Mosaic sex chromosome aneuploidy 47,XXY/46,XX versus homozygous Klinefelter syndrome (47,XXY)

Acronym: **MOSAI-XX**

English title: **Gonosomal mosaic aneuploidy 47,XXY/46,XX versus homogeneous Klinefelter syndrome (47,XXY)**

Research protocol not involving human subjects

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This study was not submitted in response to a call for proposals

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1. Abbreviations

AMH	:	Anti-Müllerian hormone
AP-HP	:	Public Health Service – Paris Hospitals
CRMR	:	Rare Disease Reference Center
DPD	:	Data Protection Officer
DPI	:	Electronic Health Record
FISH	:	*Fluorescent In Situ Hybridization*
FSH	:	Follicle-Stimulating Hormone
KS	:	Klinefelter syndrome
INSL3	:	*Insulin-Like Factor 3*
LH	:	Luteinizing Hormone
mKS-XX	:	Mosaic Klinefelter syndrome 47,XXY/46,XX
GDPR	:	General Data Protection Regulation
RNIPH	:	Research Not Involving Human Subjects

2. Synoptic Summary

Title	Mosaic 47,XXY/46,XX sex chromosome aneuploidy versus homogeneous Klinefelter syndrome (47,XXY)
Short Title/Acronym	MOSAI-XX study
Principal Investigator	Dr. Luigi MAIONE, MCU-PH, Adult Endocrinology and Reproductive Medicine Department, 78 Rue du Général Leclerc, 94270 Le Kremlin-Bicêtre, Assistance Publique – Hôpitaux de Paris Luigi.maione@aphp.fr, 01 45 21 37 09
Principal Investigator	Dr. Luigi MAIONE
Data Controller	Assistance Publique – Hôpitaux de Paris
Study Type	Multicenter retrospective study using clinical data
Number of centers	2
List of centers	1) Department of Endocrinology and Reproductive Medicine, Bicêtre Hospital, Barré-Sinoussi Building, Door 40 –2nd floor, 78 Rue du Général Leclerc, 94270 Le Kremlin Bicêtre 2) Department of Pediatric Endocrinology and Diabetology, Bicêtre Hospital, Alagille 2 Building, Door 7, 2nd floor, 78 Rue du Général Leclerc, 94270 Le Kremlin Bicêtre
Study Background	Mosaic 47,XXY/46,XX (mKS-XX) sex chromosome aneuploidy is a rare, even exceptional, variant of Klinefelter syndrome (KS, 47,XXY), with only 20 cases described in the literature. Clinical descriptions are rare. Furthermore, no comparative study comparing mKS-XX and KS has been reported to date.
Study Objective(s)	1. To describe the clinical and biological parameters of a population of patients with the 47,XXY/46,XX (mKS-XX) karyotype. 2. To describe the clinical and biological parameters of a population of patients diagnosed with homogeneous Klinefelter syndrome (non-mosaic 47,XXY). 3. Compare the clinical and biological characteristics of the mKS-XX patient population with those of patients with homogeneous Klinefelter syndrome (non-mosaic 47,XXY) in order to identify predictive factors that distinguish between these two conditions. 4. Establish correlations between certain clinical and biological parameters in the mKS-XX population, namely: a. Final height versus percentage of XX cell lines. b. Final size normalized to target size versus percentage of XX cell lines. c. Age at diagnosis versus percentage of XX cell lines. d. Concentrations of testosterone versus percentage of XX cell lines. e. LH and FSH levels versus percentage of XX cell lines.
Evaluation Criteria	1) Since the primary objective of this research is to describe a patient population, the concept of an outcome measure is not relevant in this case. Descriptive analyses including means,

	standard deviations, and percentages will be performed to characterize the two study populations. 2) Since the second objective of the study is to describe a patient population, the concept of an outcome measure is not relevant in this case. Descriptive analyses including means, standard deviations, and percentages will be performed to characterize the two study populations. 3) For continuous quantitative variables (see list in the appendix), comparative analyses will be conducted using an unpaired t-test, assuming a non-normal distribution of the data (Mann-Whitney method). For categorical variables (see list in the appendix), a contingency analysis will be performed (Fisher's exact test). 4) Linear regressions performed using the Pearson's correlation coefficient for all correlations tested.
Study Population	<u>Inclusion criteria</u>: **Group A:** patients, minors or adults, diagnosed with mosaic Klinefelter syndrome (47,XXY/46,XX) confirmed by two cytogenetic tests (2 karyotypes or 1 karyotype and 1 FISH) and followed up in the Department of Endocrinology and Reproductive Medicine and/or the Department of Pediatric Endocrinology and Diabetology at Bicêtre Hospital (AP-HP.Paris-Saclay University) between 2002 and 2023. **Group B:** adult patients with non-mosaic Klinefelter syndrome (47,XXY) confirmed by two cytogenetic tests (2 karyotypes or 1 karyotype and 1 FISH), and followed up in the Department of Endocrinology and Reproductive Medicine at Bicêtre Hospital (AP-HP.Paris-Saclay University) between 2002 and 2023. <u>Exclusion criteria</u>: • Patients or their legal representatives who object to the use of their data or their child's data for research. • Patients whose information in the medical record is too incomplete or unavailable.
Size of the study population	**Group A**: 8 patients **Group B**: approximately 120 patients
Duration of the study	12 months
Participant Information	Group and individual
Data source	Patient medical records (paper and ORBIS EHR)

3. Research Type and Regulatory Requirements

A. Nature of the research

The study *"Mosaic 47,XXY/46,XX sex chromosome aneuploidy versus homozygous Klinefelter syndrome (47,XXY)"* is a multicenter retrospective study. It is based on data from the care of patients in the Department of Endocrinology and Reproductive Medicine and the Department of Pediatric Endocrinology and Diabetology at Bicêtre Hospital (AP-HP, Paris-Saclay University). It falls under the category of research not involving human subjects (RNIPH).

B. Specifics of the Research

Is this research a genetic study?	☐ YES	☒ NO
Does the research require specific samples?	☐ YES	☒ NO
Does the research require the creation of a collection of biological samples?	☐ YES	☒ NO
The research use a biological biological that has already been assembled?	☐ YES	☒ NO
Does this project involve the import and/or export of products of human origin?	☐ YES	☒ NO

C. Data Protection

The contact information for the Data Protection Officer at Assistance Publique – Hôpitaux de Paris is:
protection.donnees.dsi@aphp.fr

No subcontractors are used for this study.

4. Scientific Rationale for the Research

Klinefelter syndrome (KS) is the most common dysgonosomy, with an incidence of 1 in 600 live-born males. Its clinical manifestations are diverse and vary with age. The diagnosis, confirmed by karyotyping, reveals a 47,XXY chromosomal pattern, which is homogeneous in more than 85% of cases. In the remaining cases, the chromosomal patterns are more complex (48,XXXY, 49,XXXXY) or involve mosaicism, defined by the coexistence of different clonal cell lines, usually 46,XY.
Mosaic 47,XXY/46,XX gonosomal aneuploidy (mKS-XX) is a very rare variant of Klinefelter syndrome, with only 20 cases described in the literature [1–8]. Consequently, there is little clinical and biological data available regarding these patients. Furthermore, clinical observations are based primarily on individual clinical cases, which are often extreme and may involve potential recruitment biases. Finally, no comparative study between mKS-XX and KS has been reported to date.

The Department of Endocrinology and Reproductive Medicine and the Department of Pediatric Endocrinology and Diabetology at Bicêtre Hospital (AP-HP, Paris-Saclay University) are designated by the DGOS as a Reference Center for Rare Genital Development Disorders: From Fetus to

Adulthood (CRMR DevGen) and specialize in the management of variations in genital development, which includes Klinefelter syndrome. By conducting a preliminary feasibility study covering the period from 2002 to 2023, we identified eight patients (minors and adults) with mKS-XX who were treated in one or the other department (sometimes in both, as these departments are located on the same campus). In addition, our active patient registry of adults with non-mosaic Klinefelter syndrome includes approximately 120 patients over the same time period (2002–2023).

5. Research Objectives and Evaluation Criteria

A. Objectives

1. To describe the clinical and biological characteristics of a population of patients with the 47,XXY/46,XX karyotype (mKS-XX).
2. To describe the clinical and biological characteristics of a patient population diagnosed with homogeneous Klinefelter syndrome (non-mosaic 47,XXY).
3. Compare the clinical and biological characteristics of the mKS-XX patient population with those of patients with homogeneous Klinefelter syndrome (non-mosaic 47,XXY) to identify predictive factors that distinguish between these two conditions.
4. Establish correlations between certain clinical and biological parameters in the mKS-XX population, namely:
    a. Final height versus percentage of XX cell lines.
    b. Final size normalized to target size versus percentage of XX cell lines.
    c. Age at diagnosis versus percentage of XX cell lines.
    d. Testosterone concentrations versus percentage of XX cell lines.
    e. LH and FSH concentrations versus percentage of XX cell lines.

B. Evaluation Criteria

1) Since the primary objective of the research is to describe a patient population, the concept of an outcome measure is not relevant in this case. Descriptive analyses including means, standard deviations, and percentages will be performed to characterize the two study populations.
2) Since the second objective of the research is to describe a patient population, the concept of an outcome measure is not relevant in this case. Descriptive analyses including means, standard deviations, and percentages will be performed to characterize the two study populations.
3) For continuous quantitative variables (see list in the appendix), comparative analyses will be conducted using an unpaired t-test, assuming a non-normal distribution of the data (Mann-Whitney method). For categorical variables (see list in the appendix), a contingency analysis will be performed (Fisher's exact test).
4) Linear regressions will be performed using Pearson's method for all tested correlations.

6. Research Methodology

A. Experimental Design

The study “Mosaic 47,XXY/46,XX sex chromosome aneuploidy versus homogeneous Klinefelter syndrome (47,XXY)” is a multicenter retrospective study. It is based on data from the care of patients in the Department of Endocrinology and Reproductive Medicine and the Department of Pediatric Endocrinology and Diabetology at Bicêtre Hospital (AP-HP, Paris-Saclay University).

B. Study Population

1. Inclusion Criteria

The study population consists of two groups:

Group A: patients, both minors and adults, with a diagnosis of mKS-XX confirmed by two cytogenetic tests (2 karyotypes or 1 karyotype and 1 FISH) and followed in the Department of Endocrinology and Reproductive Medicine and/or the Department of Pediatric Endocrinology and Diabetology at Bicêtre Hospital (AP-HP.Paris-Saclay University) between 2002 and 2023.

Group b: adult patients with non-mosaic Klinefelter syndrome (47,XXY) confirmed by two cytogenetic tests (2 karyotypes or 1 karyotype and 1 FISH), and followed up in the Department of Endocrinology and Reproductive Medicine at Bicêtre Hospital (AP-HP.Paris-Saclay University) between 2002 and 2023.

2. Exclusion Criteria

The following patients will not be included in the study:

- Patients who object to the use of their medical data for research.
- Patients whose information in the medical record is too incomplete or unavailable.

3. Feasibility Study

A feasibility study was conducted to determine the approximate number of patients to whom the study could be offered. For Group A, approximately 8 patients, including one minor between the ages of 7 and 10, were identified. For Group B, approximately 120 patients were identified.

C. Study Period

The analysis will focus on patient records from patients observed and examined between 2003 and 2023.

D. Study Procedure for Participants

The patient will not be involved in the research. They will receive information in accordance with the procedures described in the section “Procedures for Informing the Affected Population.”

E. Study Duration

The estimated duration of the study is 12 months from the date regulatory approvals are obtained.

F. Statistical Analysis

Descriptive analyses including means, standard deviations, and percentages will be conducted to characterize the two study populations.

Comparative statistics between the mKS-XX and KS populations will be performed on a number of variables:

- For continuous quantitative variables (see list in the appendix), comparative analyses will be conducted using an unpaired t-test, assuming a non-normal distribution of the data (Mann-Whitney method).
- For categorical variables (see list in the appendix), a contingency analysis will be performed (Fisher's exact test).

Finally, linear regressions will be performed to assess the relationship between certain parameters of the mKS-XX population using Pearson's method.

Statistical analyses will be performed using GraphPad Prism software, version 6.0, provided by our institution.

7. Data Management

A. Data Collection and Protection Procedures

1. Source Data

The data necessary for the proper conduct of the study (and only those data) will be collected by members of the research team from patients' medical records (ORBIS EHR and/or paper records).

2. Data Files

The software used to collect and store data extracted from the source database will be Excel. The individuals responsible for data collection will manually complete the file based on what they see in the source database. Two files will be generated by each of the two departments conducting the research:

- A pseudo-anonymized data file, in which each patient will be represented by an alphanumeric code of the form *"Department Code – 3-digit Code."* This file will be protected by a password that may be shared by both departments involved in the research.
- A lookup table file linking the patient's identity to their alphanumeric code. This file will be protected by a unique password that is different for each of the two departments involved in the research.

The coordinating investigator will be responsible for merging the data files.

The Excel files will be stored on a secure server with restricted access specific to each department.

3. Data Access

Pseudo-anonymized data will be accessible to all members of the research team.

A patient's personally identifiable data will be accessible only to research staff in the department where the patient is being treated.

B. Data Flow

Identifiable files (mapping table) remain stored in their respective departments.

The coordinating investigator, a physician in the Endocrinology and Reproductive Medicine Department at Bicêtre Hospital, retrieves the pseudonymized data file from the second department at the same hospital involved in the research, using the "DISPOSE" IT solution (the AP-HP's secure *dropbox*).

The coordinating investigator merges the two pseudo-anonymized data files.

There are no subcontractors for this research.

C. Retention Period for Documents and Data

The research data will be retained for two years following the publication of the study or the publication of the final research report. No archiving will be performed; the research investigators agree to destroy the data after this period.

8. Ethical and Legal Considerations

A. Study Sponsor

Since the study falls within the scope of research not involving human subjects (RNIPH, or a study not covered by the Jardé Law), there is no sponsor. Assistance Publique – Hôpitaux de Paris is the data controller for personal data.

B. Procedures for Informing the Affected Population

Information for patients and legal guardians will be provided at two levels: collective and individual. Regarding collective information, all AP-HP patients and their legal representatives are informed of the potential use of their data for scientific purposes (to advance biomedical research) and of their rights through notices posted in hospital departments, as well as in all correspondence such as "appointment notices" and "reports" regarding consultations and/or hospitalizations.

1. Individual Information Sheet for Adult Patients

The patient will be informed of the study via an individual information sheet that will be handed to them in person during their next routine visit to the department. If hand delivery is not possible, a letter or email will be sent to the patient. This notice will explain the context of the study, its objectives, the study protocol, the patient's rights regarding the research, and in particular the right to object to the use of their data. If, at any time prior to the publication of the final research report, the patient expresses objection to the use of their healthcare data, they will be excluded from the study. The patient's medical care will not be affected by the conduct of the study, regardless of their decision.

2. Individual Information Sheet for Minors

A minor patient between the ages of 7 and 10 may potentially be included in the study. The patient's legal guardians will be informed of the study via an information sheet that will be handed to them in person during their next routine visit to the department. If hand delivery is not possible, a letter or email will be sent to them. This information sheet will explain the context of the study, the general research hypothesis, the study's objectives, the study protocol, their rights and their child's rights regarding the research, and in particular the right to object to the use of their child's data. They will be asked, to the extent possible and taking into account the child's ability to understand, to discuss the research with their child and to respect the child's choice.

A simplified information sheet will also be provided to the minor patient. It will be drafted in accordance with the recommendations of the CERPed (http://www.cerped.fr/). Since the patient is between 7 and 10 years old, informal language will be used and drawings will be included.

If, at any time prior to the publication of the final research report, a legal representative of the patient or the patient themselves objects to the use of the data, the patient will be excluded from the study. The patient's medical care will not be affected by the conduct of the study, regardless of their decision.

C. Procedures Regarding Data Protection Regulations

The research protocol, information sheets, and an impact assessment were submitted to the Data Protection Officer (DPO) of the APHP-Paris-Saclay University Hospital Group. Based on these documents, the DPO determined that the research design complies with the "Reference Methodology for the Processing of Personal Data in the Context of Health Research" (MR-004, as amended). The AP-HP has signed a commitment to comply with this "Reference Methodology."

The data processing carried out as part of this research has been entered into the AP-HP data processing register, which is under the responsibility of the AP-HP Data Protection Officer, in accordance with the provisions of the GDPR (General Data Protection Regulation).

D. Request for an Ethical Review

An ethical review was requested from the INSERM Ethics Review Committee (CEEI).

E. Publication Guidelines

The first authors of publications shall be those who actively participated in the development of the protocol, its implementation, and the drafting of the results.

Assistance Publique-Hôpitaux de Paris must be cited as the research sponsor, and the terms "Assistance Publique-Hôpitaux de Paris" must appear in the authors' addresses.

9. References

1. Hovnik T, Zitnik E, Avbelj Stefanija M, Bertok S, Sedej K, Bancic Silva V, et al. An Adolescent Boy with Klinefelter Syndrome and 47,XXY/46,XX Mosaicism: Case Report and Review of Literature. Genes. Apr 23, 2022;13(5):744.

2. Low KJ, Abdel-Fattah S, Barton J, Crowne EC, Denbow M, Lerpiniere N, et al. Rare XXY/XX mosaicism in a phenotypic male presenting as cystic hygroma in the first trimester. Clin Dysmorphol. Apr 2017;26(2):1079.

3. Mohd Nor NS, Jalaludin MY. A rare 47 XXY/46 XX mosaicism with clinical features of Klinefelter syndrome. Int J Pediatr Endocrinol. 2016;2016:11.

4. Ozsu E, Mutlu GY, Cizmecioglu FM, Ekingen G, Muezzinoglu B, Hatun S. Ovotesticular disorder of sexual development and a rare 46,XX/47,XXY karyotype. J Pediatr Endocrinol Metab JPEM. 2013;26(7–8):789–91.

5. Pattamshetty P, Mantri H, Mohan V. Rare disorder of sexual differentiation with a mosaic 46,XX/47,XXY in an individual with Klinefelter syndrome. J Reprod Infertil. 2020;21(3):222–4.

6. Tachon G, Lefort G, Puechberty J, Schneider A, Jeandel C, Boulot P, et al. Discordant sex in monozygotic XXY/XX twins: a case report. Hum Reprod (Oxf, Engl). Dec 2014;29(12):2814–20.

7. Talreja SM, Banerjee I, Yadav SS, Tomar V. A rare case of lateral ovotesticular disorder with Klinefelter syndrome mosaicism 46, XX/47, XXY: An unusual presentation. Urol Ann. 2015;7(4):520–3.

8. Tangshewinsirikul C, Dulyaphat W, Tim-Aroon T, Parinayok R, Chareonsirisuthigul T, Korkiatsakul V, et al. Klinefelter syndrome mosaicism 46,XX/47,XXY: A new case and literature review. J Pediatr Genet. Dec 2020;9(4):221–6.

9. Velissariou V, Christopoulou S, Karadimas C, Pihos I, Kanaka-Gantenbein C, Kapranos N, et al. Rare XXY/XX mosaicism in a phenotypic male with Klinefelter syndrome: case report. Eur J Med Genet. 2006;49(4):331 7.

10. Samplaski MK, Lo KC, Grober ED, Millar A, Dimitromanolakis A, Jarvi KA. Phenotypic differences in mosaic Klinefelter patients compared with non-mosaic Klinefelter patients. Fertil Steril. Apr 2014;101(4):950 5.

11. Zitzmann M, Aksglaede L, Corona G, Isidori AM, Juul A, T'Sjoen G, et al. European Academy of Andrology Guidelines on Klinefelter Syndrome. Endorsing Organization: European Society of Endocrinology. Andrology. 2021;9(1):145–67.

12. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. Lancet (Lond, Engl). July 17, 2004;364(9430):273–83.

10. Appendices

List of Variables

The continuous quantitative variables are:

- Final height in cm
- Target height in cm
- Weight in kg
- Age in years
- Gonadal volume in mL
- Maximum gonadal volume in mm
- Serum concentrations of LH (IU/L), FSH (IU/L), total testosterone (ng/mL), estradiol (pg/mL), inhibin B (pg/mL), AMH (pmol/L), and INSL3 (pg/mL)

The categorical variables are:

- Reason for consultation (3 categories)
- Presentation pattern (4 categories)
- Presence of ovarian tissue (2 categories)
- Educational level (3 categories)
- Gynecomastia (2 categories)
- Genital atypia (2 categories)