm, which is relatively rare in a sexually healthy population. Of the 28 who had clitoral surgery, 18 found it impossible to have orgasm, compared with none among those who had not had clitoral surgery.

It was difficult to determine exactly why most of the study individuals were having difficulty with sexual function because only a questionnaire was used to obtain the data. There did not appear to be a difference among those patients recruited from the clinic versus those in support groups.

It would appear that genital surgery at a young age did not lead to satisfactory gender identity and sexual activity. However, it is not clear what the most appropriate approach should be. The authors encourage debate about the ethical issues, the development of reliable information, support of research in this area and how important it is to share this information with parents and patients who are considering clitoral surgery.

Minto CL et al. *Lancet* 2003;361:1252-1257.

**First Editor's Comment:** *The outcomes of the management of intersex are not perfect. This study following up on previously treated individuals suggests that clitorotomy does not lead to sexual satisfaction, however, neither does clitoral reduction. Clearly, more research and discussion are needed in this area.*

Judith G. Hall, OC, MD

**Second Editor's Comment:** *As the authors acknowledge, interpretation of their study is hampered by the small number of study subjects and the possibility that those electing to participate were among the more*

*dissatisfied patients contacted initially. Quite interesting are the data that indicate that clitoromegaly itself is associated with sexual dysfunction. In addition to the concept that clitoral recession will permit the child to more readily accept her female sex assignment, the procedure is performed to ease parental acceptance of their newborn child. Those who have dealt on a personal and daily basis with parents of children with ambiguous genitalia know the need to assure and reassure parents is a paramount goal which is difficult to attain. Early clitoral recession by a skilled surgeon is most often recommended by this writer in those neonates with more severe degrees of genital ambiguity.*

*Because of widespread neonatal screening for CAH, there is an increasing number of females with the most severe form of genital ambiguity known as Prader V or complete incorporation of the urethra into the phallus/clitoris. In the opinion of this writer and many others it is inappropriate to rear these genotypic and potentially fertile girls as males, thus necessitating genital surgery. Since both clitoromegaly and clitoral surgery impede sexual satisfaction, the challenge is to devise a corrective procedure that does not do so.*

*It would have been of interest to learn whether in those women with ambiguous genitalia who did not undergo clitoral surgery, clitoromegaly during childhood and young adulthood was a matter of significant concern. Counseling girls with ambiguous genitalia, whether operated upon or not, needs to begin in mid-childhood and to be conducted by individuals skilled in the management of this problem, as mentioned by Slijper in an excellent commentary regarding this article, in the same issue of *Lancet* (2003;361:1236-1237).*

*Minto's article also provides further support for the antenatal treatment with glucocorticoids of women bearing female CAH offspring at risk for development*

Table

**Sexual function of 28 participants, according to GRISS**

	Subscale scores (%)			Subscale scores (%)		
	Clitoral surgery group (n=18)			No clitoral surgery group (n=10)		
	Normal*	Difficulties†	Severe difficulties‡	Normal*	Difficulties†	Severe difficulties‡
Frequency	28%	72%	33%	30%	70%	30%
Communication	28%	72%	17%	20%	80%	20%
Satisfaction	61%	39%	0%	80%	20%	0%
Avoidance	28%	72%	22%	20%	80%	10%
Sensuality	22%	78%	22%	80%	20%	10%
Vaginal penetration§	33%	67%	33%	33%	67%	22%
Orgasm	39%	61%	28%	60%	40%	0%

\*Score of 1-4. †Score 5-9. ‡Score of 8 or 9. §Four individuals chose not to answer the question on vaginal penetration.

Adapted from Minto CL et al. *Lancet* 2003;361:1252-1257.

of ambiguous genitalia. It will be of great interest to assess the psychosexual development, orientation, and sexuality of these subjects as adult women. With the observations collected to date the impression is that they are normal little girls.

Allen W. Root, MD

**Third Editor's Comment:** The topic of intersex management, outcome, and research has received much attention in the past 2-3 years. The reader should be aware of publication of a collection of excellent papers

presented in May 2002 at a conference entitled "Genetic and Hormonal Basis of Sexual Differentiation Disorders" (*The Endocrinologist* 2003;13:175-287) and of a "Summary of a Research Workshop on Intersex" held in sequence with the above conference (to be published in *The Endocrinologist*). Furthermore an excellent review entitled "Management of Children with Intersex Conditions: Psychological and Methodological Perspectives" by S. Berenbaum was presented in *GGH* 19:1.

Robert M. Blizzard, MD

## Neonatal Exendin-4 Prevents the Development of Diabetes in the Intrauterine Growth Retarded Rat

Intrauterine growth retardation (IUGR) has been shown to be associated with significant adult morbidity, including insulin resistance, reduced pancreatic  $\beta$ -cell mass, and subsequent type 2 diabetes. Uteroplacental insufficiency, a cause of IUGR, limits the availability of substrates, growth factors, and hormones to the fetus. A rat model of IUGR can be induced with bilateral uterine artery ligation at 19 days of the 22 day gestation period. In rats during the newborn period there is extensive remodeling of the pancreas brought about by  $\beta$ -cell replication, neogenesis and apoptosis. A second wave of neogenesis occurs during weaning.

The incretin hormone glucagon-like polypeptide-1 (GLP-1) stimulates pancreatic neogenesis and increases  $\beta$ -cell mass. Therefore its administration to rat pups who have undergone 90% partial pancreatectomy results in an increase in both  $\beta$ -cell mass and improved glucose homeostasis. Exendin-4 is a long-acting GLP-1 which in addition to the aforementioned activities stimulates expression of Pancreatic Duodenal Homeobox (PDX) protein in the pancreas. PDX is critical for the early development of both the endocrine and exocrine pancreas and mediates glucose responsive stimulation of transcription of the insulin gene.

Stoffers and colleagues treated IUGR rat pups with exendin-4 during the early postnatal period to study its effects on the subsequent development of type 2 diabetes. Four groups of rat pups were studied: (1) control pups given vehicle injection, (2) control pups given exendin-4 injections, (3) IUGR pups given vehicle injections, and (4) IUGR pups given exendin-4 injections. Injections were administered on postnatal days 1 through 6. Glucose tolerance,  $\beta$ -cell mass,  $\beta$ -cell proliferation and PDX gene expression were measured at 14 days and 3 months of age. Glucose tolerance was also determined at 7 weeks and 8 months of age.

Exendin-4 decreased weights in both control and IUGR pups (Groups 2 and 4) at 2 weeks. This decrease persisted into adulthood (Table). At day 14, glucose

tolerance in the IUGR pups treated with exendin-4 was similar to that in control animals. The treated animals remained euglycemic at 8 months. Vehicle-treated IUGR pups (Group 3) developed diabetes by 3 months and died by 8 months of age. Exendin-4 treated IUGR pups (Group 4) had normal  $\beta$ -cell mass comparable to that in Group 1 as the result of normalized replication rates. While Pdx-1 mRNA levels were reduced by 60% in IUGR rats not receiving exendin-4 at 14 days, those treated with exendin-4 had normal levels.

The authors state their major finding is that a short treatment with exendin-4 during the early newborn period prevents the development of diabetes in the IUGR rat. It is not clear whether this effect is through the stimulation of Pdx-1. However, the effect is independent of  $\beta$ -cell mass, since its effects were observed prior to any reduction in the IUGR pancreatic mass. They suggest that the permanent improvement in maintenance of  $\beta$ -cell mass by exendin-4 may mean that similar drugs could be effective in reducing the risk or preventing type 2 diabetes mellitus in individuals born with IUGR. The negative part of the study was the growth inhibiting effect of exendin-4.

Stoffers DA, et al. *Diabetes* 2003;52:734-740.

Table

### Body weight at 2 weeks and 3 months

Treatment group	2 weeks (g) (n=9)	3 months (g) (n=7)
Control vehicle	27.7 $\pm$ 0.3	331.7 $\pm$ 7.0
Control Ex-4	22.2 $\pm$ 0.6*	305.3 $\pm$ 12.7*
IUGR Ex-4	13.8 $\pm$ 0.7†	311.0 $\pm$ 4.0†
IUGR vehicle	17.2 $\pm$ 0.7‡	351.7 $\pm$ 26.2‡

Data are means  $\pm$  SE. \*P < 0.05 control Ex-4 vs. control vehicle; †P < 0.05 IUGR Ex-4 vs. IUGR vehicle; ‡P < 0.05 control vehicle vs. IUGR vehicle.

Adapted from Stoffers DA, et al. *Diabetes* 2003;52:734-740.

**Editor's Comment:** These fascinating data suggest that possibly there may be a treatment available in the future for the prevention of type 2 diabetes mellitus in IUGR individuals, if treated early in the neonatal period. Stoffers and colleagues have shown using an IUGR rat model that exendin-4 given for a short period of time postnatally can prevent glucose intolerance by restoring Pdx-1 function and normalizing  $\beta$ -cell proliferation rates. One cannot read this study without thinking about other

morbidity associated with IUGR and how other treatments administered in the neonatal period might someday become available to treat those as well. The obvious example would be treatment given early to restore normal growth velocity. These authors have presented data that opens up a whole new world of possibilities.

William L. Clarke, MD

## Morbid Obesity and Mutations in Appetite Controlling Genes

It is known that the loss of function mutations of the melanocortin 4 receptor (MC4R) gene lead to severe obesity in humans and in mice. These genetic mutations disrupt the appetite control centers in the hypothalamus and lead to severe obesity. In the March 20, 2003 issue of the *New Eng J Med*, two papers were published which clarify the clinical syndrome resulting from the mutations in the appetite controlling MC4R gene.

In the first paper, Farooqi et al determined the nucleotide sequence of the MC4R gene, which is known to be a cause of a monogenic form of obesity. They studied 500 probands with severe obesity. In these families they examined the cosegregation of identified mutations, and in the subjects who were found to have MC4R deficiency they performed a metabolic-endocrine evaluation and characterized their clinical phenotype. The results were correlated with the signaling properties of mutant receptors. Twenty-nine probands (5.8%) had mutations in MC4R; 23 were heterozygous and 6 were homozygous. Mutation carriers were severely obese; their mean percentage of body fat was 43% of their body composition. Excess body weight gain was evident since the first year of life. They also presented increased lean body mass, increased linear growth, hyperphagia and severe hyperinsulinemia. The serum leptin and lipid levels, the metabolic rate, and thyroid, adrenal and reproduction function were normal. Homozygous individuals were more severely affected than the heterozygous ones. The subjects with mutations who retained some residual signaling capacity had a less severe phenotype than those with a totally absent signaling capacity. MC4R mutations resulted in a distinct obesity syndrome inherited in a co-dominant manner. The authors concluded that MC4R alterations play a key role in the development of a distinct form of severe obesity commencing in early childhood.

In the second paper, Branson et al studied the interactions of genetic and environmental factors which may have a bearing on the development of obesity in MC4R affected individuals. Four hundred sixty-nine severely obese white subjects with an average age of 42 years and with a mean body-mass index of 44, and

25 control subjects with normal weight and no history of obesity or dieting were included in this study. They sequenced (1) the complete MC4R coding region, (2) the proopiomelanocortin gene (POMC) region which encodes the  $\alpha$  melanocyte-stimulating hormone (MSH), and (3) the binding domain of the leptin receptor (LEPR) gene. They also obtained detailed data concerning phenotypes, resting energy expenditure, diet-induced thermogenesis, serum leptin levels, and eating behaviors. Twenty-four of the 469 obese subjects (5.1%) and one of the 25 controls (4%) had MC4R mutations, including 5 novel variants. All mutation carriers reported binge-eating behavior, defined as repeated rapid consumption at least twice per week of an unusually large amount of food *in the absence of hunger*, causing the subject to feel embarrassed, depressed or guilty and out of control. This 100% prevalence of binge eating in MC4R mutation patients was compared with a 14% frequency of such behavior in obese subjects without genetic mutations. The prevalence of binge eating was similar among carriers of mutations in the LEPR as among that of non carriers. No mutations were found in the region of POMC encoding  $\alpha$  MSH. The authors concluded that *binge eating* is a major phenotypic characteristic of subjects with a mutation in MC4R, a candidate gene for the control of eating behavior.

Farooqi IS et al. Clinical Spectrum of Obesity and Mutations in the Melanocortin 4 Receptor Gene. *New Eng J Med* 2003;348:1085-1095.

Branson R et al. Binge Eating as a Major Phenotype of Melancortin 4 Receptor Gene Mutations. *New Eng J Med* 2003;348:1096-1103.

**Editor's Comment:** These two papers simultaneously published in the *New Eng J Med* are landmark studies. They contribute greatly to the understanding of the pathogenesis of obesity in humans. Farooqi and colleagues determined what proportion of obesity is attributed to a mutated gene of MC4R. They found that about 6% of severely obese individuals who had obesity since early childhood had these mutations. Thus, patients carrying MC4R mutations constituted an

important subgroup of the severely overweight population. Given the high prevalence of observed MC4R deficiency, it appears that this condition represents the most common form of monogenic obesity in humans. Pediatricians and pediatric endocrinologists should be on the look out for this, especially in children who gain excess weight beginning in early childhood. Clinically, these patients differ from those with Prader Willi syndrome, who also have another form of monogenic severe obesity, by the normal stature and muscle development which are abnormal in Prader Willi syndrome.

The second study showed that overweight people who are binge eaters are more likely to harbor genetic mutations of MC4R than overweight people who constantly overeat. Until now, binge eating was considered a psychological phenomenon or disorder. For the first time a genetically driven characteristic was demonstrated. MC4R mutations appeared to disrupt brain signals governing satiety.

Both studies clearly document that there are severely obese individuals who overeat, not because of lack of will power, but because they have a genetically determined pathological syndrome.

However, these data also demonstrate that there are some individuals who have genetically determined mutations, yet are not obese. The reverse also occurs; specifically, binge eating behavior may occur and not be found to be associated with genetic mutations of MC4R. Thus, these two reports also support the thesis that the etiology of obesity is multifactorial, even in individuals who have a genetically determined alteration in the appetite control centers in the hypothalamus. In these patients, as well as in other obesities, excess

energy intake over energy expenditures must occur for obesity to develop.

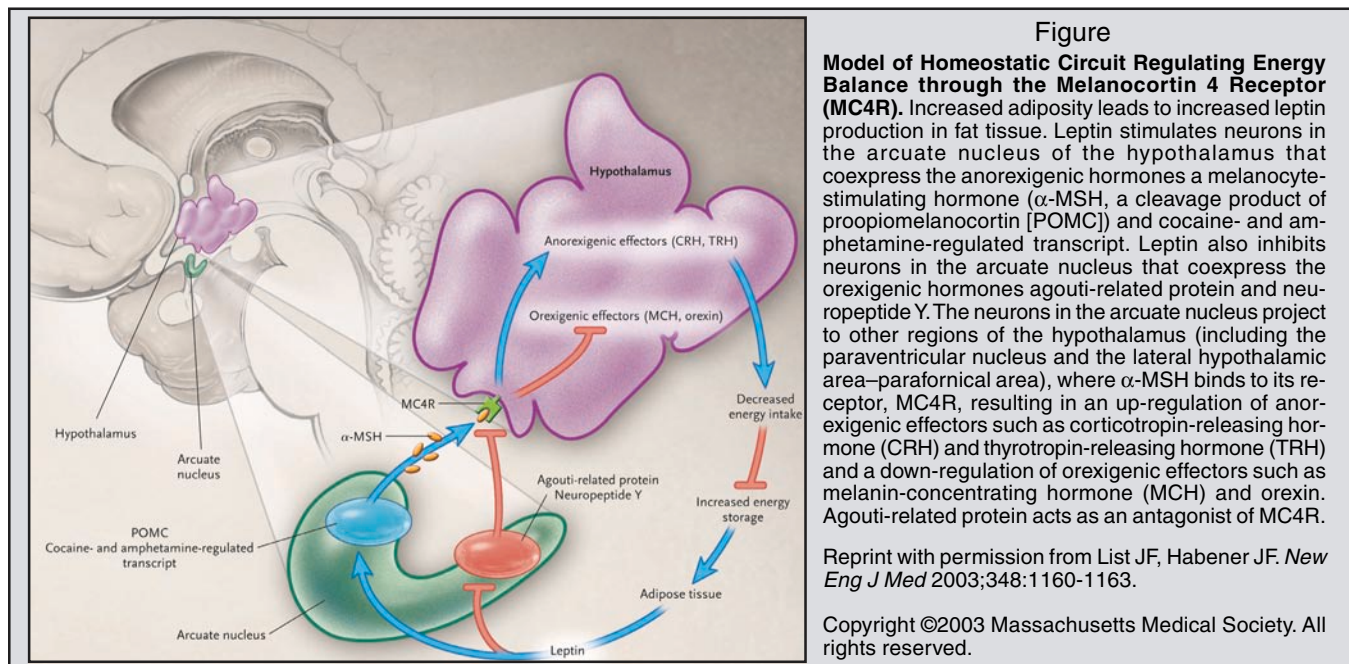
The reader is encouraged to review these two papers in detail, as well as to study the accompanying editorial by List and Habener<sup>1</sup> who clearly described the model of the homeostatic circuit regulating energy balance via the MC4 receptor. These authors point out that several hormones are known to influence the appetite control centers in the hypothalamus (Figure). MC4R deficiency is clearly implicated in the etiopathogenic mechanisms in some cases of severe obesity and binge eating, through short-circuiting the regulation of appetite in the hypothalamus. MC4R deficiency decreases the signals of anorexigenic pathways, such as CRH and TRH; and prevents the inhibition of orexigenic pathways, such as MSH and orexin. The result is increased food intake. The melanocortin agonist  $\alpha$  MSH is a peptide that is produced by the POMC, and is an agonist of MC4R. On the other hand, leptin reduces food intake through stimulation of the expression of POMC and the production of MSH, while inhibiting MC4R antagonists such as the agouti-related protein.

The abnormal molecular physiology demonstrated in MC4R deficient patients constitutes an important discovery of a missing link between genes and behavior. However, there is a lot more to be uncovered before we fully understand satiety in individuals with MC4R gene mutations, as well as in other obesity syndromes, and in normal individuals.<sup>2</sup>

Fima Lifshitz, MD

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2. Gotoda T. *N Eng J Med* 2003;349:606-609.



## Hypogonadism and Pubertal Development in Prader-Willi Syndrome

Genital abnormalities are common in Prader-Willi Syndrome (PWS) and are one of the eight major clinical criteria for diagnosis. Previous reports of the type and frequencies of these abnormalities were not necessarily from individuals with genetically confirmed PWS. Crino and associates report data from patients evaluated by the Genetic Obesity Study Group of the Italian Society of Pediatric Endocrinology and Diabetology. Eighty-four patients (42 males), mean age  $15.8 \pm 8.2$  years were studied. Sixty-three percent were over 14-years-old. All satisfied the Holm and Cassidy clinical criteria for the diagnosis of PWS and the methylation test was positive in all subjects. Microdeletion of chromosome 15(15q12-13) was demonstrated in 66%, while uniparental disomy or an imprinting defect was suspected in the others.

All males showed cryptorchidism (86% bilateral). Small testes and scrotal hypoplasia were observed in 76% and 69%, respectively. Micropenis was seen in 36%. Twenty-two of 29 males had spontaneous onset of puberty at  $14.0 \pm 3.2$  years but it was incomplete in all cases. Specifically, pubertal changes past Tanner 2-3 genital stages were rarely observed.

In females there was hypoplasia of the labia minora and/or of the clitoris in 71% and 69% of cases. Thirty-four of 39 females had spontaneous onset of puberty at  $12.6 \pm 2.7$  years, with very slow progression. Menarche occurred at a mean age of  $17.3 \pm 5.2$  years in 44% of cases over 14 years of age. Primary amenorrhea was diagnosed in 56%. Menstrual cycles were seldom regular and secondary amenorrhea occurred in 33% who had spontaneous menarche. Of note, premature

pubarche occurred in 12 subjects (6 males) and true precocious puberty in 3. It is suggested that premature pubarche might have been related to obesity. Genital and pubertal abnormalities were evenly distributed among subjects with microdeletion and UPD-imprinting defects. Treatment of various types for hypogonadism was discussed, including the use of dihydrotestosterone transdermally. However, no systematic trials on treatment with sex hormone treatment in adolescents or adults are available.

Crino A et al. *Eur J Pediatr* 2003;162:327-333.

**Editor's Comment:** *This paper provides interesting information concerning genital abnormalities in individuals diagnosed with PWS, confirmed with genetic testing. The large number of subjects in this descriptive study and the careful presentation of the findings should assist all who work with these patients and who must counsel them and their families in regard to expectations for pubertal development and fertility. It is interesting that sexual precocity was observed at a frequency that should be considered high in this group. This suggests that examination of the genitalia should be performed at each clinical visit. Whether or not current treatment with exogenous GH, which has been shown to significantly alter body composition in PWS, will affect pubertal development remains to be shown.*

William L. Clarke, MD

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## Growth and the Tyrosine Kinome

Tyrosine kinases (TKs) add phosphate moieties to tyrosine residues on proteins that typically serve as docking sites to recruit other molecules that bind and propagate signals. As such, they function as central regulators of signaling pathways that control transcription, cell cycle progression, differentiation, apoptosis and other processes that are highly relevant to growth of cells and tissues. Given this central position in regulation of growth, Bardelli et al raised the question: why have mutations in TK genes been found in only a small number of instances including certain human cancers? They speculated that mutations do exist, but have yet to be detected because the vast number of TK genes is only now becoming apparent as the human genome project unfolds. To test this idea, they took advantage of high-throughput sequencing and bioinformatics from the human genome project to search

for TK mutations in a select group of cancers, colorectal cancers.

A recent analysis organized the protein kinase complement of the human genome (the "kinome") into a dendrogram containing nine broad groups or branches of genes. Bardelli et al selected one major branch, which contained three groups including 90 TK genes, 43 TK-like genes and 5 receptor guanylate cyclase genes. Mutation analysis of 813 exons from the genomic database carried out on DNA from 35 colorectal cancer cell lines yielded 14 mutations. Further analysis of DNA from 147 tumors identified 46 novel mutations in 14 genes. All of the mutations were somatic in nature based on comparison of DNA from tumor to matched normal tissues.

The authors suggested that mutations found in seven genes, which were detected in more than one tumor,

were functional rather than coincidental. Based on the specific locations of the mutations, they further suggested that many of the mutations were activating in nature, i.e., they resided in key regions of the TK, such as the autoinhibitory activation loop. The authors concluded that at least 30% of colorectal cancers contain at least one mutation in the tyrosine kinome. They emphasized that an important reason to study TK genes is that they provide attractive targets for therapeutic intervention for growth disorders, noting the convincing success of targeting BCR-ABL tyrosine kinase in leukemia (Druker BJ. *Cancer Cell* 2002;1:31).

Bardelli A et al. Mutation Analysis of the Tyrosine Kinome in Colorectal Cancers. *Science* 2003;300:949.

**Editor's Comment:** While this paper specifically

*addresses cancer, it does not take too much imagination to see its potential relevance to growth of other tissues, such as the skeleton. Indeed, achondroplasia is due to activating mutations of the FGFR3 tyrosine kinase. Given the scope of regulation necessary to orchestrate and coordinate events in a growing bone, it seems highly probable that there are other members, perhaps many, of the tyrosine kinome involved. Accordingly, mutations of these as of yet undefined TKs may underlie disorders of skeletal growth. Considering the remarkable success of Gleevec in treating chronic myelogenous leukemia by inhibiting the BCR-ABL TK, it is not inconceivable to dream of using pharmacologic manipulation of growth-plate TKs to therapeutically manage certain bone growth disturbances in the future.*

William A. Horton, MD

## What do Craniosynostosis and Kallmann Syndrome Have in Common? *FGFR1*

Kallman syndrome is characterized by loss of the sense of smell, anosmia and hypogonadotropic hypogonadism. The anosmia results from absence or hypoplasia of the olfactory bulbs and tracts. The hypogonadism is due to a deficiency of GnRH, probably the result of failure of GnRH-synthesizing neurons to migrate from the olfactory epithelium to the forebrain along the olfactory nerve pathway. Kallmann syndrome occurs mainly in males and most often is inherited in an X-linked recessive fashion; the gene responsible for this form has been identified, *KAL1*. However, there are instances, such as failure to detect a *KAL1* mutation, that suggest an autosomal form of Kallmann syndrome.

Through segregation analysis of polymorphic markers and FISH chromosomal analysis, Dodé et al identified two *de novo* deletions of about 11 Mb at chromosome 8p11.2-p12 in two individuals affected by different contiguous gene syndromes that included Kallmann syndrome. The overlapping region of about 540 kb contained three genes, one of which, *FGFR1* (fibroblast growth factor receptor 1) was considered a strong candidate for causing Kallmann syndrome because of its known interaction with the *KAL1* gene product, anosmin-1. Southern blot analysis of 43 individuals with familial or sporadic Kallmann syndrome failed to detect additional deletions of *FGFR1*. However, sequencing of *FGFR1* in 129 unrelated patients with Kallmann syndrome revealed heterozygous mutations in four familial and eight sporadic cases. The mutations, including nonsense, frameshift and splice-site mutations, predicted loss of *FGFR1* function.

These observations suggest that Kallmann syndrome can result from haploinsufficiency or reduced dosage for *FGFR1*. The authors point out that anosmin-1 binds

to heparin sulfate proteoglycans which are required for FGF ligands to bind to FGF receptors and that *KAL1* and *FGFR1* are expressed in many of the same areas in the embryo including the region of olfactory bulb development. They offer a possible explanation for the higher prevalence of Kallmann syndrome in males even in families with autosomal inheritance which is based on the assumption that the local concentration of anosmin-1 is important to FGF signaling, and the observation that *KAL1* partially escapes X-inactivation. Accordingly, females with two *KAL1* alleles synthesize higher amounts of anosmin-1 than do males with a single *KAL1* allele. The authors propose that this may be enough in some cases to maintain FGF signaling above a critical threshold with regard to *FGFR1* signaling in the context of olfactory bulb and tract development.

Dodé C, et al. Loss-of-function Mutations in *FGFR1* Cause Autosomal Dominant Kallmann Syndrome. *Nat Genet* 2003;33:1-3.

**First Editor's Comment:** *FGFR1 joins a small group of genes for which both gain and loss of function mutations are known and associated with disease. It is not surprising that gain and loss of function mutations lead to quite different clinical consequences. Gain of FGFR1 function causes craniosynostosis, especially Pfeiffer syndrome, while loss of FGFR1 function results in Kallmann syndrome. Thus, these two syndromes are technically allelic disorders. One wonders how common this phenomenon actually is. Indeed, those of us with interest in FGFR3 have pondered if some individuals with tall stature have loss of function mutations of this gene in contrast to the gain of FGFR3 mutations that cause achondroplasia.*

The paper also illustrates the importance of gene dosage. In some instances, the precise dosage of a gene or its product does not seem to matter so much. Examples include, metabolic disorders in which half the normal amount of enzyme is more than enough to prevent disease and mutations of structural proteins, where inclusion of variable amounts of abnormal gene product can disrupt the formation of multimeric molecules containing the products of both mutant and normal alleles. When mutations involve regulation, such as mutations that affect signaling or formation of transcription factor complexes, small differences may have large effects on the outcome of the regulated events, especially if they involve thresholds as proposed for FGFR1 signaling in this report.

William A. Horton, MD

**Second Editor's Comment:** The authors have identified a second gene involved in the pathogenesis of Kallmann syndrome. The large number of subjects with Kallmann syndrome (N=116) in this study in whom mutations in neither KAL1 or FGFR1 were found indicates that there are (many) more genetic mutation which lead to this disorder. Search for involved genes might be directed toward those that encode products known to be important in neural cell migration and upon the intracellular proteins that are phosphorylated and the downstream genes whose transcription is regulated by FGFR1. It is interesting (curious?) that gain-of-function mutations of FGFR1 are associated with the Pfeiffer syndrome of craniosynostosis, but that inactivating mutations of this gene have not been linked to delayed closure of cranial sutures.

Allen W. Root, MD

## Clinical, Autoimmune, and HLA Characteristics of Children Diagnosed With Type 1 Diabetes Before 5 Years of Age

Little is known about auxologic, autoimmune, and HLA characteristics specific to children with early-onset diabetes (EOD). In this paper 40 children with a mean CA of 2.6 years who developed diabetes mellitus before 5 years of age were studied. These patients were compared with a matched subgroup of children of mean age of 9.9 years, therefore, with later onset diabetes mellitus (LOD). Auxologic data and antibody radioimmunoassay data from medical records were retrospectively analyzed. HLA diabetes related class II alleles were typed and evaluated for comparison between "whites" and "Hispanics". The frequencies of glutamic acid decarboxylase (GAD) and islet cell antibodies (ICA) were significantly lower in the EOD group than in the group developing diabetes at an older age. No significant differences were detectable for insulin auto-antibodies (IAA), thyroid peroxidase, and thyroglobulin antibodies. None of the patients of the EOD group developed hypothyroidism, whereas 20% of the

LOD patients did. There was a negative correlation between GAD antibodies and the predisposing haplotype DR3/DQ2. None of the EOD patients had either of the protective alleles (DRB1\*1501 or DQB1\*0602) for diabetes. There were significant differences in the frequencies of some diabetes related HLA alleles between EOD patients and a large non-age stratified type 1 diabetes group. The pertinent clinical information, frequency of autosomal markers and HLA data among ethnic groups are below (Tables 1-3). The authors concluded that children with EOD have different diabetes related autoimmune and genetic characteristics from those diagnosed later on in life.

Hathout EH et al. *Pediatrics* 2003;111: 860-863.

**Editor's Comment:** Very young children with diabetes mellitus are known to have a more severe course than those diagnosed later in life. The difficulties in the control

Table 1  
Clinical Information on Study Children With Type 1 Diabetes

	Early-Onset Group (Diagnosis Age <5 Years)	Late-Onset Group (Diagnosis Age >5 Years)	P Value
Concomitant illness at diagnosis	72.73%	31.80%	<.01
Documented honeymoon period	16.70%	42.10%	.24
DKA at diagnosis	80.80%	36.40%	<.01
Hemoglobin A1C at diagnosis	10.33 ± 2.20	11.27 ± 2.57	.21
No. of ICU days at diagnosis	1.82 ± 2.09	.53 ± .70	.07

DKA indicates diabetic ketoacidosis; ICU, intensive care unit.

Table 2  
Frequency of Autoimmune Markers in Study Children With Type 1 Diabetes

Marker	Early-Onset Group (Diagnosis Age <5 Years; %)	Late-Onset Group (Diagnosis Age >5 Years; %)	P Value
TpoA	6	9	1.00
TGA	9	11	1.00
IAA	50	65	.43
GAD	32	77	<.01
ICA	29	68	<.01

TpoA indicates thyroid peroxidase antibody; TGA, thyroglobulin antibody

Table 3  
HLA Data in the 2 Major Ethnic Subgroups of Study Children With Onset of Type 1 Diabetes Before 5 Years of Age

HLA Allele(s)	Percentage of Whites With Allele(s) (%)	Percentage of Hispanics With Allele(s) (%)
DRB1*0401-DQA1*03-DQB1*0302	70.6	.0
DRB1*0402-DQA1*03-DQB1*0302	.0	92.9
DRB1 0401	35.3	.0
DRB1 0405	.0	21.4

Tables 1-3 are adapted from Hathout EH et al. *Pediatrics* 2003;111: 860-863.

of the disease among the younger patients may account for more frequent and more severe complications of the disease occurring earlier in life. However, the data in this paper are suggestive that there are autoimmune and genetic differences among type 1 diabetic patients according to age (early vs late onset), and these may account for the differences in the control and the outcome of the disease. Chromosomal abnormalities (parental isodisomy of chromosome 6) also have been described among patients with the transient form of

neonatal diabetes.<sup>1</sup> Studies like these suggest that EOD probably is not classic type 1 diabetes mellitus, and thus may require unique approaches for prevention and therapy.

Fima Lifshitz, MD

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## The Thyrotropin Receptor Autoantigen in Graves Disease is the Culprit as well as the Victim

The thyrotropin (TSH) receptor (TSHR) is the only 7-transmembrane G-protein coupled receptor (GPCR) for glycosylated hormones that undergoes cleavage after its primary formation; the amino terminal extracellular domain is cleaved at/near amino acid 289 (*subunit A*) leaving a short residual extracellular amino acid sequence, the 7 transmembrane domains and extracellular and intracellular connecting loops, and the intracellular carboxyl terminal domain (*subunit B*). Subunit A then circulates and can serve as an immunogen. The role of *subunit A* of the TSHR in the pathogenesis of autoimmune hyperthyroidism and the development of TSHR stimulating immunoglobulin (TSIg) was examined by the present investigators. They constructed within adenovirus cDNA transcripts of the

amino terminal 289 amino acid sequence (*subunit A*), the wild-type (wt) *TSHR* from which amino acids 317-366 had been removed rendering the truncated TSHR resistant to cleavage, and the intact wt *TSHR*.

Adenoviruses expressing different forms of the TSHR were then administered to female mice who subsequently developed abnormalities of thyroid function and antibodies of variable biologic activity in response to these proteins. In animals receiving TSHR 1-289, clinical, biochemical, and thyroid histologic evidence (thyromegaly, hyperthyroxinemia, and follicular hyperplasia) of thyrotoxicosis developed. These animals also developed TSIg (assessed by increase in cyclic AMP formation in CHO cells expressing TSHR). In only a few mice receiving cleavage resistant TSHR or wt

TSHR were serum thyroxine levels increased and thyroid follicular hyperplasia present. In contrast, all mice, regardless of the form of TSHR received, developed high but approximately equal titers of immunoglobulins that bound to TSHR or inhibited radiolabeled TSH from binding to TSHR. TSIg did not develop in animals receiving cleavage resistant TSHR, but did appear in 30% of those injected with wt TSHR. Higher titers of thyroid blocking antibodies (assessed by their effect on TSH mediated increase in cyclic AMP generation in CHO cells expressing TSHR) were present in mice receiving the cleavage resistant form of the TSHR than in those receiving TSHR 1-289. The authors conclude that it is the extracellular segment of the TSH receptor that is ordinarily shed that serves as the immunogen for the development of TSIg in this experimental model of hyperthyroidism (and by analogy in patients with Graves disease).

Chen C-R, et al. *J Clin Invest* 2003;111:1897-1904.

**First Editor's Comment:** *This extremely interesting manuscript provides significant insight into the pathogenesis not only of thyrotoxicosis, but of autoimmune thyroid disease itself. Thus, when the ectodomain of the TSHR is cleaved, it provokes the production of TSHR stimulating immunoglobulins (as well as low titers blocking antibodies) in genetically susceptible individuals. In other at-risk patients, the intact TSHR (or perhaps other sequences or epitopes of the TSHR) or TSH itself, serves as the immunogen for development of TSHR function-blocking antibodies. Other components of the thyroid gland serve as immunogens for antibodies that are injurious to the thyroid cell. A human monoclonal antibody has been recently isolated from a patient with Graves disease, but the epitope of the TSHR to which it is directed has not been identified to date.<sup>1,2</sup> It would be of interest if it were directed to the ectodomain of the human TSHR.*

*While a number of tyrosine kinase receptors shed their extracellular domains (growth hormone binding protein, prolactin binding protein, many cytokines), it is apparently unusual for G-protein coupled receptors to do so. This is an area that merits further examination.*

Allen W. Root, MD

**Second Editor's Comment:** *In Dr. Root's editorial comment, he refers to the recent identification of a monoclonal antibody that stimulates the TSH receptor in the thyroid cell to release thyroxin.<sup>1,2</sup> This also was no small accomplishment in helping us understand Graves' disease more fully. As pointed out by Dayan, who states:*

*"So, is the final proof of the existence of thyroid-*

*stimulating immunoglobulin after a journey of 47 years of anything more than academic interest? Almost certainly the answer is "yes." First, this finding might lead to a new generation of assays for thyroid-stimulating immunoglobulin in which competition for labeled TSH is replaced by competition for specific monoclonal antibodies. If a sensitive assay can be developed, it should have close to 100% specificity for Graves' disease and replace all other antibody tests, such as antithyroid peroxidase and antithyroglobulin, in this condition. Second, it should finally allow us to understand how such antibodies, even in the monomeric Fab form, can activate the TSH receptor. Such understanding of the biology of glycoprotein-hormone receptors may lead to new small-molecule agonists and antagonists not only for thyroid disease but also for hypogonadism and infertility (via the closely related receptors for luteinising and follicle-stimulating hormones). And it may prove possible to clone a potent human TSH-receptor-blocking antibody which might provide a rapid initial treatment for thyrotoxicosis. Third, the finding may lead to a better understanding of the pathogenesis of Graves' disease. How is it that the spontaneous development of such agonist antibodies, unique in autoimmune diseases, occurs so frequently (almost 1 in 100 of the population)? Does the agonist activity itself, once it appears, promote autoimmunity in a positive feedback loop? Most intriguingly, cloning of agonist TSH-receptor autoantibodies might reveal antibodies that contribute to thyroid eye-disease, the most mysterious manifestation of Graves' disease, and perhaps lead to inhibitors for these antibodies. And finally, agonist antibodies may prove a useful therapeutic agent in their own right, such as to enhance iodine-131 uptake in thyroid cancers. Many of the holy grails of biological science, from the structure of DNA to the nature of the T-cell antigen receptor, have been found. Thankfully, once in hand, they change into pointers to the many more waiting to be discovered."*

*The findings of Chen and those of Sanders et al are linked closely and the almost simultaneous reporting of these factors which are linked should permit a logarithmic advance in our understanding of how antibodies and receptor structure and function can relate and, consequently, provide better therapy of immunological diseases.*

Robert M. Blizzard, MD

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