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CAREOMICS

X and Y Chromosome Typing in the Maltese Population: From Population Genetics to Clinical Practice

ABSTRACT

The CAREOMICS project is establishing a national framework for advanced genetic profiling in Malta that includes sex-chromosome markers. X-chromosomal Short Tandem Repeats (X-STRs) and Y-chromosomal STRs (Y-STRs) and Single Nucleotide Polymorphisms (Y-SNPs) are powerful tools in forensic casework, complex kinship analysis and population genetics. Although Maltese studies have characterised autosomal STRs, mitochondrial DNA and Y-chromosome variation, important gaps remain for sex-chromosome data in the local population. This review summarises the rationale for focusing CAREOMICS on X- and Y-chromosome typing, outlines key findings from previous Maltese Y-chromosome work, and describes the ongoing effort to generate the first systematic X-STR haplotype database for Malta. The clinical and forensic implications for healthcare professionals are discussed, particularly for kinship testing, missing-person identification and the interpretation of complex family structures in a small, historically rich island population.

INTRODUCTION: WHY SEX CHROMOSOMES MATTER IN CAREOMICS

The human sex chromosomes occupy a unique position within the genome: while autosomes recombine in both sexes, the Y chromosome is transmitted strictly along the paternal line, and its male-specific region (MSY) behaves largely as a non-recombining block that accumulates lineage-defining Y-SNPs and polymorphic Y-STRs passed from father to son, with occasional mutations generating new haplotypes. In contrast, the X chromosome recombines only in females; males inherit a single maternal X, whereas females inherit one X from each parent, producing characteristic patterns of linkage disequilibrium and haplotype structure that are highly informative in specific kinship scenarios. Within CAREOMICS (Comprehensive Approach for Rare Diseases Employing Multi-Omics Strategies), X- and Y-chromosome markers are viewed as complementary to autosomal STRs and mitochondrial DNA (mtDNA): Y-markers are particularly valuable for male-lineage reconstruction, paternal ancestry inference and the deconvolution of male components in mixed forensic samples, while X-markers, especially X-STRs arranged in linkage groups, enhance the resolution of complex kinship analyses involving daughters, paternal half-sisters and multi-generational pedigrees where autosomal data alone may be inconclusive. By integrating autosomal, X, Y and mtDNA markers in a coordinated project, CAREOMICS is laying the groundwork for a multi-layered genetic framework that reflects both the demographic history and clinical needs of the Maltese population.

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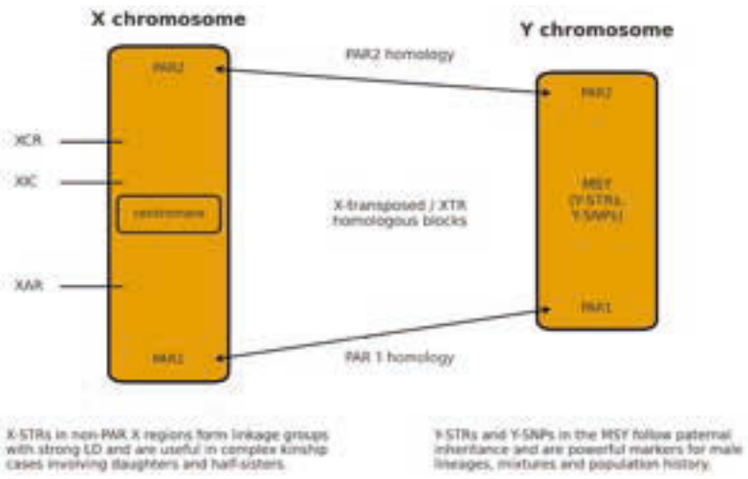
Figure 1. The homology and forensic regions of the sex chromosomes.

Visual representation of the human X and Y chromosomes, highlighting the homologous pseudoautosomal regions (PAR1 and PAR2), the X-transposed region, and chromosome-specific segments. The X chromosome is divided into the X-conserved region (XCR), X-inactivation centre (XIC), and X-added region (XAR), while the Y chromosome has the male-specific region (MSY) containing Y-STR and Y-SNP markers. PAR regions recombine between X and Y during male meiosis, whereas non-PAR regions are inherited in a sex-specific manner.



THE MALTESE Y CHROMOSOME: LESSONS FROM PAST AND ONGOING WORK

The paternal genetic history of Malta is relatively well studied and underpins the Y-chromosome arm of CAREOMICS: Capelli *et al.*, with Maltese participation by Professor Alex E. Felice, combined Y-SNPs and Y-STRs to place Maltese males within a Central–Eastern Mediterranean continuum, showing strong affinities to Sicily and Sardinia and identifying R1 and J2 as dominant haplogroups, reflecting mixed European and Near Eastern influences.[1] Cassar *et al.*, using autosomal STRs, confirmed close genetic similarity between Malta and Sicily, while Caruana's doctoral work, integrating mtDNA, Y-STRs and autosomal markers, highlighted the need for Malta-specific forensic databases rather than reliance on foreign allele frequency data.[2-3] Vella expanded Y-STR, Y-SNP and mtDNA datasets and contributed 292 Maltese Y-STR haplotypes to the Y Chromosome Haplotype Reference Database (YHRD),[4-5] a substantial resource but still below the ~400 samples recommended for a fully robust population database.

Overall, these studies show that the Maltese Y-chromosome landscape is neither isolated nor homogeneous. Instead, it reflects layered historical contacts with Sicily, North Africa and other Mediterranean ports. For forensic practice, male-lineage markers must therefore be interpreted against a locally informed background, where haplogroup frequencies and Y-STR diversity are specific to Malta. For CAREOMICS, this provides a baseline on which modern Y-typing using contemporary multiplex systems and high-resolution haplogrouping can be built.

Y-CHROMOSOME TYPING IN CAREOMICS

The Y-chromosome component of CAREOMICS, led by Daniel Brincat, is establishing a standardised, high-resolution Y-typing framework for forensic and research applications. Using the PowerPlex® Y23 System (PPY23), the analysis generates informative Y-STR haplotypes across 23 loci, well suited to a small yet genetically complex population such as Malta. In parallel, a qPCR–high-resolution melting (HRM) approach is applied to type key Y-SNPs and assign samples to major Y-haplogroups A–T and relevant subclades. Together, Y-STR haplotypes and Y-SNP haplogroups yield a two-layered dataset that improves Maltese haplogroup

frequency estimates, corrects earlier sampling biases, supports robust forensic and kinship analyses, and anchors local male lineages within a global phylogenetic framework

Practically, expanding and refining the Maltese Y-STR/Y-SNP database has immediate implications for healthcare and forensic professionals. Likelihood ratios for paternity testing, sexual offence investigations and missing-person identifications become more precise when calculated against population-specific data rather than imported references. Enriched Y-chromosome information will also enable future studies of male-specific disease susceptibilities and sex-biased demographic events in a small island population with known founder effects.

THE X CHROMOSOME: THE MISSING PIECE IN MALTESE FORENSIC GENETICS

The X chromosome remains a major gap in Maltese genetic databases, even though X-STRs are internationally recognised as key markers in forensic genetics and complex kinship testing, particularly in pedigrees involving at least one female (e.g. disputed paternity of daughters, paternal half-sisters, specific grandparent–grandchild cases), owing to their female-specific recombination and distinctive haplotype structure.[6] Studies in neighbouring Mediterranean populations highlight both the potential and complexity of these markers: Ferragut *et al*. used the Investigator® Argus X-12 kit to show subtle isolation and sex-biased gene flow in Valencia and the Balearic Islands, with Ibiza displaying low intra-population variability and strong linkage disequilibrium,[7] and broader Western Mediterranean work confirmed that X-STRs can differentiate groups with distinct migration and admixture histories while still detecting regional homogeneity.[8] Tomas and co-workers, combining X-STRs, X-SNPs and autosomal markers, demonstrated how X-linked data capture female-mediated migration and population structure not evident from autosomes alone,[9] while Robino and colleagues, focusing on Sardinia, showed how local isolation and drift can markedly shape X-STR haplotypes and linkage patterns, with important consequences for both population studies and forensic interpretation.[10-11]

Despite Malta's proximity to these populations, no dedicated X-STR database has yet been published for the Maltese population. As a result, forensic casework relying on X-linked markers, for example, in complex paternity disputes or half-sister testing, must currently use allele frequencies and haplotype patterns from other populations, often Sicilian, Italian or Iberian datasets. While broadly similar, the Maltese genetic landscape is not identical, and modest differences in allele frequencies can significantly alter likelihood ratios in borderline cases. From legal and ethical perspectives, being able to state that calculations are based on "Maltese data" rather than approximations represents a major advance.

BUILDING THE FIRST MALTESE X-STR DATABASE WITHIN CAREOMICS

The X-chromosome arm of CAREOMICS, led by Sarah Theuma, aims to generate the first population-level X-STR dataset for Malta, initially focusing on unrelated Maltese males, each carrying a single maternal X chromosome, which simplifies interpretation. Using multiplex kits such as GT XDetector (Genetek Biopharma) or the Investigator® Argus X-12 system, X-STR loci organised into four linkage groups are typed and treated as haplotypes rather than independent markers. The workflow follows standard forensic practice, DNA extraction, quantification, multiplex PCR and capillary electrophoresis, with alleles called and grouped into haplotypes (e.g. via GeneMarker® HID) and population-genetic analyses used to estimate allele/haplotype frequencies and diversity, with future female datasets enabling assessment of Hardy–Weinberg equilibrium and linkage disequilibrium within and between linkage groups.

Methodologically, the project is aligned with recommendations from the DNA Commission of the International Society for Forensic Genetics (ISFG) on X-chromosomal markers. Tillmar, Kling, and colleagues emphasise treating tightly linked X-STRs as haplotypes, accounting for linkage and linkage disequilibrium in likelihood calculations, and ensuring sufficiently large, well-characterised population datasets before casework use.[12] The CAREOMICS X-STR dataset is being designed with these principles in mind, targeting a sample size that balances practical constraints with statistical robustness.

Once established, the Maltese X-STR data will be compared to existing Mediterranean datasets, including those from Spain, the Balearic Islands, Sardinia, Greece and North Africa. This will allow Malta to be placed within the wider Mediterranean X-chromosome landscape, and to assess whether Maltese X-STR profiles are closer to those of neighbouring Sicily or show distinct patterns reflecting Malta's unique demographic history. Importantly, the dataset is intended to be formatted for integration into public or semi-public databases such as ChrX-STR.org and, where appropriate, for use within forensic software such as FamLinkX, which can perform likelihood computations for X-linked markers while accounting for linkage, recombination and mutation.

INTEGRATING X AND Y TYPING INTO A CAREOMICS FRAMEWORK

The X- and Y-chromosome projects form a unified CAREOMICS framework that integrates autosomal STRs, Y-STRs/Y-SNPs, X-STRs and mtDNA into population-specific databases and pipelines to support forensic practice and precision medicine in Malta. In casework, autosomal markers remain primary, with Y-STRs used to resolve male contributors and strengthen paternal lineages, and X-STRs boosting power in complex kinship analyses involving female relatives. More broadly, sex-chromosome data, combined with transcriptomic, epigenomic and metabolomic profiles, will enable studies of sex-biased disease, X-linked disorders and Y-related male health, while providing more accurate, locally grounded likelihood ratios that strengthen paternity and kinship testing, improve the robustness of court reports and align Maltese practice with international standards.

CONCLUSIONS

The CAREOMICS project is a major step toward a comprehensive, population-specific genetic framework for Malta, explicitly addressing two key gaps: limited resolution of existing Y-chromosome data and the previous absence of Maltese X-STR population data. Building on the work of Capelli, Vella, Cassar, Caruana and others, it applies modern multiplex kits, advanced analytical tools and ISFG-compliant guidelines to generate X- and Y-chromosome datasets that truly reflect the Maltese population.[1-5,12] For healthcare professionals and researchers, this will yield more precise and defensible genetic evidence in forensic and kinship cases, a stronger basis for multi-omics studies and a clearer view of Malta's place in the Mediterranean genetic landscape, making robust, locally derived sex-chromosome data a practical necessity rather than a luxury.

If interested in participating in this population genetics research, kindly contact Prof. Borg (joseph.j.borg@um.edu.mt).

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