ABOUT JMGG

The Journal of Medical Genetics and Genomics (JMGG) is published monthly (one volume per year) by Academic Journals.

Journal of Medical Genetics and Genomics (JMGG) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as metagenics, evolutionary anthropology, fragile X syndrome, immunotherapy etc.
The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMGG are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jmgg@acadjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Medical Genetics and Genomics will only accept manuscripts submitted as e-mail attachments.

Please read the Instructions for Authors before submitting your manuscript. The manuscript files should be given the last name of the first author.
Editors

Prof. Viroj Wiwanitkit, M.D.
Wiwanitkit House, Bangkhae,
Bangkok Thailand 10160.
Visiting Prof. Tropical Medicine,
Hainan Medical College,
Hainan China.

Dr. Israel Fernandez-Cadenas
Neurovascular Research Laboratory,
Institut de Recerca, Vall d’Hebron Hospital,
Barcelona.
Spain

Dr. Wanis H Ibrahim
Qualifications: FRCP (Edin), FRCP (Glasg), FCCP
Hamad General Hospital, Weill-Cornell Medical College
Qatar

Prof. Kenneth Blum
Institution Department of Psychiatry,
University of Florida college of Medicine,
Gainesville, Fl
USA

Prof. Debnath Bhattacharyya
Hannam University,
Daejeon,
Korea

Dr. Abd El-Latif Hesham
Genetics Department, Faculty of Agriculture,
Assiut University
Egypt

Prof. Viroj Wiwanitkit
Wiwanitkit house, bangkhae, Bangkok Thailand
10160
Thailand

Dr. Pritha Ghosh
Indian Institute of Chemical Biology
India

Dr. Khaled Abu-Amero
College of Medicine, King Saud University,
Saudi Arabia

Dr. Faiyaz Ahmed
Department of Studies in Food Science and Nutrition
University of Mysore,
India
### Editorial Board

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Rama Devi Mittal</td>
<td>Sanjay Gandhi PGI Lucknow, India</td>
</tr>
<tr>
<td>Prof. Kai Li</td>
<td>Suzhou University, Suzhou, China</td>
</tr>
<tr>
<td>Prof. Kai Li</td>
<td>Shanghai Jiaotong University, China</td>
</tr>
<tr>
<td>Dr. Aliza Amiel</td>
<td>Faculty of Life Science Bar-Ilan Ramat-Gan, Israel</td>
</tr>
<tr>
<td>Dr. Aliza Amiel</td>
<td>Bar-Ilan University, Ramat-Gan, Israel</td>
</tr>
<tr>
<td>Dr. Olufemi Oloyede</td>
<td>Department of Obstetrics and Gynaecology, Onabani University, Teaching Hospital, Sagam, Ogun State, Nigeria</td>
</tr>
<tr>
<td>Prof. Ruixing Yin</td>
<td>Department of Cardiology, Institute of Cardiovascular Diseases, Guangxi Medical University, Guangxi, China</td>
</tr>
<tr>
<td>Dr. Guangming Han</td>
<td>Georgia State University, USA</td>
</tr>
<tr>
<td>Dr. C. Emmanuel</td>
<td>Global Hospitals Group, India</td>
</tr>
<tr>
<td>Dr. Alessio Squassina</td>
<td>Department of Neuroscience, University of Cagliari, Italy</td>
</tr>
<tr>
<td>Dr. Jiexiong Feng</td>
<td>Department of Pediatric Surgery, Tongji Hospital, Huazhong University of Science and Technology, China</td>
</tr>
<tr>
<td>Dr. Magdy Abd ElRehim Sayed Aly</td>
<td>Faculty of Science, Beni Suef University, Egypt</td>
</tr>
<tr>
<td>Dr. Hamid Jafarzadeh</td>
<td>Mashhad Faculty of Dentistry and Dental Research Center, Iran</td>
</tr>
<tr>
<td>Dr. Youse Rasmi</td>
<td>Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran</td>
</tr>
<tr>
<td>Dr. Keya Chaudhuri</td>
<td>Indian Institute of Chemical Biology, India</td>
</tr>
<tr>
<td>Ivan Y. Torshin</td>
<td>Computational Center of The Russian Academy of Sciences, Russia</td>
</tr>
<tr>
<td>Dr. Wagdy K. B. Khalil</td>
<td>National Research Centre (NRC), Egypt</td>
</tr>
<tr>
<td>Vishnu Priya</td>
<td>Saveetha University, India</td>
</tr>
<tr>
<td>Dr. A. Chandrasekar</td>
<td>Anthropological Survey of India, Southern Regional Bogadi, 2nd stage, Mysore-570 026, India</td>
</tr>
<tr>
<td>Dr Raghavendra Babu YP</td>
<td>Kasturba Medical College, Mangalore, India</td>
</tr>
<tr>
<td>Dr. Shayesteh Jahanfar</td>
<td>Royal College of Medicine, Perak; University of Kuala Lumpur, Malaysia</td>
</tr>
<tr>
<td>Prof. Wei Wang</td>
<td>Capital Medical University, Beijing, China; Chinese Academy of Sciences, Beijing, China</td>
</tr>
<tr>
<td>Dr. Wei Wang</td>
<td>Capital Medical University, Beijing, China; Chinese Academy of Sciences, Beijing, China</td>
</tr>
</tbody>
</table>
Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The cover letter should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process
All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review. Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JMGG to publish manuscripts within weeks after submission.
Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author’s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author’s name should be mentioned, followed by ‘et al’. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter ‘a’ and ‘b’ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:


Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.
Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the Journal of Medical Genetics and Genomics is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Copyright: © 2015, Academic Journals.
All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Disclaimer of Warranties
In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the JMGG, whether or not advised of the possibility of damage, and on any theory of liability.
This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.
ARTICLES

Research Article

Evaluation of BNC2 as a new candidate gene for hypospadias
Anna Skarin Nordenvall, Jia Cao, Ellen Markljung, Izabella Baranowska
Körberg and Agneta Nordenskjöld
Evaluation of BNC2 as a new candidate gene for hypospadias

Anna Skarin Nordenvall1,2, Jia Cao1, Ellen Markljung1, Izabella Baranowska Körberg1 and Agneta Nordenskjöld1,2*

1Department of Women’s and Children’s Health, Center for Molecular Medicine, Karolinska Institutet, SE-171 76 Stockholm, Sweden.
2Department of Pediatric Surgery, Astrid Lindgren Children’s Hospital, Karolinska University Hospital, SE-171 76 Stockholm, Sweden.

Received 12 February, 2015; Accepted 26 March, 2015

In order to evaluate the use of the BNC2 gene in clinical practice regarding hypospadias, the presence of the impact of mutations in BNC2 gene in males who had been treated surgically for hypospadias in Sweden (N=413) and controls (N=455) were evaluated. Mutational screening was performed using Sanger sequencing and genotyping by Taqman allelic discrimination, and the findings were evaluated by disease-causing potential using Mutation Taster and PolyPhen. Nine missense mutations were identified, six of which were more common among cases than among controls, one being previously unknown. Six of these genetic variants were predicted to be possibly or probably damaging in mutational predictions and are thus potentially disease-causing. No difference was confirmed in the contribution of these findings in different severity of phenotype. Due to the complexity of the gene structure, the impact of variants was difficult to evaluate, and thus the clinical use of BNC2 in the management of 46,XY disorder of sex development (DSD) remains limited.

Key words: BNC2 gene, hypospadias, mutations, polymorphism.

INTRODUCTION

Hypospadias is one of the most frequent congenital malformations in boys. The malformation results from an arrest of the normal development of the urethra, foreskin and ventral aspect of the penis during gestational week 8 to 16. It is characterized by misplacement of the urethral opening, ventrally and proximally from the tip of the glans penis. The site of failure of urethral fold will dictate the position of the abnormal urethral meatus, and thus the severity of hypospadias. Majority of the cases of hypospadias are mild, with the meatus positioned at corona or on the glans penis (Bergman et al., 2015). More severe cases were associated with penile curvature as well as penoscrotal transposition and intersex conditions (Baskin and Ebbers, 2006).

Hypospadias is a multifactorial disorder caused by both genetic and environmental factors. Accordingly, hypospadias sometimes displays a monogenic inheritance pattern. Majority of the cases are sporadic, but in approximately 7%
of them, familial clustering, has been described, which supports the theory of an underlying genetic effect (Fredell et al., 2002a). Consequently, most hypospadias are sporadic and without a known molecular cause in individual cases. The malformation exhibits an under-masculinization in male fetuses, but mutations in genes involved in the androgen pathway, such as steroid-5-alpha-reductase (SRD5A2) and androgen receptor (AR), are rare and reported in only a small portion of affected individuals (Hiort et al., 1994; Kon et al., 2015). Other genes that have been associated with hypospadias are MAML1, which when knocked down in mice reduces testosterone production and exhibits mutations in a few percent of hypospadias patients (Kalfa et al., 2008), HOXA13, which may cause hand-foot-genital syndrome (Frisen et al., 2003), MID1, in which mutations have been associated with the Opitz syndrome, which is characterized by midline defects and WT1 gene, in which mutations may lead to 46,XY disorder of sex development (DSD), nephropathy causing renal insufficiency, and Wilms' tumor (Kalfa et al., 2010; Kaltenis et al., 2004; van der Zanden et al., 2012).

A highly conserved gene that has attracted attention lately is the basonuclein 2 (BNC2; 9p22.1). It has been established that the protein characteristics of BNC2 are similar to those of its namesake, BNC1 (Vanhoutteghem and Dijan, 2004), a zinc finger protein highly expressed in skin keratinocytes, which is associated with cell proliferation (Tseng, 1998). The function of BNC2 is, however, unknown, although it is believed to participate in mRNA processing (Vanhoutteghem and Dijan, 2006). Bhoj et al. (2011) have described the potential role of BNC2 in urethral development. They found high expression of BNC2 in fetal periurethral tissues and BNC2 knockout mice with a high prevalence of distal urethral defects. Furthermore, they performed mutational screening of the major coding exons in 48 males with hypospadias and in controls, and four potentially deleterious variants were identified in three subjects with hypospadias; whereas only one was found in the control group (Bhoj et al., 2011). This study prompted the DSD team to incorporate the BNC2 gene in mutational screening of 46,XY DSD in our hospital. Kon et al. (2015) recently found one heterozygous missense mutation in BNC2 when they analyzed the molecular basis of hypospadias among 57 patients.

In order to further evaluate the use of the BNC2 gene in clinical practice, presence of mutations was screened in it, in a large collection of DNA samples from males with hypospadias and predicted the potential disease-causing effects of candidate polymorphisms.

MATERIALS AND METHODS

Clinical material

Four hundred and thirteen (413) males who had been surgically treated for hypospadias in Sweden were included in the study. The Ethics Committee of Karolinska Institutet approved the study first in 1995. All samples were obtained after oral or written information were given to the parents that gave oral consent. These samples have been collected continuously since 1996. Initially and until 2008, the consent was only oral. The original approval of the study covered all the years that the study took place, but since 2008, when an extended application on additional congenital malformations was requested, the consent have also, according to new rules from the National Ethics Committee been documented in the patient's medical chart. According to the rules from the National Ethics Committee, there is no demand for an explicitly written consent and the Ethics Committee of Karolinska Institutet have specifically approved oral consent from parents.

Both sporadic cases and familial (one per family) ones were included with varying phenotypic severity ranging from mild glandular hypospadias to perineal hypospadias with an intersex condition. Initially, 236 cases of the ranging phenotype were sequenced for mutational analyses. Phenotypes were grouped according to severity as glandular (N=35), penile (N=55), or perineal/perianal (N=77) and. In 22 cases, the severity of hypospadias was unknown. An additional 47 males with familial hypospadias, with mainly a glandular phenotype, were included. Further analyses using Taqman were performed regarding two variants, which led to the inclusion of 177 additional cases.

Control samples of DNA from healthy volunteer anonymous blood donors and placenta tissue from the Karolinska University Hospital were analyzed using both standard sequencing and Taqman. The number of controls ranged from 76 to 455, depending on whether sequence variants that were identified in cases were controlled for by Sanger sequencing or Taqman. The Ethics Committee of Karolinska Institutet approved the study.

DNA extraction

Genomic DNA from the clinical sample was extracted from peripheral blood or penile tissue obtained during surgery using the standard phenol chloroform extraction protocol or the Gentra Purogene kit (Qiagen, Maryland, USA).

Mutational analysis

Exons 1 to 6 of BNC2 were PCR-amplified and primers were designed to cover the entire coding sequence, including flanking intronic sequences (primer-3; http://primer3.wi.mit.edu) (Vanhoutteghem and Dijan, 2007; Bhoj et al., 2011). PCR products were purified using Shrimp Alkaline Phosphatase and Exonuclease I (Fermentas Life Sciences). Sanger sequencing was performed on both strands, using standard cycle-sequencing reactions with the BigDye terminator v3.1 cycle sequencing kit (BigDye® Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems), and run on an ABI3130 Sequencer (Applied Biosystems). Chromatograms were analyzed using SeqScape v3.7 (Applied Biosystems) and compared with reference sequence ENST00000380672. All positive findings were confirmed by resequencing from the original DNA solution.

Genotyping

Genetic variations that were identified in the materials used and previously of unknown frequency in a healthy population were further analyzed using TaqMan allelic discrimination assays according to recommended protocols. The results were analyzed using the 7900 HT Fast-Real-Time PCR system (Applied Biosystems) and SDS 2.2 (Applied Biosystems). Only successful samples are reported (≥92% success rate in patients).

Four SNPs (rs62540608, TMP_ESP_9_16435990, rs3739715,
The frequency of p.P684L (0.4) was higher and allelic frequencies in the general population were (Table 2). All three variants have been described previously described p.A923V as “tolerated” using SIFT analysis. In case that was homozygous, whereas only heterozygotes hypospadias. However, p.D930G was only found in one population, suggesting that these variant s do not cause cases compared with the controls and the general populations (Table 1). Bhoj et al. (2011) have previously were found in the controls or in the previously described gene variants without rs number (c.1002, c.1239, and c.1738) were further evaluated in the cases and controls. No parents of the patients were genotyped.

**RESULTS**

By sequencing, 20 genetic variants were identified in the BNC2 gene in 115 out of 236 cases with hypospadias (Table 1 and Figure 1). Seventeen polymorphisms were previously known, thus three were novel, one of which was a missense mutation. Allelic variants were found most frequently in exon 5. Findings of this study are grouped as the results of the mutational prediction in silico subsequently.

### Possibly damaging

Three missense mutations were predicted to be possibly damaging: (c.916C>G, p.P306A), (c.1738G>A, p.G580R), and (c.1868C>A, p.P623H). In one boy with Robinow syndrome, a change was identified in p.P306A; this boy was also a carrier of p.A923V, which was predicted to be damaging. The missense mutation in p.G580R was identified in one boy with several other malformations, including anal atresia, cardiac malformations, and polydactyly. The change in p.G580R has not been described previously and did not affect any of the controls in our analyses; p.P623H was identified in two boys, one of whom also had an associated cri-du-chat syndrome.

Both p.P306A and p.P623H have been described previously and occurred less frequently or just as frequently in cases as in controls or the general population. Both of these variants were predicted to be damaging according to the SIFT analysis, as reported by Bhoj et al. (2011). However, our results indicate that they are only possibly damaging when further simulation analyses are taken into account.

### Benign

Majority of our findings were predicted benign, 11 being synonymous mutations (c.84A>T, p.A28A), (c.501C>T, p.V167V), (c.519C>T, p.S173S), (c.936C>T, p.F312F), (c.1002A>G, p.P334P), (c.1239T>C, p.D413D), (c.1545A>G, p.S515S), (c.1947C>T, p.T649T), (c.2202C>T, p.G734G), (c.2478A>G, p.L826L), and (c.2739C>T, p.R913R), and three were non-synonymous (c.171G>T, p.Q57H), (c.1240C>G, p.L414V), and (c.2929A>G, p.I974V).

The changes in p.P334P and p.D413D were previously unknown, each affecting one individual, and none of these changes were present in controls.

In total, there was an even distribution of findings among penoscrotal/perineal (64%), penile (64%) and glandular (69%) hypospadias. Mutational findings where less frequent among familial cases (43%). The frequency of findings defined as probably damaging was slightly higher in penile (9%) and penoscrotal/perineal (9%) cases than in individuals with glandular (6%) hypospadias.

We tested for statistical difference between the phenotypes using the Fischer exact test; there was no significant difference. The findings in the familial cases were all predicted to be benign.

### DISCUSSION

In this study, mutational screening of the BNC2 gene in 413 males with hypospadias was performed to evaluate the potential clinical use of BNC2 in molecular diagnostics of hypospadias. By sequencing BNC2 in 236 affected males, nine missense mutations were found with six of
Table 1. Data on all mutational findings, including calculated allelic frequencies among cases and controls. Disease-causing potential prediction based on PolyPhen and Mutation Taster.

<table>
<thead>
<tr>
<th>Protein</th>
<th>RSnumber</th>
<th>Case N (%)</th>
<th>Control N (%)</th>
<th>Population rate</th>
<th>Disease-causing potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.A28A</td>
<td>rs76485966</td>
<td>4/236 (1.7)a</td>
<td>0/80 (0)</td>
<td>4.5/0.3</td>
<td>Benign</td>
</tr>
<tr>
<td>p.Q57H</td>
<td>rs145011045</td>
<td>1/236 (0.2)</td>
<td>0/83 (0)</td>
<td>0.0/0.1</td>
<td>Benign</td>
</tr>
<tr>
<td>p.V167T</td>
<td>rs143821778</td>
<td>1/236 (0.2)</td>
<td>0/95 (0)</td>
<td>0.0/0.1</td>
<td>Benign</td>
</tr>
<tr>
<td>p.S173S</td>
<td>rs149019822</td>
<td>1/236 (0.2)</td>
<td>0/95 (0)</td>
<td>0.0/0.1</td>
<td>Benign</td>
</tr>
<tr>
<td>p.P306A</td>
<td>rs114964332*</td>
<td>1/236 (0.2)</td>
<td>0/117 (0)</td>
<td>0.2/0.0</td>
<td>Possibly damaging</td>
</tr>
<tr>
<td>p.F312F</td>
<td>rs77464990</td>
<td>1/236 (0.2)</td>
<td>0/117 (0)</td>
<td>1.1/0.0</td>
<td>Benign</td>
</tr>
<tr>
<td>p.P334P</td>
<td>n.a.</td>
<td>1/236 (0.2)</td>
<td>0/361 (0)</td>
<td>n.a</td>
<td>Benign</td>
</tr>
<tr>
<td>p.D413D</td>
<td>n.a.</td>
<td>1/236 (0.2)</td>
<td>0/455 (0)</td>
<td>n.a</td>
<td>Benign</td>
</tr>
<tr>
<td>p.L414V</td>
<td>rs148873573*</td>
<td>7/236 (1.5)</td>
<td>3/94 (1.6)</td>
<td>0.2/0.4</td>
<td>Possibly damaging</td>
</tr>
<tr>
<td>p.S515S</td>
<td>rs117470554</td>
<td>17/236 (3.8)</td>
<td>9/94 (4.8)</td>
<td>2.6/5.1</td>
<td>Benign</td>
</tr>
<tr>
<td>p.G580R</td>
<td>n.a.</td>
<td>1/236 (0.2)</td>
<td>0/443 (0)</td>
<td>0.0/0.0</td>
<td>Benign</td>
</tr>
<tr>
<td>p.P623H</td>
<td>rs114596065*</td>
<td>2/236 (0.4)</td>
<td>1/82 (0.6)</td>
<td>0.4/0.0</td>
<td>Possibly damaging</td>
</tr>
<tr>
<td>p.T649T</td>
<td>rs62540608</td>
<td>7/384 (0.9)</td>
<td>8/444 (0.09)</td>
<td>0.2/0.7</td>
<td>Benign</td>
</tr>
<tr>
<td>p.P684L</td>
<td>rs138187836</td>
<td>2/236 (0.4)</td>
<td>0/76 (0)</td>
<td>0.1/0.0</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>p.G734G</td>
<td>TMP_ESP_9_16435990</td>
<td>1/236 (0.2)</td>
<td>0/361 (0)</td>
<td>0.0/0.0</td>
<td>Benign</td>
</tr>
<tr>
<td>p.L826L</td>
<td>rs3739715</td>
<td>65/406 (8.4)c</td>
<td>48/356 (6.9)d</td>
<td>6.8/6.7</td>
<td>Benign</td>
</tr>
<tr>
<td>p.R913R</td>
<td>rs188985388</td>
<td>1/236 (0.2)</td>
<td>0/361 (0)</td>
<td>0.0/0.0</td>
<td>Benign</td>
</tr>
<tr>
<td>p.A923V</td>
<td>rs117452684*</td>
<td>12/236 (2.5)</td>
<td>14/159 (4.4)</td>
<td>1.2/3.0</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>p.D930G</td>
<td>rs41268965</td>
<td>1/236 (0.4)a</td>
<td>0/159 (0)</td>
<td>0.2/0.7</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>p.I974V</td>
<td>rs3739715</td>
<td>6/236 (1.3)</td>
<td>2/159 (0.6)</td>
<td>0.3/0.7</td>
<td>Benign</td>
</tr>
</tbody>
</table>

*Previously described by Bhoj et al. (a). All cases homozygous. One case homozygous. Three cases homozygous. One control homozygous.

Table 2. Detailed information on phenotype of the cases with variants predicted damaging in mutational simulation.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Detected non-synonymous variant</th>
<th>Disease-causing potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>c.2051 C&gt;T p.P684L</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H2</td>
<td>c.2051 C&gt;T p.P684L</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H3</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H4</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H5</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H6</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H7</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H8</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H9</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H10</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H11</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H12</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H13</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H14</td>
<td>c.2768 C&gt;T p.A923V</td>
<td>Probably damaging</td>
</tr>
<tr>
<td>H15</td>
<td>c.2768 A&gt;G p.A923V</td>
<td>Probably damaging</td>
</tr>
</tbody>
</table>

which occurred more frequently in cases than in the controls, and one was previously unknown. Six of the genetic variants were predicted to be possibly or probably damaging and potentially disease-causing; however, some of these findings occurred just as frequently or even less frequently in cases than in controls, implying that the variants are not causative regarding hypospadias. Four of these missense mutations have been described previously.
Figure 1. The black filled boxes represent the coding regions, and the light gray filled boxes denote the untranslated regions. The positions of the variants found in the BNC2 are shown. Numbers indicate the number of exons in BNC2. Conclusion indicating disease-causing potential: filled with white background, benign; filled with gray, possibly damaging; filled with black, probably damaging.

previously in patients with hypospadias (Bhoj et al., 2011). No difference was confirmed in the distribution of mutational findings between different phenotypes.

Disturbances in developmental pathways, such as midline fusion and skin development, could, apart from defects in androgen signaling, cause hypospadias. Malformations involving the midline, such as cleft-lip-palate and congenital heart malformations, are known to be more frequently associated with hypospadias (Fredell et al., 2002b; Wu et al., 2002). BNC2 is mainly expressed in renal, intestinal, uterine, and testicular tissue (Vanhoutteghem and Djian, 2006), but also in keratinocytes (Romano et al., 2004) and periurethral tissue during development (Bhoj et al., 2011). It has been confirmed that BNC2 is important for the proliferation of craniofacial mesenchymal cells during embryogenesis in mice and it has been suggested that human craniofacial abnormalities may result from a lack of BNC2 (Vanhoutteghem et al., 2009). Previous studies have demonstrated the importance of BNC2 in urethral development, since knockout mice develop typical hypospadiac features (Bhoj et al., 2011), potentially due to defects in skin development and midline fusion.

The BNC2 gene spans 461 kb on chromosome 9 (9p22.1) and possesses 6 promoters (1 major and 5 minor), 4 polyadenylation sites, and a total of 23 exons (1, 1d, 1b, 1c, 2, 2f, 2b, 2a, 2g, 2b, 2a, 2g, 2b, 2l, 2c, 2e, 3, 3a, 4, 4a, 5, 5c, 5d, 5a, 5b, 6). Each promoter, splice site, and polyadenylation addition site has been suggested to be used independently, with the potential to generate up to approximately 90,000 variants encoding >2000 different proteins (Vanhoutteghem and Djian, 2007). Thus, none of the 23 exons is present in all BNC2 mRNA isoforms, all exons can therefore be considered as alternative. The most abundant BNC2 mRNA variants are stable and translated into proteins as demonstrated by PCR and transient transfection in HeLa cells (Vanhoutteghem and Djian, 2007).

With regard to the unknown function and large number of potential transcripts of BNC2, the significance of occasional mutational findings and their role in the pathogenesis of hypospadias are difficult to evaluate. Among the three variants predicted probably damaging in this study only one was more common among cases compared to controls (p.P684L); it was present in two cases with penile hypospadias and none of the controls. Thus, it is believed that the use of BNC2 in current clinical practice regarding hypospadias is limited.

To further evaluate the potential pathogenic effect of sporadic variants in BNC2, it would first of all be desirable to determine which isoforms of BNC2 are expressed in penile tissue during development. Secondly, functional studies on BNC2 would be useful to assess the effect of our findings regarding the pathogenesis of hypospadias.

Conclusion

In this study, BNC2 have been screened for mutations in order to investigate its potential clinical use in the diagnostics of XY, DSD. Several potential disease-causing SNPs that are more abundant in males with hypospadias than in controls have been identified, but we have not identified any certain phenotype-causing mutations. Due to the complexity of the gene structure and our occasional findings, the clinical use of BNC2 was assessed in the management of 46,XY DSD as being limited.

ACKNOWLEDGEMENTS

The Swedish Research Council, the Foundation Frimurare Barnhuset Stockholm, the Stockholm City Council, the Swedish Society for Medical Research and Karolinska Institutet supported this work.
Conflict of interests

The authors have no conflicts of interest to declare.

REFERENCES


Journal of Medical Genetics and Genomics

Related Journals Published by Academic Journals

- Journal of Medical Laboratory and Diagnosis
- Journal of Metabolomics and Systems Biology
- Journal of Neuroscience and Behavioral Health
- Journal of Physiology and Pathophysiology
- Journal of Public Health and Epidemiology
- Journal of Petroleum Technology and Alternative Fuels