

CASE REPORT

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Identification of satellited markers by microdissection and fluorescence in situ hybridization: a clinical case of isodicentric chromosome 22

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Abstract

Background: The presence of small supernumerary marker chromosomes (sSMCs) in a karyotype leads to diagnostic questions because the resulting extra material may cause abnormal development depending on the origin of the duplication/triplication. Because SMCs are so small, their origin cannot be determined by conventional cytogenetic techniques, and new molecular cytogenetic methods are necessary. Here, we applied a target approach to chromosome rearrangement analysis by isolating a chromosome of interest via microdissection and using it in fluorescence in situ hybridization (FISH) as a probe in combination with whole-chromosome painting probes. This approach allows to identify origins of both the euchromatin and repeat-rich regions of a marker.

Case presentation: We report a case of an adult male with congenital atresia of the rectum and anus, anotia, and atresia of the external auditory canal along with hearing loss. Karyotyping and FISH analysis with whole-chromosome painting probes of acrocentric chromosomes and the constructed microdissection library of the marker chromosome reliably identified an additional chromosome in some metaphases: mos 47,XY, +idic(22)(q11.2)[14]/46,XY [23].

Conclusion: We propose to use whole-chromosome libraries and microdissected chromosomes in FISH to identify SMCs enriched with repeated sequences. We show that the methodology is successful in identifying the composition of a satellited marker chromosome.

Keywords: Bisatellite isodicentric, Microdissection, Tetrasomy, Supernumerary marker chromosome, Congenital atresia, Cryptorchidism, Mosaicism, Acrocentric, Chromosome 22, Repeats

Background

Small supernumerary marker chromosomes (SMCs) are structurally abnormal chromosomes that are generally equal in size to or smaller than chromosome 20 of the same metaphase spread [1]. SMCs are found in ~0.043% of newborns and ~0.077% of prenatal cases and are tenfold more prevalent among patients with intellectual disability and fourfold more prevalent among subfertile

individuals [2]. Most SMCs (~70%) originate from short arms and pericentromeric regions of acrocentric chromosomes [3]. SMCs derived from nonacrocentric autosomes are rarer and arise at a frequency of ~30% of all markers [4]. It has been previously reported that satellite markers derived from chromosome 22 pose a high risk of a phenotypic abnormality [5]. On the contrary, markers derived from chromosomes 13, 14, 15, and 21 as well as small ring autosomal markers derived from both alphoid and satellite II or III pericentric heterochromatin of chromosomes 1, 9, 15, and 16 are all associated with a low risk of a phenotypic abnormality [5].

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Approximately one-third of small SMC (sSMC) cases have a specific clinical picture, and most of the remaining sSMCs are not yet correlated with clinical syndromes. Specific SMCs have been associated with Pallister–Killian syndrome involving isochromosome 12p, inverted duplication 15q12–q13 syndrome, isochromosome 18p syndrome, and cat eye syndrome (CES) [1].

Conventional karyotyping can certainly detect large aberrations, but origin identification for small supernumerary elements is beyond its resolution. For identification of sSMCs carrying heterochromatin or repeated sequences, the common method of GTG staining is not suitable. Fluorescence in situ hybridization (FISH) with human-sorted chromosome-specific painting probes is informative only when the fragment of a q arm with euchromatin is present in the marker. Thus, FISH designed to help with the identification of marker origin is powerless in cases of chromosomes derived from repeat-rich fragments. Here, we use microdissection of an sSMC followed by direct and reverse painting of affected and unaffected human metaphases to identify the origin of the sSMC and to determine the region of homology up to the cytogenetic-band level.

Case presentation

An adult male patient (19 years old at the time of the study) with intellectual disability has a large number of congenital malformations. Clinical findings in the proband included congenital atresia of the rectum and anus (corrected after birth), anotia, and atresia of the external auditory canal together with mixed conductive and sensorineural hearing loss. Computed tomography of temporal bones revealed signs of atresia of the right external auditory canal, hypoplasia of the tympanic cavity, and aplasia of cells of the right mastoid process. No other malformations were detectable, e.g., iris coloboma was absent.

FISH with whole-chromosome painting probes of acrocentric chromosomes revealed a narrow band from chromosome 22 on an sSMC and faint signals from all acrocentric chromosome probes on both arms of the marker. We then carried out microdissection of the chromosome of interest. By FISH of this microdissected whole-chromosome probe onto normal metaphases and metaphases with a marker, we determined the origin of the sSMC as bisatellite and dicentric $\text{idic}(22)(\text{pter} \rightarrow \text{q11.2}::\text{q11.2} \rightarrow \text{pter})$, which causes partial tetrasomy by inverted duplication of an HSA22 fragment (Fig. 1). FISH and the microdissection were performed as described earlier [6–10]. The isodicentric bisatellited chromosome formation model was proposed in the last century [11]. The isodicentric chromosome 22 [$\text{idic}(22)$] formation scheme in a simplified outline is shown in Fig. 2. No rearrangements were found in the proband's mother's metaphases, and the father's karyotype was not available.

Discussion

The majority of sSMCs (65%) originate from chromosome 15, whereas sSMCs derived from other acrocentric chromosomes constitute only 7% [12]. A subset of SMCs(22) confers tri- or tetrasomy onto the cat eye chromosomal region [1]. Chromosome 22 partial tetrasomy is present in several syndromes such as CES and $\text{inv dup}(22)(\text{q11.2})$ syndrome. Approximately 40% of CES patients present with the classic triad of symptoms: anal anomalies, preauricular skin tags, and coloboma of the iris, which may make an eye look like a cat eye [12]. In contrast to other satellite markers, an sSMC derived from chromosome 22 commonly has serious phenotypic effects [5].

Approximately 23% of SMCs are inherited, either from the mother (16%) or father (7%), and the rest are formed de novo [4]. In early studies, there were reports about

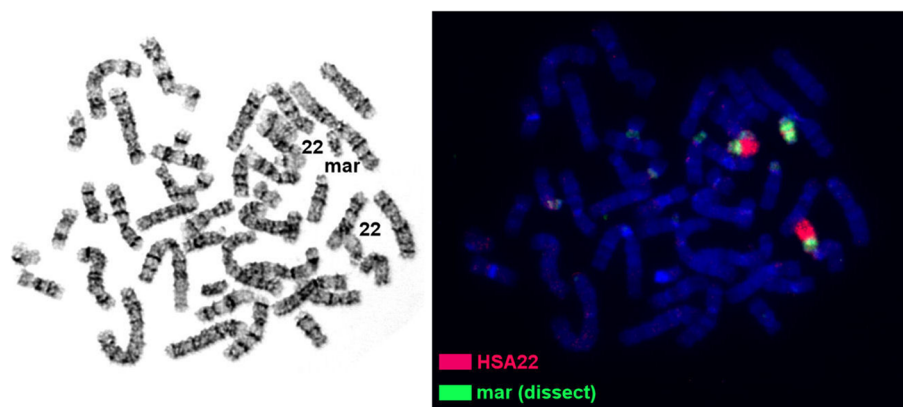


Fig. 1 FISH with painting probes of human chromosome 22 (red signal) and of the marker microdissected chromosome (green signal) on metaphase chromosomes of the proband

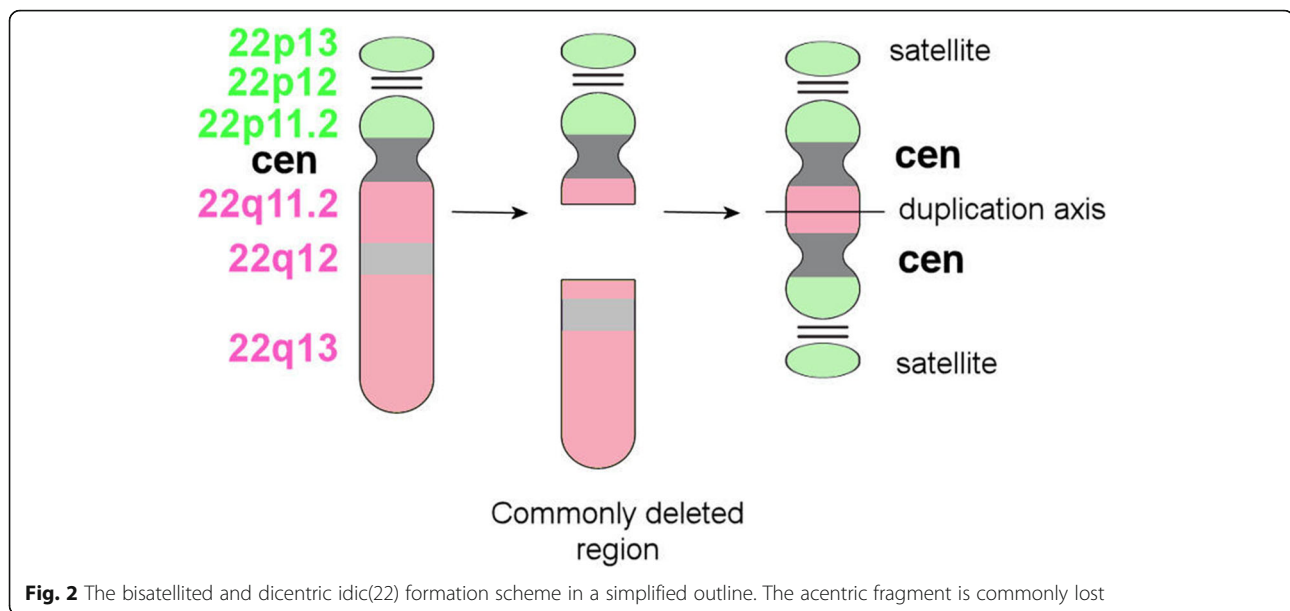


Fig. 2 The bisatellited and dicentric idic(22) formation scheme in a simplified outline. The acentric fragment is commonly lost

direct transmission of CES [13–17]. Rarely, CES is transmitted as a result of a balanced translocation in one of the parents [18, 19]. Currently, it is generally accepted that most cases of chromosome 22 partial tetrasomy are not inherited and arise *de novo*. The condition generally occurs sporadically as a random event during oogenesis or spermatogenesis. The *de novo* origin of the idic(22) described here is possible, being supported by the mosaic nature of the rearrangement. The growing amount of information on clinical and laboratory data from previously described carriers of sSMCs is valuable. Detailed reporting of every case may help future clinicians and parents to make proper prognostic assessments and reproductive decisions.

Here, repeat-rich p arms of the marker chromosomes prevented their reliable identification by FISH with a standard whole-chromosome painting probe alone, producing a faint background signal on short arms of all acrocentrics and in the marker. The combined application of direct FISH with a sorted whole-chromosome DNA probe and reverse painting with DNA of a microdissected repeat-rich marker was developed here as an efficient way to identify isodicentric markers formed from short p arms and pericentromeric regions of human acrocentric chromosomes. The marker was identified as dicentric idic(22)(pter→q11.2::q11.2→pter). The region affected by the rearrangement is composed of a gene-poor and repeat-rich p arm and of the euchromatic region of proximal 22q.

Conclusions

Microdissection is an efficient technique for constructing specific probes for marker chromosomes and is

especially valuable in cases of small repeat-rich elements. The constructed microdissection library of idic(22) is now available and included in the array of FISH probes for cytogenetic diagnosis of sSMCs.

Abbreviations

CES: Cat eye syndrome; FISH: Fluorescence in situ hybridization; idic: Isodicentric chromosome; sSMC: Small supernumerary marker chromosome

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Authors' contributions

NAL—cell culturing and chromosome fixation, karyotyping, FISH analysis, study concept, manuscript preparation, financial support. SAR—microdissection, manuscript preparation. YVM—clinical study of patients and sampling. ARS—clinical study of patients and sampling. DVY—manuscript preparation, financial support. All of the authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article. Additional information is available from the corresponding author on request.

Ethics approval and consent to participate

The involvement of patients, their relatives in the study was strictly designed in accordance with international standards, which include the awareness of a subject (or his/her representative) and his or her informed consent to participate in the study in its entirety and guarantees of confidentiality. All the analyses conformed to ethical standards developed in accordance with the Helsinki Declaration of the World Medical Association, as amended in 2000. In addition, the analyses were supervised by the Ethics Committee on

Animal and Human Research of the Institute of Molecular and Cellular Biology, the Siberian Branch of the Russian Academy of Sciences (IMCB, SB RAS), Russia (protocol №01/21 from 26 January 2021). Consent was obtained from the patient, but only in Russian.

Consent for publication

Written informed consent was obtained from the legal representative of the patient.

Competing interests

The authors declare no conflict of interest.

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References

1. Liehr T, Mrasek K, Weise A, Dufke A, Rodríguez L, Martínez Guardia N, et al. Small supernumerary marker chromosomes – progress towards a genotype-phenotype correlation. *Cytogenet Genome Res* 2006;112:23–34. DOI: <https://doi.org/10.1159/000087510>.
2. Liehr T, Weise A (2007) Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics. *Int J Mol Med* 19:719–731. <https://doi.org/10.3892/ijmm.19.5.719>
3. Reddy KS, Aradhya S, Meck J, Tiller G, Abboy S, Bass H (2013) A systematic analysis of small supernumerary marker chromosomes using array CGH exposes unexpected complexity. *Genet Med* 15:3–13. <https://doi.org/10.1038/gim.2012.78>
4. Crolla JA, Youings SA, Ennis S, Jacobs PA (2005) Supernumerary marker chromosomes in man: parental origin, mosaicism and maternal age revisited. *Eur J Hum Genet* 13:154–160. <https://doi.org/10.1007/s004390100571>
5. Callen DF, Eyre H, Yip MY, Freemantle J, Haan EA (1992) Molecular cytogenetic and clinical studies of 42 patients with marker chromosomes. *Am J Med Genet* 43:709–715. <https://doi.org/10.1002/ajmg.1320430412>
6. Young BD, Ferguson-Smith MA, Sillar R, Boyd E (1981) High-resolution analysis of human peripheral lymphocyte chromosomes by flow cytometry. *Proc Natl Acad Sci U S A* 78:7727–7731. <https://doi.org/10.1073/pnas.78.12.7727>
7. Ferguson-Smith MA, Yang F, O'Brien PCM (1998) Comparative mapping using chromosome sorting and painting. *ILAR J* 39:68–76. <https://doi.org/10.1093/ilar.39.2-3.68>
8. Telenius H, Carter NP, Bebb CE, Nordenskjöld M, Ponder BA, Tunnacliffe A (1992) Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. *Genomics* 13:718–725. [https://doi.org/10.1016/0888-7543\(92\)90147-k](https://doi.org/10.1016/0888-7543(92)90147-k)
9. Graphodatsky AS, Yang F, Serdukova N, Perelman P, Zhdanova NS, Ferguson-Smith MA (2000) Dog chromosome-specific paints reveal evolutionary inter- and intrachromosomal rearrangements in the American mink and human. *Cytogenet Cell Genet* 90:275–278. <https://doi.org/10.1159/000056788>
10. Yang F, O'Brien PC, Milne BS, Graphodatsky AS, Solanky N, Trifonov V et al (1999) A complete comparative chromosome map for the dog, red fox, and human and its integration with canine genetic maps. *Genomics* 62:189–202. <https://doi.org/10.1006/geno.1999.5989>
11. Van Dyke DL, Weiss L, Logan M, Pai GS (1977) The origin and behavior of two isodicentric bisatellited chromosomes. *Am J Hum Genet* 29:294–300
12. LLiehr T (2012) Small supernumerary marker chromosomes (sSMC): a guide for human geneticists and clinicians. Heidelberg, Dordrecht, London, New York: Springer. <https://doi.org/10.1007/978-3-642-20766-2>
13. Schachenmann G, Schmid W, Fraccaro M, Mannini A, Tiepolo L, Perona GP, et al. Chromosomes in coloboma and anal atresia. *Lancet* 1965;286:290. DOI: [https://doi.org/10.1016/s0140-6736\(65\)92415-3](https://doi.org/10.1016/s0140-6736(65)92415-3).
14. Dallapiccola B (1971) Dermatoglyphics and chromosomes in cat-eye syndrome. *Br Med J* 3:767–768. <https://doi.org/10.1136/bmj.3.5777.767-c>
15. Schinzel A, Schmid W, Fraccaro M, Tiepolo L, Zuffardi O, Opitz JM, et al. The cat eye syndrome: dicentric small marker chromosome probably derived from a No. 22 (Tetrasomy 22pter(q11) associated with a characteristic phenotype. *Hum Genet* 1981;57. DOI: <https://doi.org/10.1007/bf00282012>.
16. Lüleci G, Bağcı G, Kivran M, Lüleci E, Bektaş S, Başaran S. A hereditary bisatellite-dicentric supernumerary chromosome in a case of cat-eye syndrome. *Hereditas* 1989;111:7–10. DOI: <https://doi.org/10.1111/j.1601-5223.1989.tb00369.x>.
17. Van Hove JLK, McConkie-Rosell A, Chen Y-T, Kimberly lafolla A, Lanman JT, Hennessy MD et al (1992) Unbalanced translocation 46,xy,-15, der(22)t(15;22)(q13;q11)pat: case report and review of the literature. *Am J Med Genet* 44:24–30. <https://doi.org/10.1002/ajmg.1320440107>
18. Ing PS, Lubinsky MS, Smith SD, Golden E, Sanger WG, Duncan AMV et al (1987) Cat-eye syndrome with different marker chromosomes in a mother and daughter. *Am J Med Genet* 26:621–628. <https://doi.org/10.1002/ajmg.1320260317>
19. Kosztolányi G, Bühler EM (1985) Partial trisomy 22—an old case reexamined. *Hum Genet* 69:193–194. <https://doi.org/10.1007/BF00293299>

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