

Full Length Research Paper

Detection of genomic instability in hypospadias patients by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) method

Adnan A. Alsulaimani¹, Nabil S. Awad^{2*} and Adel E. El-Tarras²

¹College of Medicine, Taif University, Taif, Kingdom of Saudi Arabia.

²College of Medicine, Biotechnology and Genetic Engineering Unit, Taif University, Taif, Kingdom of Saudi Arabia.

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Hypospadias is a urogenital malformation, and it is a common inborn disorder in male individuals. The etiology of hypospadias is still unsolved. The present study is aimed to identify the genetic instability in hypospadias patients. Random amplified polymorphic DNA (RAPD), a polymerase chain reaction (PCR) based technique, was adopted using ten random primers in twelve cases and twelve controls. The primer detectability on genomic instability in 12 samples ranged from 25% with primer OPA-01 to 66% with OPA-08. Case 2 showed the highest genomic instability (80%). The lowest genomic instability was (10%) case 6. The results determined numbers of genomic instabilities among hypospadias patients. In addition, the RAPD-PCR technique is a powerful tool for detection of genomic instability in hypospadias patients. Further larger studies are needed, which include low and high grade of patients to: 1) Obtain RAPD markers useful for hypospadias early diagnosis; 2) investigate different genes directly involved in the etiology of hypospadias; 3) analyze chromosomal instability among hypospadias patients.

Key words: Hypospadias, random amplified polymorphic DNA (RAPD), genomic instability.

INTRODUCTION

Hypospadias is one of the most common congenital disorders (Sutherland et al., 1996) characterized by opening on the ventral side of the penis (Manson and Carr 2003). Hypospadias occurs in 1 out of 300 live male births (Kalfa et al., 2008). Different studies on the etiology of hypospadias were conducted and most of them have focused on a number of candidate genes that control androgen action and metabolism including the *SRD5A2*, *HSD17B3* and the *AR* genes (Manson and Carr., 2003) and *CXorf6* gene mutations (Fukami et al., 2006; Kalfa et al., 2008).

Measurement of genomic instability has been performed by techniques like flow cytometry, fluorescent

in situ hybridization, comparative genomic hybridization (CGH) and allelotyping, which, although informative, are cumbersome to perform and hence, impractical in the assessment of clinical cases (Basik et al., 1997).

Random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR) based fingerprinting technique that amplifies random DNA fragments with single short primers of arbitrary nucleotide sequence under low annealing stringency (Ong et al., 1998; Williams et al., 1990). The applications of RAPD technique have been found among several kinds of organism including bacteria (Sahoo et al., 2010), fungi (Motlagh and Anvari, 2010), plants (El-Tarras et al., 2004), animals (Güneren et al., 2010), insects (Awad et al., 2010) and humans (Saleh et al., 2010). The usefulness of the RAPD technique for the detection of genomic instability in various types of human tumors has been widely documented (Singh and Roy, 2001; Papadopoulos et al., 2002; Zhang et al., 2004; Xian et al., 2005; Saleh et al., 2010). No evidence for an association between genomic instability and hypospadias has been

*Corresponding author. E-mail: nabilfaris151@yahoo.com.

Abbreviations: CGH, Comparative genomic hybridization; RAPD, random amplified polymorphic DNA; PCR, polymerase chain reaction; EDTA, ethylenediaminetetraacetic acid.

investigated. So the specific aims of this study are to investigate the association between genomic instability and hypospadias, as well as to evaluate the usefulness of the RAPD technique for the detection of genomic instability in hypospadias patients.

MATERIALS AND METHODS

Blood samples collection and DNA extraction

Whole blood samples of 12 hypospadias patients and 12 healthy individuals were drawn from a peripheral vein into ethylenediaminetetraacetic acid (EDTA) tubes and stored at -20°C until DNA extraction step. Genomic DNA was extracted from blood samples according to instructions of Blood DNA Preparation Kit (Jena Bioscience; Germany).

DNA amplification

Total reaction volume of 25 µl of 2x superhot PCR Master Mix (Bioron; Germany) was used and contains 10 Pmol of each 10 different arbitrary 10-mer primers and 25 to 50 ng of genomic DNA. The names and sequences of these oligoprimers are listed in Table 1. The RAPD-PCR amplification reactions were performed in Eppendorf® thermal cycler using the following PCR program: 1 cycle at 94°C, 4 min; 35 additional cycles consisting of 94°C 5 s, 37°C 20 s and 72°C 20 s. After the amplification, the PCR reaction products were electrophoresed with 100 bp ladder marker (Fermentas, Germany) on 10 x 14 cm 1.5%-agarose gel (Bioshop; Canada) for 30 min using Tris-borate- EDTA Buffer. The gel was stained with 0.5 µg/ml of ethidium bromide (Bioshop; Canada).

Data analysis

All gels were visualized and documented using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene; Frederick, Maryland, USA). Each RAPD-PCR amplified DNA fragment was assumed to represent a single locus. The digital image files were analyzed using Gene Tools software from Syngene. The densitometric scanning of each based on its three characteristic dimensions was carried out. Each band was recognized by its length, width and intensity. Accordingly, the relative amount of each band was measured and scored. The absence or addition of an amplified product in at least one hypospadias sample and its corresponding control sample was used as a criterion of genomic instability.

RESULTS AND DISCUSSION

During the present study, 12 hypospadias patients and corresponding normal individuals were examined using RAPD-PCR method to detect genomic instability which appears as banding pattern changes between patients and normal amplified DNA with ten different RAPD primers. Results of genomic instability detected by RAPD-PCR analysis are shown in Table 1 and Figure 1. Among all studied cases, genomic instability was demonstrated with at least one primer. Among all studied cases with all primers, the detectability of genomic instability ranged from 25% with primer OPA-01 to 66.6% with OPA-08 primer. With all used primers, case 2 showed the highest genomic instability (80%), whereas, the lowest

genomic instability was (10) with case 6.

There are three types of hypospadias glandular or coronal (mild), penile (moderate), scrotal and perineal (severe) which are also called first, second and third grade, respectively (Ghirri et al., 2009). The powerful technique that detects genomic alteration correlated with human tumor is microsatellites analysis (Odenthal et al., 2009; Janavicius et al., 2010). However, this methodology is time consuming and can only detect base-pair expansion or contraction in specific microsatellite loci (Ong et al., 1998). On the contrary, for genomic instability analysis, it is important to investigate genetic alterations in the entire genome besides microsatellite loci. In contrast, the RAPD method can simply and rapidly detect genetic alterations in the entire genome without knowledge of specific DNA sequence information (Papadopoulos et al., 2002; Ibrahim et al., 2010). In the RAPD method, genetic alterations appeared as either loss or gain of a band, shift of a band, or decrease or increase of intensities of a band of cancer tissue DNA relative to the corresponding normal tissue DNA (Maeda et al., 1999). Obtained results indicated that RAPD-PCR is an effective tool for identifying genetic alteration and genomic instability which is in agreement with several various studies (Ibrahim et al., 2010; Wang, 2001). Figure 1 shows the banding profiles of hypospadias and corresponding normal DNAs and demonstrate the detected genetic alteration by RAPD technique among hypospadias patients in comparison with normal control group. Banding shifts, missing bands and/or banding intensity changes, which indicate genomic instability, were demonstrated in this figure.

These results might be due to mutations that occurred at the primer - template interaction sites (Maeda et al., 1999). The summarized results which are illustrated in Table 1 indicated that, there are differences in genetic instability among the studied cases which ranged from 10% with case number 6 to 80% for case number 2. These differences might be due to differences in studied hypospadias types (mild, moderate or severe). Data in Table 1 and Figure 1 reflects the ability of each primer to detect genomic instability which range from 25% with primer OPA-01 to 66.6% with OPA-08 primer. This finding might be due to the fact that some loci in the DNA or chromosome were apt to changes of nucleotide sequences like the sequences which amplified by primer OPA-08, while the sequences amplified by primer OPA-01 would tend to remain stable among hypospadias patients (Jianxun et al., 2002). Although, the number of hypospadias samples used in this study is relatively small, these results seem to indicate that there is a close relationship between genomic instability detected by RAPD analysis and this is in agreement with (Ong et al., 1998).

Conclusion

From the obtained results, it could be concluded that,

Table 1. Primer code, their sequences and % of primer detectability of genetic instability among hypospadias cases in comparison with controls.

Primer code	Primer sequence	Samples												Primer detectability (%)
		1	2	3	4	5	6	7	8	9	10	11	12	
OPA-01	CAGGCCCTTC	-	+	-	-	-	-	-	-	+	-	-	+	25
OPA-02	TGCCGAGCTG	-	+	-	-	+	-	-	+	+	+	-	-	41.6
OPA-03	AGTCAGCCAC	+	+	-	-	-	-	-	+	-	+	-	-	33.3
OPA-04	AATCGGGCTG	+	-	-	+	+	+	-	-	+	+	-	-	50
OPA-05	AGGGGTCTTG	+	-	-	+	-	-	-	-	+	-	+	-	33.3
OPA-06	GGTCCCTGAC	-	+	+	+	-	-	-	+	+	-	-	+	50
OPA-07	GAAACGGGTG	-	+	-	+	+	-	+	-	-	+	+	-	50
OPA-08	GTGACGTAGG	+	+	+	+	-	-	+	+	-	+	-	+	66.6
OPA-09	GGGTAACGCC	-	+	-	+	-	-	-	+	-	-	-	+	33.3
OPA-10	GTGATCGCAG	-	+	-	+	-	-	-	+	-	-	-	+	33.3
Total		40	80	20	70	30	10	20	60	50	50	20	40	

+: Genomic instability detected; -: no genomic instability detected.

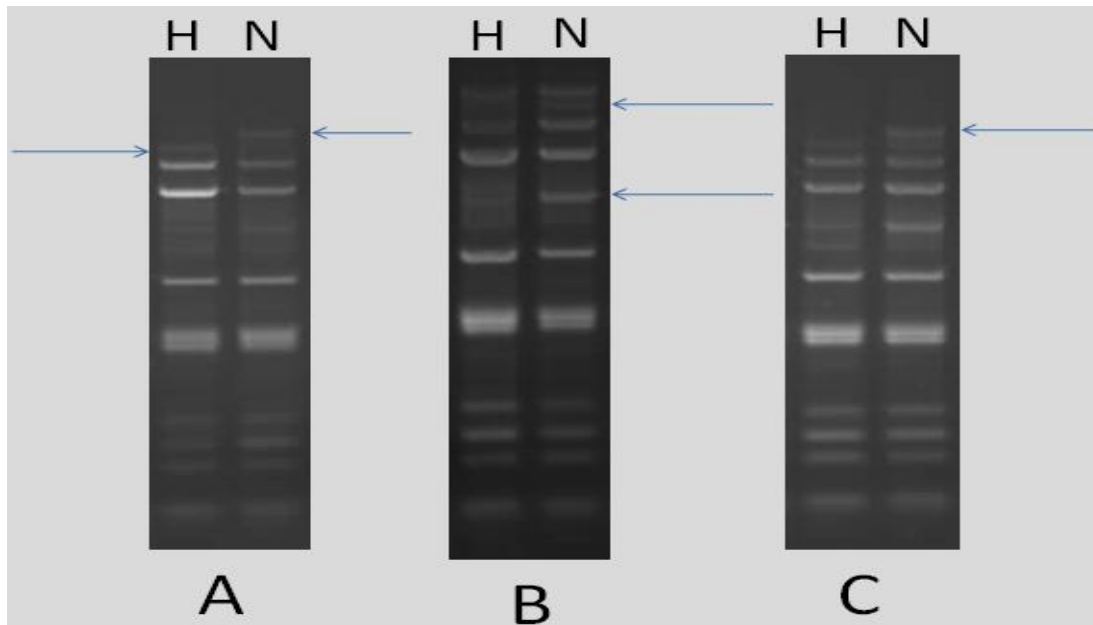


Figure 1. Genetic alteration revealed from RAPD-PCR analysis of hypospadias (h) and corresponding control (n). A, with primer OPA-02; B, with primer OPA-03 and C, with OPA-05.

among hypospadias patients, a number of genetic instabilities represented as loss or gain, shift and/or decrease or increase of intensities of a DNA bands were identified. RAPD-PCR method is suitable for detecting genomic changes among hypospadias patients. Further, deep molecular genetic case control studies with high and low hypospadias grade are needed to: 1) Determine and identify specific DNA markers that could be used in early detection and molecular diagnosis programs of hypospadias; 2) investigate different genes directly involved in the etiology of hypospadias and 3) analyze chromosomal instability among hypospadias patients.

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