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Case Report

Azoospermia Counting by Flow Cytometry Reveal X-Linked Mutation

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Abstract

We report a case of aneuploid DNA content in the female parent with 3 male siblings been azoospermic. A single, rapid and reproducible diagnostic test to predict the type of azoospermia and outcome of sperm retrieval is not yet available. So, the feasibility of employing DNA flow cytometry for rapid investigation of X-linked deletions is proposed.

Keywords: Azoospermia; DNA; Infertility

Introduction

Azoospermia, a type of male infertility associated with absence of measurable spermatozoa in the semen, accounts for 20% to 30% of all male infertility cases [1] affecting about 1% of the male population [1,3].

Azoospermia is the medical condition of a man whose semen contains no sperm [3]. Although it has been associated with miscarriage, many types are responsive to target therapeutic strategies. According to epidemiological studies azoospermia is reported in 1% of the male population [4] and may be contribute in up to 20% of male infertility cases [5].

According to scientific literature, several X-linked genes are detected as potential contributors for infertility based on their testicular expression and participation in spermatogenesis. The replacement of clone libraries to high throughput sequencing technologies enabled the identification of additional genetic factors in azoospermic patients either by Targeted Amplicon Sequencing (TAS) or Whole Exome Sequencing (WES). The documented large load of X-linked deletions has been postulated to be an outcome of a high frequency of genomic mutations, which may also end up to medical health challenges. This hypothesis might explain the epidemiological observations showing a link between abnormal spermatogenesis and a higher incidence of common causes of morbidity (including cancer) along with lower life expectancy (Jensen et al. 2009; Salonia et al. 2009; Eisenberg et al. 2014).

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Case Presentation

We report an otherwise normal Greek family with 3 male siblings found to be azoospermic in diagnostic testing during family counseling. The parents did have any fertility problems and according to their knowledge no fertility issues were recorded in any family tree. For that reason, genetic analysis using flow cytometry of blood specimens was performed in each family member to identify possible genetic defects that might be associated with siblings' azoospermia.

Materials and Methods

Both parents of the presented family were queried about their parental origin, family ancestry, and fertility history which revealed no familial history of infertility in either of them. All analyzed siblings had azoospermia, with normal testicular volume and normal Follicle-Stimulating Hormone (FSH) levels. Medical history of the affected siblings was insignificant regarding sexual history, past medical and surgical history, past exposures to sexually transmitted infections and treatment, past and current use of drugs as well as smoking and alcohol intake history. All the study participants consented to undergo genetic evaluation (Figures 1 and 2).

Samples were analyzed on a Cytomics FC 500 flow cytometer (Beckman Coulter), set up with an argon-ion laser emitting at 488 nm (400 m W) for aneuploidy. A minimum number of 17.000 nuclei from each specimen were analyzed.

Results

The data were displayed as histograms. The flow cytometry analysis revealed an aneuploid DNA content in the female parent.

Discussion

Although, validated X chromosome-linked monogenic causes of male infertility are surprisingly rare, various genes are distinctively attained on the human X chromosome and are solely expressed in the testis in physiological conditions. Based on their genetic and modulation interaction and their possible involvement in cell proliferation, these genes are expected to play a role in spermatogenesis.

Flow cytometry enables rapid quantification of DNA content of individual cells, and the cellular DNA content provides useful

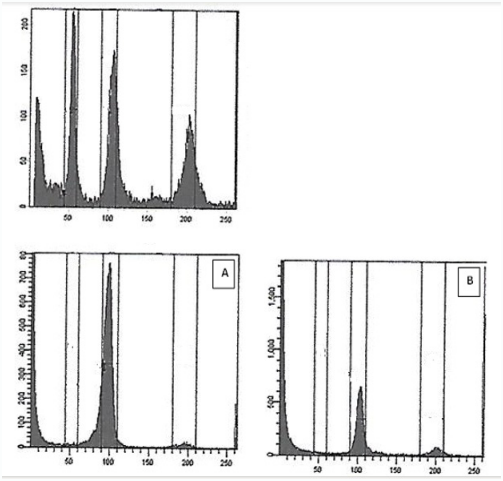

Figure 1: Control.

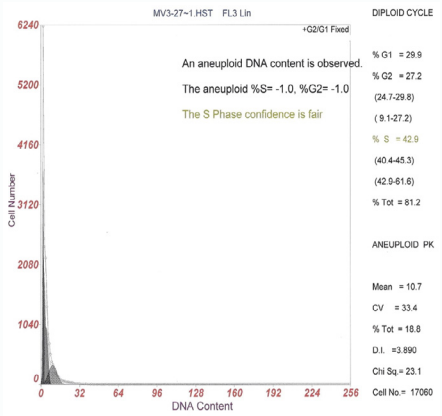

Figure 2: An aneuploid DNA (Diploid) content after Flow Cytometry analysis revealed, (A & B).

information about the ploidy, expressing the modal DNA value, and the proliferative activity in a sample. The ability of flow cytometry to estimate cellular DNA content is based on the measurement of fluorescence from dyes which bind in a stoichiometric manner to DNA [6,7]. As the DNA content is duplicated prior to cell division, mathematical models have been derived which can estimate the percentage of cells in different phases of the cell cycle.

In cancer research, interest has centered on the percentage of cells in the DNA synthetic phase of the cycle (SPF). The performed genetic analysis of our samples disclosed an aneuploid DNA content in the female parent (S=-1.0% & G2=-1.0%), with a 42.9% of cells in the DNA synthetic phase confirming the above assumption. DNA analysis by flow cytometry provides fast results, permits multiparameter analysis correlating DNA content with antigen expression, and provides the sensitivity for detecting near-diploid aneuploid peaks [6].

A diagnostic approach based on flow cytometric assessment for X-linked deletions should be initially considered in genetic investigation of azoospermia, regardless of the patient's age. More work must be done to identify other genetic causes of male infertility.

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