Two Unique Androgen Receptor Mutations Causing Complete Androgen Insensitivity

Jeromy Miller

Writer's comment: When I first considered the subject of CAIS, I was only interested in its genotypic aspects: could the condition be nailed down to a point mutation? how many? how was it inherited? what's the clear genetic contribution to the disorder? When I finally unearthed research articles that described how the CAIS genetics fell into place, I widened my focus and took in CAIS as a human condition. There were faces behind all subjects involved. I looked at my research through rose-colored glasses, hoping that Subjects one and two were both there because their families needed to know how to help them get through life with the least amount of discomfort. I hoped they were there to get freely-offered help, and not just to get a nibble off their observer's research grant. I looked at the scientists involved as true investigators who were able to both educate and aid CAIS patients, and not as just another batch of college brains aiming for clean-typed notoriety. I'm happy to have explored CAIS, written this paper, and experienced, first hand, how scientists get wrapped-up in genetic/physiologic/anatomic studies—they really can work to overwhelm your senses.

— Jeromy Miller

Instructor's comment: Jeromy's paper was written for my NPB 131 "Physiological Genomics" class. My instructions are that the paper should focus on a problem of human physiology where genomics has been used to understand the underlying biology. Students pick a topic that interests them and tell a story of increasing understanding and complexity, such as might be done in a textbook. My primary goal for this writing assignment is for students to learn how to search and read peer-reviewed scientific literature. Most scientists use PubMed and online PDF files to search and read the scientific literature. Thus, I help students to learn that that PubMed is a better search engine for the scientific literature than Google. Jeromy's paper is an excellent example from the class. He chose the topic, found the papers, wrote accurately and clearly, and asks excellent questions in his conclusions.

—Craig Warden, Neurobiology, Physiology, and Behavior

Abstract: Several molecular studies since the early 1950s have concluded that complete androgen insensitivity syndrome (CAIS) is an X-linked recessive disease where the affected are born with a 46 XY karyotype but develop overtly female external features by adulthood. People with CAIS most often are devoid of internal female reproductive structures leaving them sterile. The affected maintain normal male levels of androgen hormones that promote anatomical maleness but said hormones are invalidated due to androgen receptor (AR) and / or 5 alpha-reductase type 2 (SRD5A2) gene mutations. This study focuses on the similarities and differences between two cases of complete androgen insensitivity syndrome and their particular rare causal agents: an androgen receptor mutation as a part of a multiple congenital anomaly syndrome and an androgen receptor mutation caused by an X chromosome inversion.

Keywords: complete androgen insensitivity syndrome, androgen receptor, congenital anomaly syndrome, X chromosome inversion.

Introduction

Androgens regulate many developmental and homeostatic processes in vertebrates. These processes range from the production of axillary hair and male vocal tones, in the development of a normal male phenotype, to spermatogenesis in normally functioning adult males[3]. In mammals the major circulating androgens are testosterone and 5 alpha dihydrotestosterone (DHT)—two steroid hormones. They are considered major androgens because they are required for the entire spectrum of androgen-related processes[3]. These androgens must associate with properly functioning androgen receptor (AR) proteins for growth and development to be adequately affected by them. AR proteins are encoded on the human X chromosome and each contains specific binding domains for their specific androgen ligand(s). A wide range of mutations that alter androgen receptor proteins gives rise to many degrees of androgen insensitivity. Individuals with completely non-functional androgen receptors are said to have complete androgen insensitivity syndrome[3].

According to the literature on CAIS, the first person to report a series of individuals with androgen insensitivity was John Morris in 1953. Morris described "Morris' Syndrome" or "Androgen-Insensitivity Syndrome" to be present in individuals who, despite having inter-

nalized bilateral testes, expressed a clearly female phenotype[1]. The characteristics of individuals with complete androgen insensitivity syndrome are external female genitalia with a short, stunted vagina that does not connect to a cervix or uterus—sometimes called a "blind ending vagina"[1;] lack of male reproductive structures such as an epididymes, vas deferens, seminal vesicles, or a prostate gland (known collectively as mullerian derivatives); and the absence of pubic hair. Morris also noted that, at puberty, the affected individuals had high levels of testosterone and lutenizing hormones (concerning hormone levels, in the reference materials the age group and developmental level of Morris's original subjects was unclear in his first analyses concerning androgen insensitivity); these findings further backed his idea of complete androgen insensitivity: androgens were being produced but were causing no phenotypic maleness[1].

More current analyses have shown androgen receptors to be ligand-dependent transcription factors[3]. Missing or defective receptors prevent the normal development of external as well as internal male structures in individuals with 46 XY genotypes[5]. The androgen receptor is a 110-114 kilo Dalton protein encoded by a single copy gene located at Xq11-12 (on the X chromosome). The AR gene has a total of eight coding exons with organization similar to genes that code for steroid receptors. In normal human populations the first exon of the AR gene contains a highly polymorphic short tandem repeat (STR). The sequence is an in-frame CAG that repeats 11 to 31 times[1]. A wide range of AR gene mutations has been documented; they include: frame-shift mutations that arose from nucleotide deletions or insertions, point mutations that caused premature stop codons or amino acid substitutions, and partial or complete gene deletions. These mutations can potentially affect any of the four functional domains of the AR protein: (exon 1) the N-terminal domain that regulates transcription, (exons 2 and 3) the DNA binding domain, (exon 4) the hinge region that contains nuclear localization signals, and (the three prime end of exon 4 along with exons 5 through 8) the ligand binding domain[2]. Mutant genotypes are expressed in individuals with only a single active X chromosome, making CAIS classified as an X-linked recessive disease[1].

The phenotypes of 46 XY individuals range from an outwardly normal female phenotype (in the case of CAIS), through myriad altered female phenotypes that can include ones with cliteromegaly or reduced vaginal length due to fusion of the labia minora, all the way to outwardly male phenotypes with ambiguous genitalia or infertility (in

PAIS, partial androgen insensitivity syndromes)[2]. According to the most up-to-date information gathered for this analysis, CAIS is still considered a relatively rare disorder; occurring once in every 13,158 to 40,800 live births worldwide[4].

The Subjects

Subject one was 3 months old, the second child of a non-related couple in their 20's. The subject's weight was 5.6 kg, length 57.7 cm at time of testing, having normal female external genitalia, no palpable perineal and / or inguinal masses. Physical findings were normal, overall[6]. During a surgery to repair an inguinal hernia noticed just after birth, a biopsy was taken of suspicious tissue, tissue later identified as being testicular in nature. Normal pituitary function was also noted as normal; testosterone, dihydrotestosterone (DHT), follicle stimulating hormone (FSH), and luteinizing hormone (LH) levels were obtained via laboratory studies. After an abdominal ultrasound, it was noted that Subject one lacked a uterus but had "uniform echogenic ovoid masses in the inguinal canals, consistent with testes[6]" and no Mullerian derivatives. After CAIS was diagnosed, the subject, now 3.5 months old, underwent a bilateral gonadectomy due to heightened concern about gonadal malignancy later in life. The subject recovered from surgery without incident[6].

Later it was discovered that one of Subject one's maternal aunts was diagnosed with a general androgen insensitivity syndrome (AIS) at the age of five. Coincidentally, surgeons had discovered internalized testes while performing corrective surgery on the aunt's juvenile inguinal hernia. This aunt was found to have a 46 XY karyotype and underwent a bilateral gonadectomy at the age of six[6]. At age 16 the aunt had primary amenorrhea and a height of 180 cm. Cytogenetic analysis of Subject one's mother showed a 46 inverted-X, X karyotype. The mother was reported to have normal menarche that started at the age of 13 as well as the development of pubic hair, but completely lacked axillary hair. A younger aunt of Subject one was investigated and found to be phenotypically and cytogenetically normal[6].

Subject two was the first daughter of an unrelated Egyptian couple. Directly after birth this subject was found to be a normal and healthy female. The only obvious abnormality was the subject's larger than average ocular globes. At two weeks of age the subject was diagnosed with bilateral congenital glaucoma and given immediate corrective

surgery[2]. Investigation into the subject's family history uncovered that remarkably large ocular globes ran in the family—on the father's side. Some family members were diagnosed with glaucoma, but none of the nine familial cases of enlarged globes presented in early infancy. At two weeks of age the subject was also diagnosed with congenital hypertrophic pyloric stenosis[2]. This was discovered after investigators observed the subject's frequent and increasingly severe bouts of vomiting and performed ultrasound evaluations of the subject's abdomen and pelvis. Subject two had a normal weight (3.75 kg) and length (50.5 cm) at the time of testing, expressing female external genitalia. These included the presence of separate urethral and vaginal openings, a normal clitoris, labia majora and labia minora (no Mullerian derivatives were observed). More out of the ordinary was the fact that Subject two had bilateral inguinal swellings found to be internal testicles[2].

Questioning the subject's family and investigating their medical records revealed no familial cases of infertility or sexual ambiguity. Subject two was karyotyped and identified as a 46 XY individual[2]. Hormonal evaluation that included an estimation of plasma testosterone and DHT levels fell into normal range. The subject's development was tracked for the next 18 months as the subject passed standard milestones for normal development and was deemed devoid of any mental retardation[2].

Materials and Methods

For all cytogenetic studies concerning Subject one and her family, "metaphase chromosomes were obtained from phytohemagglutinin (PHA) stimulated peripheral blood lymphocytes, and analyzed by G-banding using standard techniques"[6].

Androgen receptor genes were located on Subject one and her tested family members via fluorescent in situ hybridization (FISH). This technique used a single copy 70.6 kb BAC probe (RP11-479J1) that maps to the Xq11.1-Xq13.3 of the X chromosome and contains a 90kb segment of the androgen receptor locus. A standard kit from Puregene, Gentra Systems, (Minneapolis, MN) was used to isolate DNA. Nick-translation incorporating digoxigenin-11-dUTP was used to label the BAC probe. A common spectrum orange labeled SRY probe was mixed with spectrum green labeled CEP X probe and used as a control. The two aforementioned techniques were applied to 20 metaphase spreads and FISH was performed as instructed by the manufacturer[6].

For Subject two, DNA was extracted using a technique analogous to the one performed on Subject one[2]. The eight exons of the AR gene as well as the five exons of the SRD5A2 gene were amplified via polymerize chain reaction (PCR). Primers specific to the genes were commercially obtained. Agarose gel electrophoresis was used to verify the length of PCR products before use[2]. Said products were also purified using "Qiaquick PCR columns and sequenced using the ABI Prisim Dye terminator sequencing kit and the ABI 301 apparatus from Applied Biosystems, Courtaboeuf, France"[2].

Results

In *Subject one*, all 20 of the metaphase cells analyzed carried the abnormal karyotype: 46, inverted-X (q11.2q27),X. FISH analysis uncovered two digoxigenin labeled BAC probe signals at either end of the subject's X chromosome, flanking the region of inversion. This indicated that one of the inversion breakpoints was within the flanked region. FISH analysis of the subject's chromosomally normal younger aunt uncovered one digoxigenin labeled BAC probe signal as expected[6].

Subject two: After being stimulated with human chorionic gonadotropins (HCG) subject two's testosterone levels shot up from 0.1 to 14 ng/mL (this is considered a good response) and the subject's ratio of testosterone to DHT greatly increased, exceeded 100 (a normal value would have been around 14)[2]. Sequence analysis of the subject's SRD5A2 genes five exons showed no mutations whatsoever. The AR gene on the other hand had a single C-to-T mutation on exon 6. This substitution caused a phenylalanine residue to be replaced with a leucine residue at position 804 on the X chromosome. The subject's mother exhibited a normal sequence in exon 6 of the AR gene, so the aforementioned mutation was considered de novo[2].

Discussion

If the phenotypes of Subjects one and two are compared to the parameters for detection of complete androgen insensitivity syndrome set by John Morris[1], it's clear that both subjects have CAIS. Both subjects started off life appearing to be normally developed newborn females. They had external female genitalia along with normal heights, weights, and testosterone levels. But, soon after birth, outward abnormalities in anatomy and physiology (an inguinal hernia present at birth and

enlarged ocular globes with frequent vomiting) prompted separate investigations that unearthed further commonalities between subjects[2,6]. When ultrasound technology was employed by investigators, both subjects were found to have a typical CAIS internal anatomy. Both lacked Mullerian derivatives, a uterus, and a cervix but had bilateral testes in their inguinal canals. From this point genetic analysis had to be undertaken to definitively diagnose the subjects with CAIS[2,6].

Molecular studies showed that both subjects had a 46 XY karyotype with a mutation in an area of their X chromosome that coded for their respective androgen receptor proteins. With this information added to previously gained data a clear diagnosis of CAIS could be made for both subjects[2,6]. What is more interesting, though, is the information obtained concerning the glaring differences in genetic mutation and syndrome inheritance that resulted in a common CAIS phenotype.

When Subject one was diagnosed with CAIS, investigators soon found an X-linked pattern of inheritance common in CAIS patients. The subject's older aunt was found to have gone through the same hernia surgery at a young age, had the same CAIS internal and external anatomy, and underwent the same type of gonadectomy as her niece[6]. Other female family members were not affected by the syndrome, but genetic analysis revealed that the subject's mother carried her daughter's specific mutation but didn't express the mutant phenotype herself[6]. This occurred because the mother is a genetic as well as a phenotypic female having a second wildtype X chromosome to negate the effects of the X-linked recessive disorder. Overall this mutation followed its expected X-linked course of inheritance.

When Subject two was diagnosed with CAIS, no familial history of sexual ambiguity or intersex conditions was found, only an ocular size disorder inherited from the subject's father's side of the family. Investigators sequenced the subject's mother's androgen receptor gene as well as her SRD5A2 (another previously mentioned gene linked to CAIS) and found no mutation in either. The subject was said to have a de novo mutation, one that's completely new and not inherited[2].

The two subjects had two very different types of AR gene mutations. Subject one had a X chromosome inversion where the q domain split in the region of q11.2-12 and at q27. This DNA section rotated 180 degrees relative to the X chromosome it came from, then rejoined the chromosome segment. This produced two fluorescent signals upon

FISH analysis indicating the inversion mutation split the (well-documented) AR gene region of the X chromosome in two pieces, making it non-functional[6]. The loss of function in Subject two's AR gene came from a single mutation that caused a phenylalanine residue to be replaced with a leucine residue at position 804 on the X chromosome (the aforementioned F804L mutation). The F804 region is reportedly part of a hydrophobic cluster that holds together four helices that make up the AR protein. Researchers speculate that a mutation in F804 could cause the ligand binding domain of the AR to misfold, causing non-functionality[2]. These two specific mutations most likely caused complete androgen insensitivity in their respective subjects and were both considered novel mutations by the researchers who identified them.

Researchers studying Subject one drew up a pedigree and traced the subject's mutation rather easily[6]. Researchers studying Subject two had a harder time explaining where their novel point mutation came from. What the researchers could trace was the subject's paternally inherited case of glaucoma. But since a few of the subject's family members had glaucoma but not CAIS, the researchers could not say definitively that the subject's ocular condition was linked to her androgen insensitivity[2]. Instead the researchers theorized a link between the subject's congenital hypertrophic pyloric stenosis (CHPS) and a disruption of androgenic action. They say that a study by Hernanz-Schulman suggests that a well-know antiandrogen, cyproterone acetate, induces adenomas in the pyloric antrum in over 85% of mice given the antiandrogen. Even though this hints at a possible connection, a lot more evidence is needed to directly link CHPS to CAIS[2]. Overall Subject two was considered to have a multiple congenital anomaly syndrome since no previously described syndrome ensconced all three conditions affecting this individual.

A loose end concerning both studies: In Subject two a very high ratio of testosterone to DHT was measured and noted by investigators. It was also noted that this high ratio has been reported in other CAIS patients and shown to most likely be associated with a secondary mutation in the 5 alpha reductase gene, producing reduced levels of the reductase needed to reduce testosterone to DHT. Subject two's investigators sequenced her SRD5A2 gene and found no evidence of mutation, so they had to leave the high ratio of testosterone to DHT as an unexplained phenomenon. It was unexplained but at least it was explored. The researchers who studied Subject one didn't even explore

the possibility of an SRD5A2 gene mutation. They measured normal ratios of testosterone to DHT in their infant subject but didn't follow up with measurements of hormone levels in the subject at a later date—when the subject's androgens might be produced in greater quantities. Since SRD5A2 gene mutations have been reported in past cases of CAIS, their presence should be explored in conjunction with any supposed novel AR mutation before said novel mutation is reported as a specific causative agent for CAIS.

Questions arise when looking at these two different studies: why was Subject one given a gonadectomy, as a preventative measure, while the surgery wasn't mentioned as part of treatment for Subject two? (Was Subject two too fragile, due to having a multiple anomaly syndrome, to undergo preventative surgeries?) Are gonadectomies really necessary for CAIS or PAIS patients in general? What is the rate of gonadal malignancy in CAIS and PAIS patients who do not undergo surgery to remove their internalized bilateral testies? These are areas of study that could further inform the aforementioned test subjects' condition as well as CAIS and PAIS syndromes in general, therefore warranting additional investigation.

Conclusion

In 1988 the androgen receptor gene was cloned after being mapped to the q11.2-q12 region of the X chromosome. For over a decade interested researchers have known that the receptor is 90 kb long with 8 exons with 2,750 base pairs that code for a 919 amino acid long protein[6]. Research concerning AR gene mutations has been done over and over again, to the point that over 300 specific AR gene mutations have been identified and entered into Gottlieb's androgen receptor gene mutation database[6]. Various levels of androgen insensitivity have been identified, studied, and most often linked to single base mutations of the AR gene that lead to prematurely terminated AR proteins[6]. It's the aforementioned perplexing novel mutations and unexplained links between syndromes that keep a lot of researchers on the path toward a more complete understanding of androgen insensitivity syndromes. In doing research for this project, I was pleased to see studies being carried out that examine the sexual difficulties and gender association issues many CAIS patients confront while trying to fully understand their condition[4]. It's always important to take a step back from research and observe the big picture. The work done by the aforementioned scientists goes toward explaining sexual ambiguities that may cause severe

emotional and physical distress in the affected individuals. The therapy provided in these scientific studies suggests that CAIS individuals may have been affected mentally and physically although they never felt the need to question their ability to have children or why they developed differently than most.

References

1. Fogu, G., Bertini, V., Dessole, S., et. al. 2003. Identification of a mutant allele of the androgen receptor in a family with androgen insensitivity syndrome: detection of carriers and prenatal diagnosis. *Archives of Gynecology and Obstetrics* 269(1): 25–29.

2. Gad, YZ., Mazen, I., Lumbroso, S., et. al. 2003. A novel mutation of the androgen receptor (F804L) in an Egyptian newborn with complete androgen insensitivity associated with congenital glaucoma and hypertrophic pyloric stenosis. *Clin Genet* 63: 59–63.

3. McPhaul, MJ. 2002. Androgen receptor mutations and androgen insensitivity. *Molecular and Cellular Endocrinology*. 198 (1–2): 61–67.

4.Minto, CL., Liao, LM., Conway, GS., et. al. 2003. Sexual function in women with complete androgen insensitivity syndrome. *Fertility and Sterility* 80(1): 157–164.

5. Van, YH., Lin, JL., Huang, SF., et. al. 2003. Novel point mutations in complete androgen insensitivity syndrome with incomplete mullerian regression: two Taiwanese patients. *European Journal of Pediatrics* 162(11): 781–784.

6. Xu, W., Robert, C., Thornton, P., et. al. 2003. Complete androgen insensitivity syndrome due to X chromosome inversion: a clinical report. *American Journal of Medical Genetics*. 120A: 434–436.