Original Article

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Activating transcription factor 3 is not up-regulated in hypospadias patients in Japan

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ABSTRACT

Background: The aetiology of hypospadias is largely uncharacterized. Some of the researchers have advocated that activating transcription factor 3 (ATF3), an oestrogen-responsive transcription factor, is up-regulated in patients with hypospadias. The purpose is to evaluate the universality of this fact; we studied the expression of ATF3 protein in prepuce tissue obtained from hypospadias and phimosis patients living in metropolitan Tokyo. **Materials and Methods:** Prepuce tissue was obtained from outer foreskin at the time of surgery, quickly prepared for paraffin-embedded sectioning and stained immunohistochemically for ATF3. Two researchers blindly evaluated immunoreactivity and scored it semi-quantitatively as nil = 0, weak = 1, or strong = 2, to give a final staining intensity score (SIS). Subjects were 18 hypospadias patients and 17 phimosis patients (as controls) who had surgery between January, 2009 and March, 2010. **Results:** All subjects lived in metropolitan Tokyo, Japan. Mean ages at surgery were 2.9 ± 1.0 and 3.9 ± 2.4 years, respectively ($P > 0.05$). SIS was not statistically different between hypospadias patients (1.4 ± 0.5) and controls (1.5 ± 0.5), ($P > 0.05$). **Conclusions:** Our data suggest that ATF3 is not highly associated with hypospadias in metropolitan Tokyo. Differences in ethnicity might have influenced our results.

Key words: Activating transcription factor 3, environmental factor, hypospadias

INTRODUCTION

Hypospadias is one of the most common urogenital malformations, but its aetiology is still uncharacterised. Environmental factors such as *in utero* exposure to estrogenic or anti-androgenic compounds have been considered to be associated with the development of this anomaly, and some researchers have advocated a potential role for up-regulated expression of activating transcription factor 3 (ATF3), an oestrogen-responsive transcription factor.[1-3] To evaluate the universality of this fact, we studied the expression of ATF3 protein in prepuce tissue obtained from Japanese hypospadias and phimosis patients living in metropolitan Tokyo.

MATERIALS AND METHODS

Medical records of patients undergoing curative surgery for hypospadias and elective circumcision for phimosis were reviewed focusing on severity and demographic data (residential address and parental occupation).

For evaluation of ATF3 protein expression in prepuce tissue, prepuce specimens were obtained from 18 hypospadias patients (coronal [4], distal [2], mid-shaft [5], proximal [3], penoscrotal [4]) and 17 phimosis patients (as controls) between January, 2009 and March, 2010. All subjects lived in metropolitan Tokyo, Japan. Parental occupation was recorded as office worker in all except for one military personnel, one shopkeeper, and one care giver for a hypospadias group. There were no farmers or manual labourers. Prepuce specimens were obtained from outer foreskin at the time of surgery, quickly prepared for paraffin-embedded, and sectioning. ATF3 protein was stained immunohistochemically. Briefly, sections were incubated with rabbit anti-human ATF3 (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA), then further incubated with peroxidase-labelled polymer-horseradish peroxidase (HRP) conjugated to goat anti-rabbit immunoglobulins (Envision + System HRP; DAKO, Carpinteria, CA, USA). Development of peroxidase was achieved using freshly prepared 33′-diaminobenzidine tetra hydrochloride (Sigma, St. Louis, MO, USA). Sections were counterstained with Meyer's haematoxylin,

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dehydrated and mounted. Light microscopy was used for all examinations. Two researchers blindly evaluated immunoreactivity and scored it semi-quantitatively as nil = 0, weak = 1, and strong = 2, to give a final staining intensity score (SIS) [Figure 1]. The results were expressed as mean ± standard deviation Data were analysed using Chi-square, two-way analysis of variance and Mann-Whitney tests. A $P < 0.05$ was considered to be significant. This study was approved by the Juntendo University School of Medicine Ethics Committee and complies with the Helsinki Declaration of 1975 (revised 1983).

RESULTS

Mean ages at surgery were 2.9 ± 1.0 and 3.9 ± 2.4 years, respectively ($P > 0.05$). SIS in stromal cell nuclei and vascular endothelium were not statistically different between hypospadias patients (1.4 ± 0.5) and controls (1.5 ± 0.5), ($P > 0.05$) [Figure 2]. In the hypospadias group, severity of hypospadias according to location of the native meatus did not affect SIS; coronal 1.3 ± 0.5; distal 1.0 ± 0.0, mid-shaft 1.2 ± 0.8, proximal 1.8 ± 0.3, penoscrotal 1.5 ± 0.4 ($P > 0.05$) [Figure 3].

DISCUSSION

ATF3 is a transcription factor involved in mesenchymal-epithelial interactions.[1] Its expression has been shown to be significantly higher in hypospadias compared with age-matched controls and increases with severity.[2] Thus, ATF3 is considered to be strongly associated with the development of hypospadias. It also responds to environmental oestrogen exposure, proven by *in vitro* research using human foreskin fibroblasts where ATF3 increased in response to oestrogen exposure.

Meanwhile, reports from Europe and the US would suggest there is a geographical difference in incidence of hypospadias.[3] Parental occupation is thought to be another factor contributing the development of hypospadias; e.g., the offspring of farmers or gardeners have a higher risk for hypospadias, indicating the effects of exposure to agricultural endocrine disruptors.

Are these facts universal? To the best of our knowledge, there are no Japanese studies examining ATF3 expression in and parental occupation of hypospadias patients compared with a control group.

In the present study, we examined the expression of ATF3 protein in patients with hypospadias living in metropolitan Tokyo. In contrast to reports in the literature, we could not find any difference in ATF3 expression between hypospadias patients and controls, and no association with severity.[2,4] Occupation could not be used

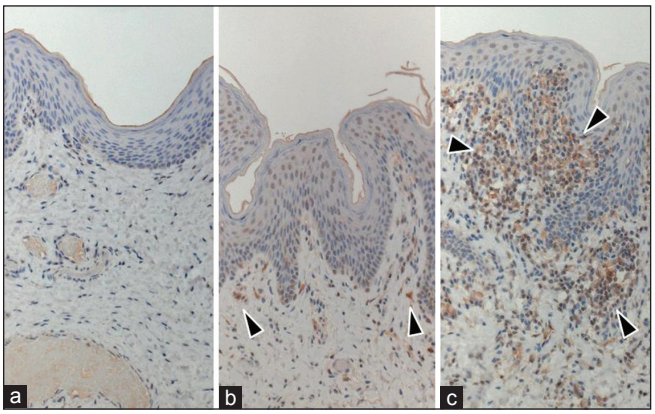


Figure 1: Activating transcription factor 3 immunostained cross-section of hypospadias foreskin. Two researchers blindly evaluated immunoreactivity and scored it semi-quantitatively as nil = 0 (a), weak = 1 (b), and strong = 2 (c), to give a final staining intensity score. Stromal cell nuclei and vascular endothelium are stained in b and c (arrowheads)

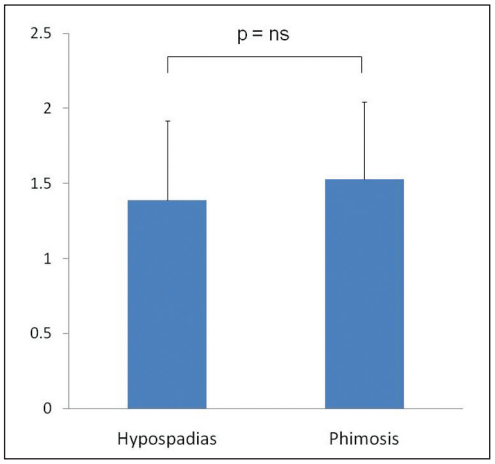


Figure 2: Staining intensity score in stromal cell nuclei and vascular endothelium were not statistically different between hypospadias patients and controls

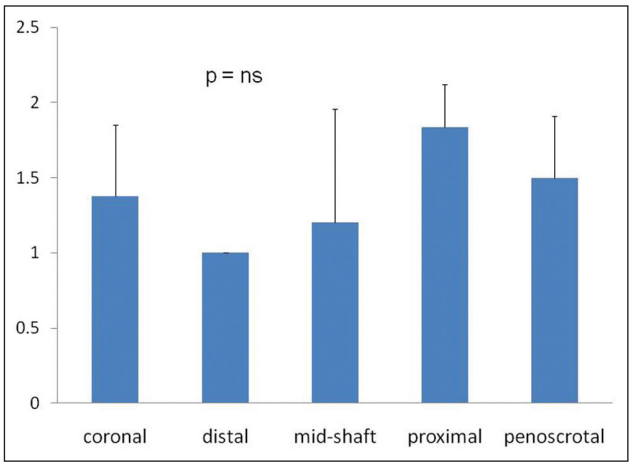


Figure 3: Severity of hypospadias according to location of the native meatus did not affect staining intensity score

for classification because almost all were office workers. It has been considered that the expression of ATF3 varies with the area of the penis.[5] Prepuce specimens were obtained from outer foreskin in our study, so differences in the area of the penis where we obtained as the specimens might have influenced our results.

Our data suggest that ATF3 is not highly associated with the occurrence of hypospadias in metropolitan Tokyo, but ATF3 expression in our controls was relatively higher than we expected and this could be an important point. Differences in ethnicity might have influenced our results. The subjects in this study might have been less exposed to the estrogenic compounds than in previous reports. Further investigations using other methods of analysis would be helpful to clarify a relationship between ATF3 and hypospadias, as well as similar studies from other cities because hypospadias is a multifactorial condition.[6]

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