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SHORT COMMUNICATION

A novel Alu-mediated microdeletion at 11p13 removes *WT1* in a patient with cryptorchidism and azoospermia

Catarina M Seabra [a,b], Sofia Quental [a], Ana Paula Neto [c], Filipa Carvalho [c], João Gonçalves [d], João Paulo Oliveira [c], Susana Fernandes [c], Mário Sousa [e], Alberto Barros [c], António Amorim [a,f], Alexandra M Lopes [a,*]

[a] *Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal*; [b] *Autonomous Section of Health Sciences, University of Aveiro, Aveiro, Portugal*; [c] *Department of Genetics, Faculty of Medicine, University of Porto, Porto, Portugal*; [d] *Human Genetics Center, National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal*; [e] *Laboratory of Cell Biology, UMIB, ICBAS, University of Porto, Porto, Portugal*; [f] *Faculty of Sciences, University of Porto, Porto, Portugal*

* Corresponding author. *E-mail address:* alopes@ipatimup.pt (A Lopes).

Alexandra M Lopes obtained her PhD in Biology in 2006 from the faculty of science of the University of Porto, Portugal. Following her PhD, she was a postodoctoral fellow at the University of Cambridge, UK. She currently works at the Institute of Molecular Pathology and Immunology of the University of Porto, Portugal as a post-doctoral researcher. Her research has focused on the evolution of sex chromosomes and the impact of chromosome structural rearrangements in the patterns of genetic diversity in human populations and in disease, namely male infertility.

Abstract This article describes a patient with cryptorchidism and nonobstructive azoospermia presenting a novel microdeletion of approximately 1 Mb at 11p13. It was confirmed by multiplex ligation-dependent probe amplification that this heterozygous deletion spanned nine genes (*WT1*, *EIF3M*, *CCDC73*, *PRRG4*, *QSER1*, *DEPDC7*, *TCP11L1*, *CSTF3* and *HIPK3*) and positioned the breakpoints within highly homologous repetitive elements. As far as is known, this is the smallest deletion as-yet described encompassing the *WT1* gene and was detected only once in a total of 32 Portuguese patients with isolated uni- or bilateral cryptorchidism. These findings suggest that molecular analysis in patients with genitourinary features suggestive of *WT1* impairment, namely cryptorchidism and renal abnormalities, may reveal cryptic genetic defects.

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KEYWORDS: azoospermia, cryptorchidism, multiplex ligation-dependent probe amplification, nonallelic homologous recombination, *WT1* cryptic deletion

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Introduction

The Wilms tumour 1 (*WT1*) gene encodes a transcription factor with a preponderant role in male sexual differentiation and kidney development. *WT1* haploinsuficiency results in genital abnormalities such as cryptorchidism and hypospadias, as well as kidney malformations (Little and Wells, 1997). This study group has previously detected by microarray analysis a cryptic deletion at 11p13, spanning *WT1*, in a patient with nonobstructive azoospermia and cryptorchidism (Lopes et al., 2013). The current article presents detailed clinical data on this patient and the molecular characterization of this deletion. To further evaluate the contribution of microdeletions removing *WT1* to the aetiology of cryptorchidism, a group of 31 Portuguese patients with uni- or bilateral forms of the disease were screened by multiplex ligation-dependent probe amplification (MLPA).

Materials and methods

Patient description

The proband, a 37-year-old man, was referred for genetic testing and counselling, following the diagnosis of azoospermia on the workup of primary infertility. His past clinical history was relevant as bilateral cryptorchidism noted at birth and right orchidopexy in early childhood. The proband is the youngest of eight siblings: two of his brothers and his only sister had children but the other four brothers were childless bachelors. He was not aware of any further cases of infertility among his relatives.

On physical examination, besides a small right testis palpable in the lower inguinal canal and the absence of left testis, there were no other relevant abnormalities, including the secondary sexual characteristics. Imaging evaluation by Doppler ultrasonography and magnetic resonance showed empty scrota, a hypoplastic testicle (length 3 cm) in the right inguinal canal and a small solid mass (diameter 2 cm) anterolaterally to the left psoas, below the aortic bifurcation, which was suggestive of undescended left testis. The seminal vesicles were atrophic and cystic, and the veins of the pampiniform plexus appeared dilated. The kidneys were of asymmetric size, with lengths of 13.0 cm and 9.5 cm on the left and the right, respectively. The left kidney was malrotated, with the renal pelvis located more anteriorly and laterally than normal, and contained a simple cortical cyst (diameter 1 cm). One small 5–6 mm calculus was visible in each kidney.

Routine laboratory blood tests, including kidney function, were within normal limits. Total and free testosterone concentrations were respectively 2.82 ng/ml (normal range 2.8–8.0 ng/ml) and 14.09 pg/ml (normal range 8.8–27 pg/ml), while FSH and LH were notably elevated to 66.38 mUI/ml (normal 1.5–12.4 mUI/ml) and 28.12 mUI/mL (normal 1.7–8.6 mUI/ml), respectively. The testicular germ cell tumour markers β-subunit of human chorionic gonadotrophin and α-fetoprotein were not increased. The urinalysis revealed mild albuminuria, with an albumin/creatinine ratio of 63.4 mg/g. The peripheral blood karyotype was normal for a male (46,XY) and there were no microdeletions of the AZF a, b or c regions of the Y chromosome.

A group of 31 infertile males with clinical history of uni- or bilateral cryptorchidism was selected following physical examination, hormonal testing (FSH, testosterone) and standard clinical genetic screening for karyotypic anomalies and Y chromosome microdeletions. The study was included in the project 'Copy number variation in infertile men genomic regions: screening in the Portuguese population' (PTDC/SAU-GMG/101229/2008), approved by the INSA Ethics Committee (Lisbon, Portugal on 6 November 2007).

Genetic analyses

Long-range PCR was performed with ExpandLong Range dNTPack (Roche, Basel, Germany), using 5% DMSO and 250 ng DNA template. Primer sequences are given in Table S1 in the online version at doi:10.1016/j.rbmo.2014.04.017 (available online).

MLPA was performed using the SALSA MLPA P219 PAX6 probemix kit (MRC-Holland, Amsterdam, The Netherlands), according to the manufacturer's recommendations with 200 ng genomic DNA per reaction. The block normalization method was performed, in which the final ratio is obtained by dividing the peak area of each amplification product by the total area of the reference probes only.

Results

MLPA with P219 PAX6 probemix kit, which includes four probes in the region of interest, confirmed the presence of a heterozygous deletion of approximately 1 Mb at 11p13 spanning nine genes: *WT1*, *EIF3M*, *CCDC73*, *PRRG4*, *QSER1*, *DEPDC7*, *TCP11L1*, *CSTF3* and *HIPK3* (Table S2 in the online version at doi:10.1016/j.rbmo.2014.04.017). In order to precisely map the breakpoints of this deletion, long-range PCR was performed and the resulting fragment was sequenced using a primer-walking approach (**Figure 1**). No mutations were found in the single allele in this patient, indicating that the phenotype resulted from *WT1* haploinsufficiency. Examination of the breakpoint showed that it lay within highly homologous Alu Y elements (89% nucleotide identity; chromosome 11 positions 32344139–32344450 and 33385196–33385460), indicating repeat-mediated nonallelic homologous recombination at 11p13 as the likely mechanism for this deletion.

The finding of a cryptic 11p13 deletion involving *WT1* in an infertile patient presenting no major signs of an underlying genetic syndrome prompted this work to assess the impact of *WT1* deletions in isolated cryptorchidism in the study cohort. A total of 31 patients with nonsyndromic uni- or bilateral cryptorchidism were screened for *WT1* deletions using the same MLPA probemix kit (P219 PAX6) but no additional deletions were found.

Discussion

Genomic rearrangements within 11p12-p14 typically remove a large number of genes (Crolla et al., 1997; van Heyningen

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