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OBM Genetics

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Case Report

sSMC Characterization in a Male with Turner Syndrome Stigmata

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Academic Editor: Joep Geraedts

Special Issue: Applications of Fluorescence in Situ Hybridization

OBM Genetics
2018, volume 2, issue 3
doi:10.21926/obm.genet.1803033

Received: March 23, 2018
Accepted: August 22, 2018
Published: September 07, 2018

Abstract

Background: Small supernumerary marker chromosomes (sSMC) are rare cytogenetic findings in general, but especially in Turner syndrome so called sSMC^T in a karyotype 46, X,+mar are even more scarce. According to the literature, sSMC^T are derived from one of the Y-chromosomes in ~70% of the cases. Thus, to identify the presence of Y-chromosomal material is imperative, since these cases have an increased risk of gonadoblastoma deriving from the streak gonads.

Methods: A 24-year-old short statured male presented with ambiguous genitalia, undescended testicles and elevated serum testosterone level. Karyotyping and an SRY-gene specific polymerase chain reaction (SRY-PCR) test were done. After detection of the sSMC^T in

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metaphase directed fluorescence in situ hybridization (FISH), sex-chromosome-specific microsatellite analysis and interphase-FISH were additionally applied.
Results: In initial cytogenetic analyses the patient's karyotype was reported as 45, X [30]; however, the presence of SRY-sequences was detected by in parallel performed SRY-PCR analysis of peripheral blood. Applying FISH, a karyotype of mos 46, X, der(Y) (:p11.2->q11.1:)[7]/45,X[13] was found. However, microsatellite analysis using gonosome specific markers indicated the presence of SRY in Yp11.31 and sY84 in Yq11.21, as well. Finally, interphase FISH revealed the presence of the SRY-region in ~8% of the cells. Thus, the final karyotype was defined as: mos 46,X,der(Y)(pter->p11.31::p11.2->q11.1:)[19]/46,X,der(Y)(:p11.2->q11.1:)[32]/45,X[189].
Conclusions: In the sSMC^{T} case presented here stepwise diagnostics, using sequentially molecular cytogenetic and molecular genetic tests was necessary to finally characterize the genetic condition comprehensively.

Keywords
Turner syndrome; small supernumerary marker chromosomes in Turner syndrome sSMC^{T}; Y-chromosome; gonadoblastoma; microsatellite analyses

1. Introduction

Small supernumerary marker chromosomes (sSMC) are defined as derivatives smaller than a chromosome 20, which origin and content can only be characterized by molecular cytogenetic and/ or molecular genetic approaches. They are present in about 2 million of phenotypically normal probands and another 1 million of clinically affected patients worldwide [1]. An sSMC can be observed additionally to an otherwise normal karyotype (i.e. 47,+mar) but also in a numerically aberrant karyotype, like in Down syndrome cases (karyotype 48,+21,+mar). Besides, in 11.4% of reported cases an sSMC is present in Turner syndrome (karyotype 46,X,+mar) [1, 2]; here the sSMC is abbreviated as sSMC^{T} [1]. Still majority of the sSMC^{T} is not well characterized.

Here we report a yet unique case of male TS where karyotyping missed the sSMC^{T}. However, Y-chromosomal material was identified by SRY-specific polymerase chain reaction (PCR) and later the presence of sSMC^{T} confirmed by Y-chromosome specific metaphase fluorescence in situ hybridization (FISH), followed by microsatellite analysis. Finally additional interphase-FISH analyses matched the results from cytogenetics, molecular cytogenetics and molecular genetics.

2. Materials and Methods

A 24-year-old phenotypic male was referred for evaluation and genetic counseling due to ambiguous genitalia. On physical examination, short stature, a broad and coarse face with masculine appearance, short, webbed neck and undescended testes were diagnosed. Endocrine evaluation revealed hypergonadotropic hypogonadism suggesting primary gonadal insufficiency. Laparoscopic evaluation showed presence of a uterus, fallopian tubes and streak gonads; the

latter were surgically removed. Sample collection and written informed consent was obtained as per institutional ethics committee and Helsinki declaration.

Metaphase chromosomes were prepared from phytohemagglutinin stimulated 72-hour lymphocyte cultures and karyotyping was done by GTG-banding at 550-band resolution. Taking into consideration the patient's male phenotype and elevated serum testosterone levels, SRY-gene specific polymerase chain reaction (SRY-PCR) was done as well [3]. FISH was performed, using subsequently mentioned probes and probe sets. First, a commercially available centromeric probe for the Y chromosome together with a probe for the SRY-gene (Abbott/Vysis, Wiesbaden, Germany) and then a subcentromeric-specific multicolor-FISH (subcenM-FISH) probe set for the Y-chromosome [4] were applied, and 20 metaphases each were evaluated. After further microsatellite analyses [5] applying 18 STR markers covering the human sex-chromosomes (see Table 1), 240 interphase cells were evaluated re-using the FISH slide hybridized with centromeric probe for the Y-chromosome and the SRY-probe.

3. Results

Chromosomal analysis from 10 routinely analyzed metaphase spreads revealed a karyotype 45, X in the index patient. However, the result of the in parallel performed SRY-PCR analyses was positive and indicated the presence of Y chromosomal material (Figure 1A).

Afterwards, FISH using a centromeric probe for the Y-chromosome revealed a mosaic karyotype with presence of an sSMC^T in 7 of 20 analyzed cells as mos 45,X[13]/46,X,+der(Y)[7]. However, no SRY-specific signal could be found, neither in the 7 cells with, nor in the 14 cells without sSMC^T (Figures 1B and C).

To check SRY-presence and also integrity of the X-chromosome, microsatellite analysis was done using 18 markers – the results are depicted in Table 1. As SRY-presence was now confirmed by two independent molecular genetic approaches, the first FISH-slide was reanalyzed - now taking into account 240 interphase nuclei. Yet in 19/240 cells (8%) an SRY-signal together with a signal for Y-centromeric probe were seen, while additional 32/240 cells (13%) only had a centromere-Y-specific signal; the remainder 189 cells (79%) had no specific signal for any of the two Y-chromosomal probes (Figure 1D). Thus, the final result was suggested to be most likely as follows: mos 46,X,der(Y)(pter->p11.31::p11.2->q11.1:)[19]/46,X,der(Y)(:p11.2->q11.1:)[32]/45,X[189] (Figure 1E).

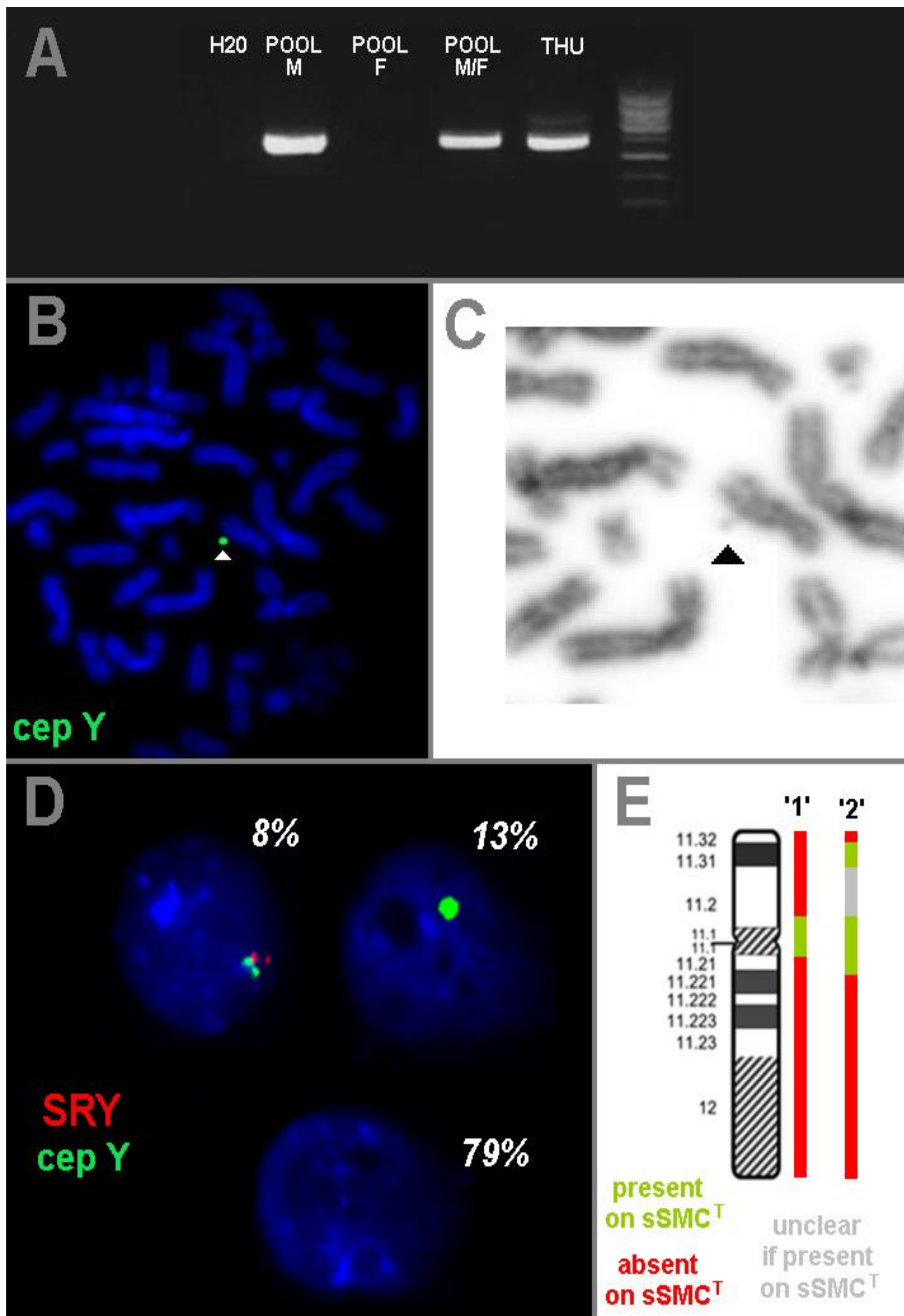

Figure 1 A) PCR gel image showed presence of SRY gene. Lane 1: Negative control 1 (H_20), Lane 2: Positive control 1 (Pooled male = POOL M)), Lane 3: Negative control 2 (Pooled female = POOL F), Lane 4: Positive control 2 (Male:Female, 10:90 = POOL M/F), Lane 5: Patient sample (THU), Lane 6: Size Ladder (100bp). B) The $sSMC^T$ was only visible after fluorescence in situ hybridization (FISH) using a centromeric probe for the Y-chromosome (cep Y = green – marked by arrow-head). C) Enlarged depiction of metaphase from Figure 1C; the $sSMC^T$ which was not identified in GTG-banding due to its small size; i.e. it was considered as “background” there. D) Interphase-FISH results

revealed a mosaic only partially detectable in metaphases; in 79% of 240 cells no Y-chromosome-specific signals could be obtained, in 13% only a signal for the centromere of the Y-chromosome (green; cep Y) was present and in 8% SRY (red) and cep Y (green) signals being colocalized could be detected. One typical nucleus is depicted here for each of the three subclones. E) The results from FISH and molecular genetics are summarized schematically here. der (Y)(:p11.2->q11.1:) is denominated here as ‘1’ was seen by FISH in metaphase and interphase; der(Y)(pter->p11.31::p11.2->q21.1:), denominated as ‘2’ was detectable only in interphase-FISH and by molecular analyses. As there were no microsatellite markers available for distal part of Yp11.2 it is not clear if this region is present or absent on the sSMC^T.

Table 1 18 sex-chromosome specific STR-markers established in house (Jena, Germany), their localization and also the result of microsatellite analyzes in the here tested patient DNA are given.

STR marker	localization	result
Amelogenin	Xp22/Yq11.2	homozygote (only X)
DXS6807	Xp22.32	homozygote (only X)
DXS9898	Xq21.11	homozygote (only X)
DXS6797	Xq22.3	homozygote (only X)
DXS7133	Xq22.3	homozygote (only X)
DXS6854	Xq25	homozygote (only X)
HPRTB	Xq26	homozygote (only X)
SRY	Yp11.31	present
DYS392	Yq11.22	absent
DYS439	Yq11.21	present
ZFY	Xp22.11	present
sY86	Yq11.21	absent
sY127	Yq11.222	absent
sY254	n.a.	absent
SRY	Yq11.31	present
sY84	Yq11.21	present
sY134	Yq11.223	absent
sY255	Yq11.223-q11.23	absent

4. Discussion

In >99% of the cases sSMC^{T}s are gonosomal derivatives. The ones derived from X-chromosome present predominantly as ring chromosomes, while sSMC^T with Y-chromosome-origin mostly are inverted-duplicated/isodicentric chromosomes [1, 2].

As the present case highlights, a really small sSMC^T may easily be missed. Thus, the cytogenetically estimated frequency of 5.5% for Turner syndrome cases with a cell line containing a normal or structurally altered Y-chromosome maybe too small [1, 6, 7]. Besides an overlooked sSMC^T, the following situations might explain the presence of Y-chromosomal sequences : low resolution of applied banding techniques, mosaicism restricted to other tissues than normally studied blood, and/or translocation of Y-chromosome material onto the X-chromosome or

autosomes [6, 7]. Thus, molecular (cyto)genetic approaches need to be applied in all Turner syndrome cases [8, 9]. Clinically the identification of Y-chromosome sequences is indicated in the following two Situations: either the presence of Y-chromosome material is suggested due to the phenotype of the patient, like in the here presented case, and/or it has to be decided if the patient's streak gonads need to precautionary removed in individual cases. The latter is indicated, as an enhanced risk for gonadoblastoma as reported for streak gonads in case the Turner-syndrome patient; developed due to (partial) loss of a Y-chromosome – here such a malignancy has been seen in >30% of the cases [10, 11].

In the present case, two different $sSMC^T$ derived from a Y-chromosome have been detected – as smaller and a larger one. Both derivatives are most likely derived from each other and resulted from incorrectly triggered trisomic rescue [12]. As recently so-called discontinuous sSMC were especially detected in Turner syndrome cases [13] the karyotype was given here as well as with a discontinuous form of the larger sSMC. Due to lack of material this could not be further elaborated.

In conclusion an accurate molecular cytogenetic characterization of $sSMC^T$s and detection of possibly present Y-chromosomal sequences are imperative for patient management and care in Turner syndrome.

Acknowledgments

We are thankful to Dr. Sunil Trivedi for critical evaluation of the manuscript for publication. We are also thankful to the patient and the family for consent and cooperation.

Author Contributions

Concept and experimental design: FS, JA, KM, TL. Clinical analysis: FS and ST. Cytogenetic and molecular analysis: FJ, JA, KM, TL. First draft manuscript: NL, ST, JJS. Critical revisions and approval of final version: TL. Final manuscript was reviewed and approved by all authors.

Funding

No funding support was received for this part of the work.

Competing Interests

The authors have declared that no competing interests (financial or non-financial).

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