Dihydrotestosterone induces minor transcriptional alterations in genital skin fibroblasts of children with and without androgen insensitivity

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Abstract. Endogenous and exogenous androgens induce masculinization of external genitalia through binding to the androgen receptor (AR). The target genes of androgens in external genitalia remain to be determined, although previous studies have shown that the apolipoprotein D gene (APOD) was significantly upregulated by dihydrotestosterone (DHT), the most potent androgen in humans. In the present study, we performed microarray analysis for genital skin fibroblasts obtained from four boys with buried penis (the control individuals) and a patient with partial androgen insensitivity syndrome (PAIS) due to a hypomorphic mutation in AR (the PAIS patient). We identified 24 transcripts that were upregulated or downregulated by DHT in all samples of control individuals and, to a lesser extent, in the sample of the PAIS patient. Differences between DHT-treated and -untreated samples were small; the results of 24 transcripts did not reach statistical significance. The 24 transcripts included CYP1B1, a gene possibly involved in the development of genital tubercle in mice, and APOD, as well as several genes that have been reported as androgen targets in prostate or other tissues. The results of this study indicate that androgen-mediated masculinization of external genitalia is unlikely to depend on massive transcriptional changes in specific AR target genes. Rather, minor transcriptional changes of several genes, and/or a complex molecular network may play a major role in penile development. Importantly, our data suggest the possible involvement of CYP1B1 in human genital development and confirm the clinical importance of APOD as a biomarker for AR function.

Key words: Apolipoprotein D, Androgen insensitivity syndrome, Androgen receptor, Gene expression, Microarray

During male embryogenesis and puberty, androgens induce masculinization of external genitalia via binding to the androgen receptor (AR) [1]. Upon the binding of androgens, such as testosterone and dihydrotestosterone (DHT, the most potent androgen in human), AR translocates from the cellular membrane to the nucleus [2]. In the nucleus, AR binds to androgen response elements (AREs) in the genome to regulate the expression of adjacent genes [3]. Since injection of exogenous androgens to children with micropenis often results in a significant increase in penile length [4], the androgen-AR signaling pathway appears to be preserved even in the prepubertal period when physiological masculinization does not progress. Mutations in AR lead to androgen insensitivity syndrome (AIS), a relatively common form of 46,XY disorders of sex development (DSD) [5]. Patients with AIS exhibit variably impaired masculinization of external genitalia, depending on residual activity of the mutant AR proteins [6]. Male-assigned patients with partial AIS (PAIS) are frequently treated with relatively high dose androgens to induce masculinization [7].

To date, analyses of transcript expression profiles in cultured genital skin fibroblasts with and without androgen treatment have been performed to determine the androgen target genes in external genitalia. Holterhus et al. reported that DHT treatment caused no significant transcriptional changes in samples of both unaffected
men and AIS patients [8]. Subsequently, Appari et al. documented that only three transcripts were significantly upregulated by DHT in fibroblasts of healthy men [9]. Of the three transcripts, only the apolipoprotein D gene (APOD) encodes a protein, while the others were a pseudogene and an uncharacterized transcript. DHT treatment did not affect expression levels of APOD in labia majora of patients with complete AIS [8], suggesting that APOD is the true target of the androgen-AR signaling pathway. However, the function of APOD in genital formation is unclear; it is possible that other androgen-target transcripts play a significant role in penile growth. In this regard, previous studies identified several androgen-responsive genes in prostate [10], other human tissues [11] and in the developing genital tubercle of mice [12-14], although involvement of these genes in human penile growth remains to be investigated. Here, we studied DHT-induced transcriptional alterations in genital skin fibroblasts obtained from boys with and without AR abnormality, and analyzed all transcripts including those with modest changes.

**Materials and Methods**

**Ethical approval**

This study was approved by the Institutional Review Board Committee of the National Research Institute for Child Health and Development and performed after obtaining written informed consent from the parents of participants.

**Subjects**

Genital skin fibroblasts were obtained from four boys with apparently normal AR activity (the control individuals) and one patient with DSD and a hemizygous p.Ala597Thr mutation in AR (the PAIS patient). The p.Ala597Thr mutation in AR is known to encode a mutant receptor whose residual activity is approximately 1/3 of the wildtype protein [15]. The four control individuals had buried penis, a hormone-independent genital malformation in which the penis of normal size is hidden due to abnormal attachment of the foreskin to the penile shaft [16]. These four boys had no endocrinological abnormalities or mutations in AR. They underwent corrective surgery for buried penis between 6 and 21 months of age. The PAIS patient exhibited hypospadias and underwent surgery at 18 months of age.

Fibroblasts were obtained from surgically removed foreskin. Fibroblasts of each individual were seeded in 10 cm dishes (5 × 10⁵ cells/dish) and grown in DMEM (Nacalai Tesque, Kyoto, Japan) containing 1 g/L glucose with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, USA), and penicillin and streptomycin. After 24 hr incubation, the cells were treated with DHT (final concentration of 1 nM, Sigma-Aldrich) and maintained for further 72 hr before harvesting. This DHT dose was determined based on a previous study [17]. As DHT-untreated control samples, we utilized methanol-treated cells.

**Microarray analysis**

Total RNAs were extracted from fibroblasts using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany). Gene expression profiles were analyzed using a catalog one-color microarray (SurePrint G3, Human Gene Expression 8 x 60 k v3.0, Agilent Technologies, Santa Clara, USA) according to the manufacturer’s instruction. The experiments were performed by Takara Bio (Shiga, Japan). Microarray data are accessible at NCBI’s Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) through GEO accession number GSE121712.

Microarray data were normalized using GeneSpring software (Agilent Technologies) with the 75th percentile shift algorithm. Transcripts with low expression levels or low signal quality were filtered out using the Agilent Feature Extraction Software. In this study, we sorted transcripts tagged with a “detected” flag in all control samples and one or two of the patient’s samples and yielded a raw signal intensity of over 100 in more than three of the control samples and one or two of the patient’s samples. Subsequently, we calculated the fold change (FC) of each transcript by dividing the normalized signal of the DHT-treated sample by that of the untreated sample. FCs were converted to the log2 scale.

We searched for putative androgen-regulated transcripts. The inclusion criteria were: (i) the mean [log2 FC] value of the four control samples was ≥0.3, (ii) three or four of the control samples showed a [log2 FC] value of ≥0.3, (iii) the [log2 FC] value of the sample of the PAIS patient was lower than the mean of the control samples, (iv) protein coding transcripts, and (v) all four control samples showed the same changes (upregulation or downregulation). The [log2 FC] cut-off value was determined based on a previous study [11]. Differences in transcript expression levels between the DHT-treated and -untreated control samples were examined statistically using Student’s t-test.

**Putative androgen-regulated transcripts**

We performed database search for putative androgen-regulated transcripts. We referred to previous reports in PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) to determine whether the identified transcripts have been linked to genital development or androgen action.

We searched for putative AREs either in intronic or exonic regions of the identified genes using previously
reported chromatin immunoprecipitation data [3]. We selected 15-mer sequences with less than three base mismatches with the consensus ARE sequence (AGAACAGNNNTGGTCT).

We also examined previous microarray data of Next-Bio (https://www.nextbio.com/) (Illumina, San Diego, USA) concerning gene expression profiles in genital skin fibroblasts of healthy men and AIS patients [18]. The original data of NextBio were obtained from NCBI Gene Expression Omnibus (accession number GSE3872, GSE6796 and GSE6797). Gene ontology analysis was performed using DAVID 6.8 (https://david.ncifcrf.gov/) [19, 20].

Results

Microarray analysis

Signals of 58,341 transcripts were obtained from the ten samples. After filtration of transcripts with low expression levels or low signal quality, we obtained 16,705 transcripts in eight control samples and 16,511 in two patient samples.

A total of 24 transcripts satisfied aforementioned inclusion criteria for the putative androgen-regulated transcripts. Of the 24 transcripts, 16 were upregulated by DHT with the mean log2 FC values of control individuals ranging from 0.30 to 0.74, whereas eight were downregulated with the mean log2 FC values of control individuals ranging from −0.30 to −0.99. Of these, APOD, KLAA1598, HSPB3, and SRMS were upregulated in the control samples but downregulated in the patient sample. Likewise, CNIH3 was downregulated in the control samples but slightly upregulated in the patient sample. None of these transcripts showed statistically significant differences between DHT-treated and -untreated samples.

Putative androgen-regulated transcripts

The 24 transcripts contained several genes that have previously been associated with genital development, sex hormone actions, and/or male reproduction (Table 1) [21-28]. In particular, APOD is known as an androgen-target gene in human genital fibroblasts, and Cyp1b1 has been implicated in genital development of mice.

Putative AREs were detected in 7 transcripts (Table 1). In particular, KLAA1598 and CNIH3 had multiple AREs in their flanking regions.

Eight of the 24 transcripts showed results consistent with those of previous microarray analyses on DHT-untreated genital skin fibroblasts; these transcripts were less strongly expressed in AIS patients than in unaffected individuals (Table 1).

Gene ontology analyses suggested that five transcripts, including APOD, CYP1B1, and HS6ST1, were potentially involved in angiogenesis (Table 2). In addition, several transcripts were associated with cell apoptosis or proliferation. RGCC appeared to be implicated in various biological processes.

Discussion

DHT treatment of genital skin fibroblasts of control individuals caused no statistically significant changes in the expression levels of any genes. These results are consistent with those of previous studies [8] and indicate that androgen-induced masculinization of external genitalia is not dependent on massive transcriptional changes of specific transcripts. The results can be explained by assuming that relatively minor changes of several transcripts contribute cooperatively to genital masculinization.

Indeed, microarray analysis identified 24 transcripts that were mildly upregulated or downregulated by DHT in all control samples and, to a lesser extent, in the sample of the PAIS patient.

The 24 transcripts included CYP1B1, which encodes a testosterone metabolizing enzyme [29]. Nishida et al. demonstrated that Cyp1b1 is strongly expressed in the mesenchyme of the proximal region of the genital tubercle in mice, where androgens exert their effects on tissue differentiation [13]. The authors also showed that the expression level of Cyp1b1 in the genital tubercle was significantly higher in male than in female mice. Notably, Cyp1b1 was initially identified as a target of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a teratogen known to cause reproductive toxicity in laboratory animals [30]. Previous studies suggested that Cyp1b1 is involved in the anti-estrogenic effects of TCDD in mice [31]. The results of our microarray analysis indicate that CYP1B1 may play a role in androgen-induced genital development in humans. Since Cyp1b1 is known to promote angiogenesis [32], CYP1B1 may control vascular development in genital tissues.

DHT treatment of control samples resulted in mild upregulation of APOD, which encodes a broadly expressed carrier protein [22]. APOD was predicted to be involved in angiogenesis and regulation of cell adhesion [33]. Lin et al. reported APOD as the most inducible gene after artificial arteriogenic insufficiency in rodent penis [34]. Our data support the previously proposed notion that APOD is the major target of androgens in genital skin fibroblasts [9]. Hornig et al. proposed that quantification of APOD mRNA in genital skin fibroblasts would be a useful marker to assess the function of mutant AR proteins of AIS patients [35]. Nevertheless, it is uncertain whether APOD plays a role in genital development. Thus, the functional significance of APOD in
| Gene symbol | Mean Log2 FC of control individuals | Log2 FC of the AIS patient | Putative AR-binding motif in the gene flanking region | Association with sex hormone$^a$ | Putative role in male reproduction$^a$ | Previous microarray data$^b$ |
|-------------|-----------------------------------|--------------------------|-----------------------------------------------------|--------------------------------|---------------------------------|--------------------------------|
| **Upregulated genes** | | | | | | |
| RGCC (RGC32) | 0.74 | 0.21 | none | regulated by androgens in human neural cells [11] | higher expression in unaffected males than AIS patients | |
| SPTA21 (SPE RGEN2) | 0.62 | 0.38 | none | possibly involved in spermatogenesis [26] | | |
| ADH1C | 0.53 | 0.03 | AGAAGACAGTTTCA | possibly regulated by estrogens in pig prostate [21] | higher expression in unaffected males than AIS patients | |
| IMH2 | 0.46 | 0.27 | GGAACATCTGTCT | associated with maturation of human endometrium [25] | higher expression in unaffected males than AIS patients | |
| SQD1L (SQOR) | 0.45 | 0.24 | none | higher expression in unaffected males than AIS patients | | |
| CYP1B1 | 0.45 | 0.05 | none | involved in androgen metabolism [29]; potential target of androgens in mice genital tubercle [13] | lower expression in unaffected males than AIS patients | |
| AP0D | 0.34 | −1.16 | none | regulated by androgens in human genital skin fibroblasts [9]; binds to cholesterol, progesterone; and pregnenolone [22] | higher expression in unaffected males than AIS patients | |
| KIA1598 (SHTN1) | 0.34 | −0.04 | AGAAGACATTTT; TGGAACAGATTTCT | involved in mouse spermiogenesis [24] | | |
| RBMP3 | 0.34 | 0.15 | none | higher expression in unaffected males than AIS patients | | |
| NFE2 | 0.33 | 0.01 | none | higher expression in unaffected males than AIS patients | | |
| CD14 | 0.32 | 0.31 | none | higher expression in unaffected males than AIS patients | | |
| HSP8ST1 | 0.31 | 0.17 | none | involved in androgen-induced prostate development [36] | | |
| SPT25 | 0.31 | 0.15 | none | | | |
| HSPB3 | 0.31 | −0.18 | none | higher expression in unaffected males than AIS patients | | |
| MMP19 | 0.30 | 0.06 | none | regulated by estrogens in mouse ovary [27] | | |
| SRMS | 0.30 | −0.04 | none | | | |
| **Downregulated genes** | | | | | | |
| PODXL | −0.99 | −0.01 | none | overexpressed in prostate cancer [23] | | |
| SYNDIG1 | −0.57 | −0.13 | TGTACAGCATTTCT | | | |
| KRIT6 | −0.54 | −0.11 | none | | | |
| ESM1 | −0.53 | −0.10 | none | correlated with AR expression in the prostate [10] | higher expression in unaffected males than AIS patients | |
| CNIH3 | −0.36 | 0.02 | AGAAGACATTTTAT; AGAAGTTGCTTTCT | | | |
| KIF24 | −0.35 | −0.03 | AGAAGACATTTCT | possibly associated with ovarian failure [28] | higher expression in unaffected males than AIS patients | |
| DICK5 | −0.30 | −0.25 | none | | | |
| GPER1 | −0.30 | −0.04 | TGAACACTCTTTCT | regulated by androgens in rat [14] | | |

FC, fold change; AIS, androgen insensitivity syndrome; and AR, androgen receptor.

$^a$ previous reports in PubMed (https://www.ncbi.nlm.nih.gov/pubmed/).

$^b$ previous microarray data of genital skin fibroblasts (NextBio; https://www.nextbio.com/).
genitalia remains to be clarified.

This study identified 22 additional transcripts whose expression in genital skin may be regulated by androgens. About one-third of these transcripts were previously shown to be expressed more strongly in unaffected men than in AIS patients (Table 1), although in the previous study, the effects of exogenous androgens were not examined. The 22 transcripts included several genes reported as targets of androgens in prostate or other tissues. For example, RGCC, which showed the largest change by DHT treatment in this study, is known to be induced by DHT in male neural stem cells [11]. Likewise, HS6ST1 has been implicated in androgen-induced prostate development [36]. Gene ontology analyses indicated that three of the 22 transcripts are involved in angiogenesis. In particular, RGCC is known as a hypoxia-regulated angiogenic inhibitor [37], and knock-out of HS6ST1 in mice was shown to result in defective angiogenesis [38]. Since previous studies revealed that exogenous androgens cause neovascularization in external genitalia [39], these transcripts, together with CYP1B1 and APOD, may contribute to genital masculinization by regulating angiogenesis. In addition, RGCC and other transcripts may control cell proliferation in the developing penis. However, considering the small FCs of these transcripts, this notion remains speculative. Further in vitro studies, such as siRNA-induced knockdown or forced overexpression of the identified genes, would help to clarify the role of each gene in genital development.

The present study has some limitations. First, because of an easy access to clinical samples, we used cultured genital skin fibroblasts instead of developing genital tissues. Thus, the results of this study may not reflect the physiological condition of external genitalia. Indeed, previous studies suggested that dynamic cell-cell interactions are necessary to produce adequate genital masculinization. Second, we focused on transcripts whose expression levels were altered by short-term exposure to exogenous androgens. It is possible that genital masculinization results primarily from serial developmental events rather than simple transactivation of some AR-target genes. Moreover, AR may contribute to penile growth by modulating the function of other transcription factors or cofactors [1]. These issues deserve further study.

In conclusion, the results of this study indicate that androgen-mediated masculinization of external genitalia is unlikely to depend on massive transcriptional changes in specific AR target genes. Instead, minor transcriptional changes of several genes and/or a complex molecular network may play a major role in penile development. Importantly, our data suggest the possible involvement of CYP1B1 in human genital development and confirm the clinical importance of APOD as a biomarker for AR function.

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Disclosure

All authors report no conflict of interest.
References

1. Heemers HV, Tindall DJ (2007) Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. Endocr Rev 28: 778–808.

2. Jenster G, Trapman J, Brinkmann AO (1993) Nuclear import of the human androgen receptor. Biochem J 293 (Pt 3): 761–768.

3. Wilson S, Qi J, Filipp FV (2016) Refinement of the androgen response element based on ChIP-Seq in androgen-insensitive and androgen-responsive prostate cancer cell lines. Sci Rep 6: 32611.

4. Nerli RB, Guntaka AK, Patne PB, Hiremath MB (2013) Penile growth in response to hormone treatment in children with micropenis. Indian J Urol 29: 288–291.

5. Batista RL, Costa EMF, Rodrigues AS, Gomes NL, Faria JA Jr, et al. (2018) Androgen insensitivity syndrome: a review. Arch Endocrinol Metab 62: 227–235.

6. Gottlieb B, Beitel LK, Nadarajah A, Paliouras M, Trifiro M (2012) The androgen receptor gene mutations database: 2012 update. Hum Mutat 33: 878–894.

7. Holterhus PM, Hiort O, Demeter J, Brown PO, Brooks JD et al. (2003) Differential gene-expression patterns in genital fibroblasts of normal males and 46,XY females with androgen insensitivity syndrome: evidence for early programming involving the androgen receptor. Genome Biol 4: R37.

8. Appari M, Werner R, Wunsch L, Cario G, Demeter J, et al. (2009) Apolipoprotein D (APOD) is a putative biomarker of androgen receptor function in androgen insensitivity syndrome. J Mol Med (Berl) 67: 623–632.

9. Lai CY, Chen CM, Hsu WH, Hsieh YH, Liu CJ (2017) Overexpression of endothelial cell-specific molecule 1 correlates with glascon score and expression of androgen receptor in prostate carcinoma. Int J Med Sci 14: 1263–1267.

10. Quartier A, Chatrousse L, Redin C, Keime C, Hausmess N, et al. (2018) Genes and pathways regulated by androgens in human neural cells, potential candidates for the male excess in autism spectrum disorder. Biol Psychiatry 84: 239–252.

11. Zheng Z, Armfield BA, Cohn MJ (2015) Timing of androgen receptor disruption and estrogen exposure underlies a spectrum of congenital penile anomalies. Proc Natl Acad Sci U S A 112: E7194–E7203.

12. Nishida H, Miyagawa S, Matsumaru D, Wada Y, Satoh Y, et al. (2008) Gene expression analyses on embryonic external genitalia: identification of regulatory genes possibly involved in masculinization processes. Congenit Anom (Kyoto) 48: 63–67.

13. Menad R, Fernini M, Smai S, Bonnet X, Germigon-Sychalowicz T, et al. (2017) GPER1 in sand rat epididymis: effects of seasonal variations, castration and efferent ducts ligation. Anim Reprod Sci 183: 9–20.

14. Klocker H, Kaspar F, Eberle J, Uberreiter S, Radmayr C, et al. (1992) Point mutation in the DNA binding domain of the androgen receptor in two families with Reifenstein syndrome. Am J Hum Genet 50: 1318–1327.

15. Crawford BS (1977) Buried penis. Br J Plast Surg 30: 96–99.

16. Vottero A, Minari R, Viani I, Tassi F, Bonatti F, et al. (2011) Evidence for epigenetic abnormalities of the androgen receptor gene in foetuses from children with hypospadias. J Clin Endocrinol Metab 96: E1953–E1962.

17. Kupershmidt I, Su QJ, Grewal A, Sundaresh S, Halperin I, et al. (2010) Ontology-based meta-analysis of global collections of high-throughput public data. PLoS One 5: e13066.

18. Huang da W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4: 44–57.

19. Huang da W, Sherman BT, Lempicki RA (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37: 1–13.

20. Kradolfer D, Floter VL, Bick JT, Furst RW, Rode K, et al. (2016) Epigenetic effects of prenatal estradiol-17beta exposure on the reproductive system of pigs. Mol Cell Endocrinol 430: 125–137.

21. Rassart E, Bedirian A, Do Carmo S, Guinard O, Sirois J, et al. (2000) Apolipoprotein D. Biochim Biophys Acta 1482: 185–198.

22. Heath EI, Heilbrun LK, Smith D, Schopperle WM, Ju Y, et al. (2018) Overexpression of the pluripotent stem cell marker podocalyxin in prostate cancer. Anticancer Res 38: 6361–6366.

23. Zhou J, Du YR, Qin WH, Hu YG, Huang YN, et al. (2009) RIM-BP3 is a manchette-associated protein essential for spermiogenesis. Development 136: 373–382.

24. Burmenskaya OV, Bozhenko VK, Smolnikova VY, Kalinina EA, Korneeva IE, et al. (2017) Transcription profile analysis of the endometrium revealed molecular markers of the personalized ‘window of implantation’ during in vitro fertilization. Gynecol Endocrinol 33: 22–27.

25. Iida H, Urasoko A, Doiguchi M, Mori T, Toshimori K, et al. (2003) Complementary DNA cloning and characterization of rat spgern-2, a spermatogenic cell-specific gene 2 encoding a 56-kilodalton nuclear protein bearing ankyrin repeat motifs. Biol Reprod 69: 421–429.

26. Nalvarte I, Tohonen V, Lindeberg M, Varshney M, Gustafsson JA, et al. (2016) Estrogen receptor beta controls MMP-19 expression in mouse ovaries during ovulation. Reproduction 151: 253–259.

27. Jaillard S, Akloul L, Beaumont M, Hamdi-Roze H, Dubourg C, et al. (2016) Array-CGH diagnosis in ovarian failure: identification of new molecular actors for ovarian
30. Gray LE Jr, Kelce WR, Monosson E, Ostby JS, Birnbaum LS (1995) Exposure to TCDD during development permanently alters reproductive function in male Long Evans rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. *Toxicol ApplPharmacol* 131: 108–118.

31. Takemoto K, Nakajima M, Fujiki Y, Katoh M, Gonzalez FJ, *et al.* (2004) Role of the aryl hydrocarbon receptor and Cyp1b1 in the antiestrogenic activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Arch Toxicol* 78: 309–315.

32. Palenski TL, Gurel Z, Sorenson CM, Hankenson KD, Sheibani N (2013) Cyp1B1 expression promotes angiogenesis by suppressing NF-kappaB activity. *Am J Physiol Cell Physiol* 305: C1170–C1184.

33. Pajaniappan M, Glober NK, Kennard S, Liu H, Zhao N, *et al.* (2011) Endothelial cells downregulate apolipoprotein D expression in mural cells through paracrine secretion and Notch signaling. *Am J Physiol Heart Circ Physiol* 301: H784–H793.

34. Lin CS, Ho HC, Gholami S, Chen KC, Jad A, *et al.* (2001) Gene expression profiling of an arteriogenic impotence model. *Biochem Biophys Res Commun* 285: 565–569.

36. Buresh-Stiemke RA, Malinowski RL, Keil KP, Vezina CM, Oosterhof A, *et al.* (2012) Distinct expression patterns of Sulf1 and Hs6st1 spatially regulate heparan sulfate sulfation during prostate development. *Dev Dyn* 241: 2005–2013.

37. An X, Jin Y, Guo H, Foo SY, Cully BL, *et al.* (2009) Response gene to complement 32, a novel hypoxia-regulated angiogenic inhibitor. *Circulation* 120: 617–627.

38. Habuchi H, Kimata K (2010) Mice deficient in heparan sulfate 6-O-sulfotransferase-1. *Prog Mol Biol Transl Sci* 93: 79–111.

39. Bastos AN, Oliveira LR, Ferrarez CE, de Figueiredo AA, Favorito LA, *et al.* (2011) Structural study of prepuce in hypospadias—does topical treatment with testosterone produce alterations in prepuce vascularization? *J Urol* 185: 2474–2478.