The Y chromosome and its use in forensic DNA analysis

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Originally relatively ignored in forensic investigations because its genetic analysis lacks inference of individual identification, the value of Y chromosome analysis has been proven in cases of sexual assault, particularly where the amount of material left by a male assailant is limited in comparison with female DNA. All routine analysis of autosomal DNA, however, targets a gene (*AMELY*) on the Y chromosome in order to identify the sex of the DNA source and this is discussed in the context of the genetic structure of this male-specific chromosome. Short-tandem repeat markers on the chromosome are tested in dedicated multiplexes that have developed over time and these are described along-side international guidance as to their use in a forensic setting. As a marker of lineage, the Y chromosome provides additional tools to assist in the inference of ancestry, both geographical and familial and the value of Y chromosome testing is illustrated through descriptions of cases of criminal and historical interest. A decision to analyse the Y chromosome has to be considered in the context, not only of the circumstances of the case, but also with regard to the ethical questions it might raise, and these are discussed in relation to the cases that have been described in more detail in the accompanying online supplementary material.

Introduction

Genetic characteristics on the human Y chromosome provide a lineage marker in the form of a single haplotype transferred directly from father to son [1]. A haplotype is the set of short-tandem repeat (STR) alleles typed on a single Y chromosome. Analysis of the Y chromosome has been relatively ignored for some time in forensic casework because it does not provide the near certainty of identification that autosomal DNA can offer. The uniparental nature of this marker can be useful in certain circumstances: sexual assault; missing persons; disaster victim identification; complex kinship analysis; and population inference [2].

The Y chromosome is one of a pair, with the X chromosome, of the human sex chromsomes located in the nucleus of human cells along with 22 pairs of autosomal chromosomes. Individuals with an X and Y chromosome normally are phenotypically male, and those with two X chromosomes, female, although there are both rare differences in the numbers of these chromosomes, or other distinct mutations that can influence that phenotype [3].

Figure 1 illustrates the inheritance typical of males in the population in which the single Y chromosome is passed down virtually intact (barring mutations) from father to son.

The human Y chromosome has two pseudoautosomal regions (PARs) with a short arm Yp and long arm Yq, separated by a centromere (Figure 2). The PARs, the short arm and the proximal part of the long arm consist of lightly packed chromatin enriched with genes PAR1 and PAR2, both recombine with areas on the X chromosome; the remaining regions are non-recombining and contain about 70 genes in the male-specific region (*MSY*). Of particular interest in forensic DNA typing is the detection of markers associated with the amelogenin (*AMEL*). The locus has two homologous genes on the X and Y chromosomes [4]: *AMELY* [5] encodes a protein involved in the demineralisation of tooth
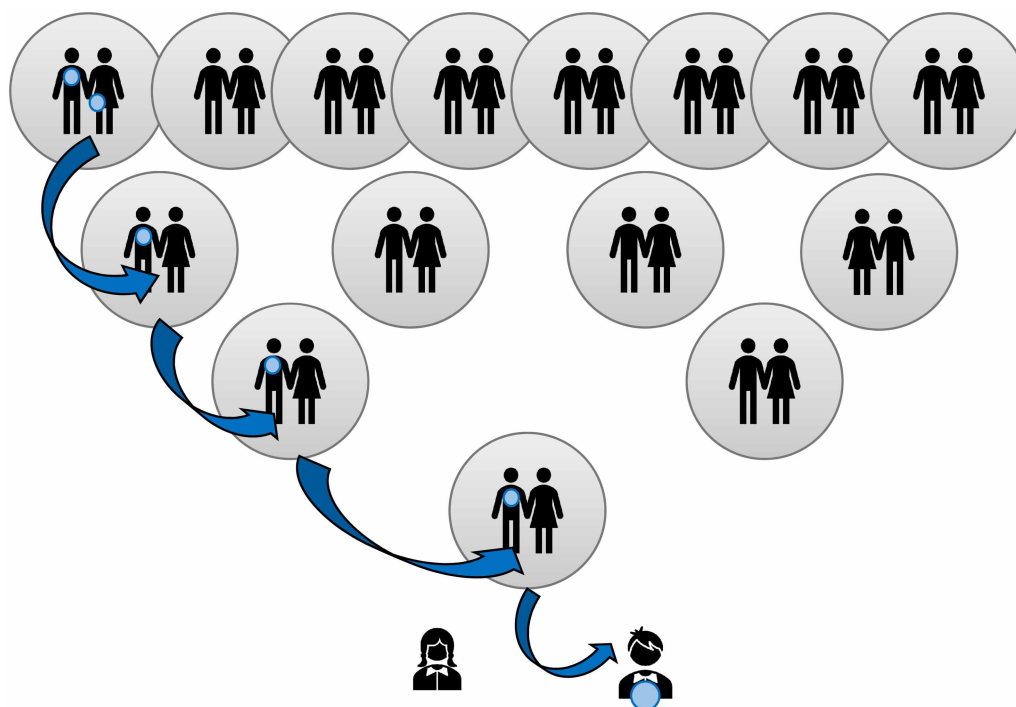
Figure 1. Illustration of Y chromosome inheritance over four generations ⬤ **indicates ancestral males that share the same Y chromosome haplotype as the child at the base.**

enamel and has a paralogue on the X chromosome (*AMELX*). The first intron of *AMELX* is six base-pairs (bp) [6] shorter and primers that target both genes are included in many forensic DNA kits in order to reveal the sex of the donor. As early as 1998 [7] there were reports in the literature of 2/24 males in a Sri Lankan population that lacked *AMELY* (*AMELY*null) and others since have reported different classes of deletions involving the gene [8], seen predominantly in South Asians with a frequency of between 1% and 6.5% in other larger studies of this population group [9–13]. Sometimes the deletion will encompass a larger area resulting in incomplete Y chromosome STR loci in profiles routinely used in Y chromosome forensic analysis. This has led to other markers, such as DYS391 (shown in bold in Figure 2), being included in standard autosomal DNA analysis kits as this marker is on the long arm of the Y chromosome and not involved in the above-reported deletion event.

The inability of most of the Y chromosome to recombine with the X chromosome is a feature that has developed over time [14] and the sequenced chromosome is full of small repeats, inversions, palindromic sequences (where nucleotide sequences are identical with that on the complementary strand) [15]. The latter, in particular, can facilitate gene conversion and increase independent deletion and mutation events in certain areas [16,17]. Where these impact on genes involved in spermatogenesis this can impact on male fertility, but the chromosome can tolerate quite large deletions without consequence.

STRs on the Y chromosome are no more likely to mutate than those selected for forensic analysis on autosomes but, not only are they are individually selected based on significant diversity measures [18], the more regular appearance of mutations between father and son is likely to reflect the high number of mitotic events in spermatogenesis. Because of this Y-STR haplotypes will be much more variable than seen in a single autosomal STR and Y-STR haplotype reference databases are required to be significantly larger than autosomal STR allele reference databases in order to provide reliable frequency estimates.

Mutations are not randomly distributed along the chromosome; some regions are more vulnerable to a mutational event than others. Those that mutate more frequently on average (often referred to as rapidly mutating STRs) have some advantages if they are included in forensic multiplexes, particularly in the distinction of closely related males.
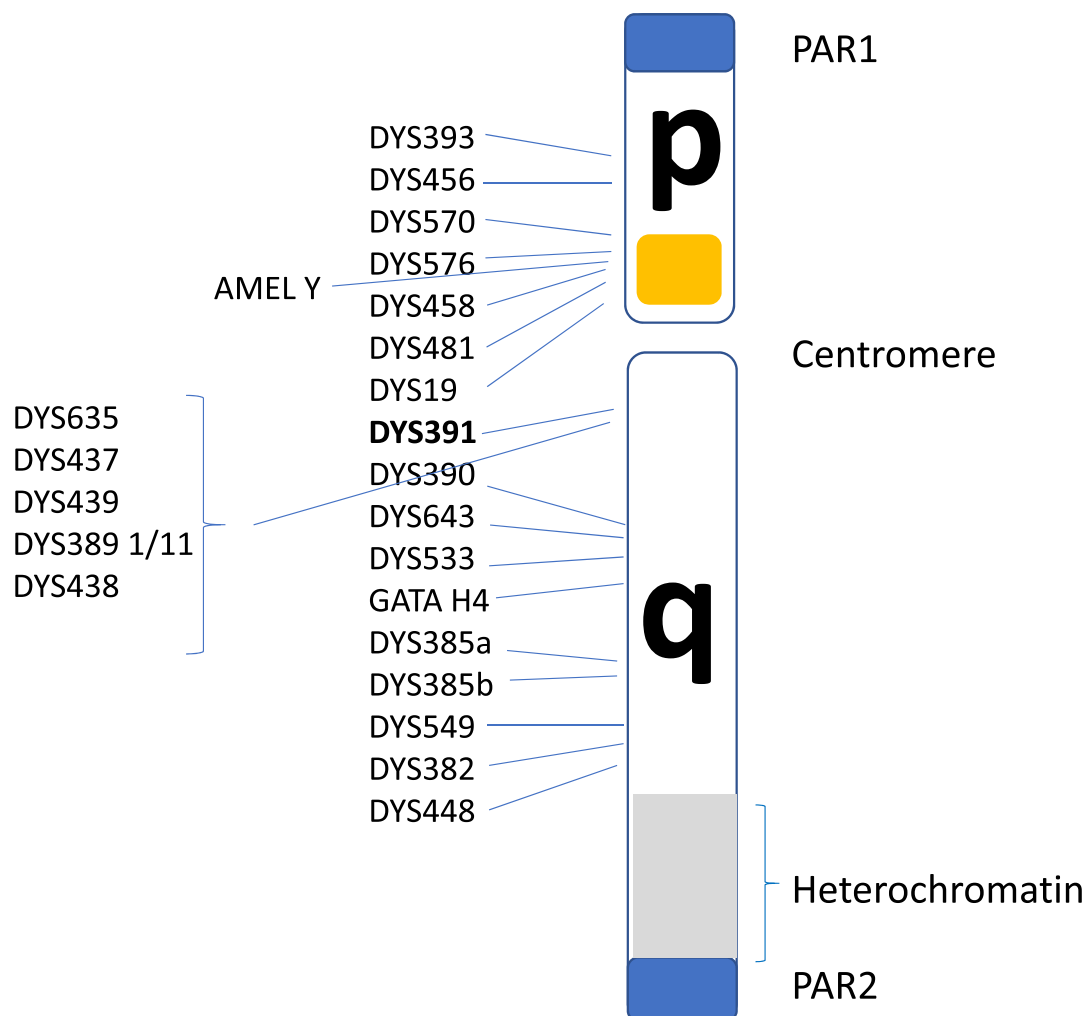
Figure 2. Structural diagram of the human Y chromosomes identifying the relative locations of PowerPlex Y23short tandem repeat (STR) loci used routinely in forensic DNA casework.
The approximate region ▮ identified in the diagram is sometimes the subject of variable size deletions.

In 2014, in a worldwide collaborative effort [19], many contributors typed almost 20 thousand samples in total from their respective populations using the Y23 loci, confirming high diversity and revealing many null (missing), duplicate (repeated) and off-ladder (not coincident with expected size) alleles. The number of alleles at any one locus varied from 10 to 31, with a median of 16, not including DYS385 which had 146 different unordered variants; 92.9% of the 19 630 haplotypes observed were unique.

In 2018, Aliferi et al. [20] published a collection of over 3000 Y23-STRs from the U.K. and Ireland. In addition to white British ($n = 1062$) and Irish ($n = 720$) the population set included black British ($n = 977$) and British Asians ($n = 369$) providing 97.9% different haplotypes with shared haplotypes being seen within the different population sets, or between the Irish and white British. Adding the five additional loci from Y filer Plus increased that to 99.5%.

Duplications and deletions were observed in eleven and eight loci, respectively, in the U.K. and Irish set. These are shown in Figure 3. The duplication of DYS448 in 11 black British suggests a population frequency of around 1% for this variation in the black population and has previously been described in the study by Purps et al. [19].

Deletions were seen predominantly associated with the short arm and in the British (south) Asian population group, with two individuals demonstrating a large deletion of most of the short arm and DYS448 on the distant end of the long arm in addition. The observed deletions in this dataset are typical of those including
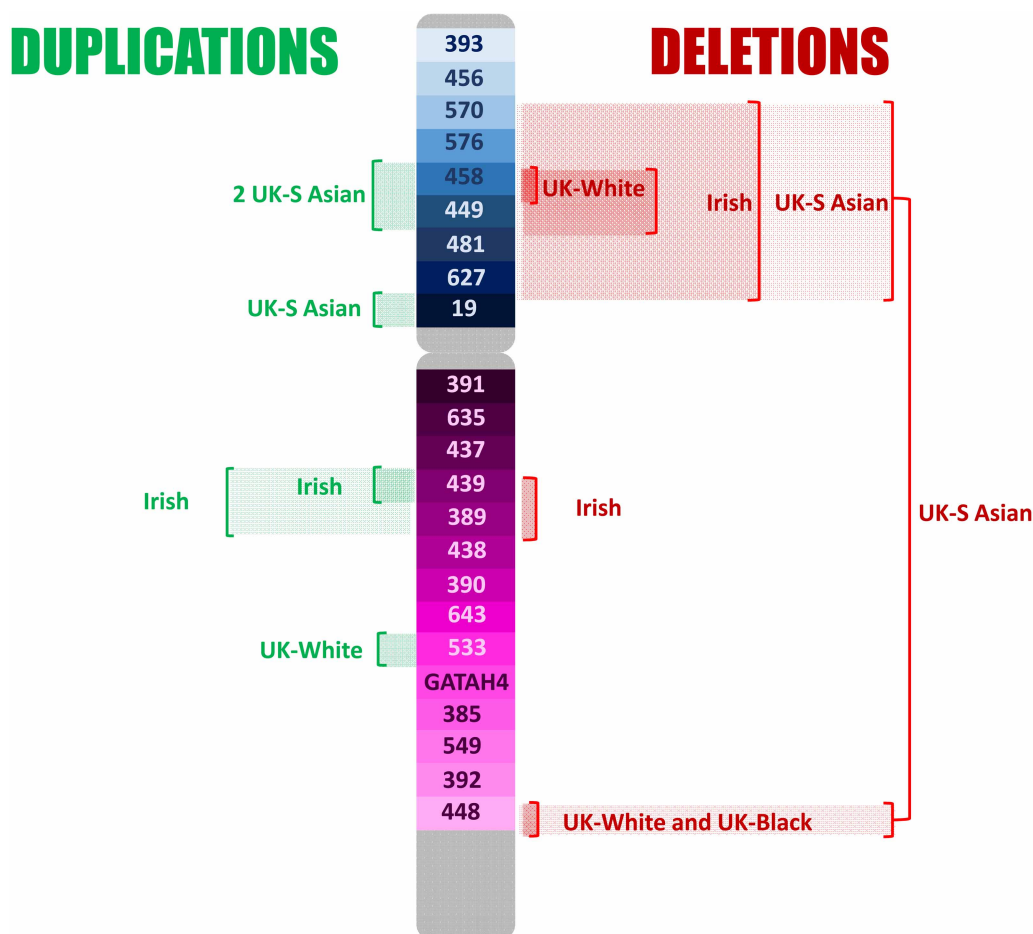
Figure 3. Duplications and deletions reported in a U.K. (three ancestries) and Irish population set of 3654 individuals typed for the Y23 set of Y-STRs [20].

AMELY that are seen at higher frequency in south Asians reported in other studies. These findings suggest a single origin for these variants clustered on the short arm of the chromosome that has gained prominence due to genetic drift. In YHRD deletions are seen in 1/9200 worldwide, but 1/148 of individuals in the Indian meta-population. In contrast, deletions of DYS448 and DYS458, seen in 17/37 and 10/37 of the total observed deletions, suggest a form of instability in these areas of the chromosome. Both areas show multiple different forms of structural variation occurring in different populations.

Y single nucleotide polymorphisms (SNPs) were also seen in the Aliferi study at two loci (DYS392 and DYS576) resulting in null alleles. The latter is a recognised SNP (rs757752030) with a frequency of around 1.4% in the South Asian 1000 genomes study [21]. Additionally, mutations upstream of DYS481 produced mobility shifts in the electropherogram that led to false off-ladder allele reports. The G > A SNP, which is 16 bp upstream of the CTT locus repeat, is hypothesised to enlarge the local secondary structure [22] and appears to be population specific, being seen in 7.3% of British South Asians, predominantly associated with allele 25. A further shift was seen associated with this locus in another population, but involving a G > T SNP further upstream, suggesting that any mutation in the flanking region of this locus may produce a change in the secondary structure, resulting in a mobility shift.

Forensic Y chromosome DNA analysis

Routine autosomal DNA analysis makes use of STR loci that comprise a standard set. In Europe, these are the twelve European Standard Set (ESS) loci, last defined in 2008, and in 2015 the United States increased their standard set to twenty in the combined DNA index system (CODIS) [23]. The main manufacturers of

Table 1. Autosomal STR kits supplied by Promega (blue), Qiagen (green) and ThermoFisher (pink) that include additional Y loci in their kits.

Locus	A	B	C	D	E	F	G
Amelogenin							
Y indel							
DYS391							
DYS570							
DYS576							

Multiplex keys: B, PowerPlex Fusion 6C; C, Versaplex 27PY; E, Investigator 24plex; F, Investigator 26plex; H, AmpFLSTR NGM Detect; I, Globalfiler; J, Verifiler Plus.

autosomal multiplex kits have always included *AMEL* as an indicator of sex but are now adding additional markers in order to avoid incidences of *AMEL*null in casework. These additional markers are usually Y-STRs but a Y-specific insertion/deletion (Y_{indel}) rs2032678 locus is used within the kits from one manufacturer [24]. Where Y-STRs have been included, all include DYS391 which is distant from the common deletion site. Table 1 summarises the current set of autosomal multiplex kits that include additional Y chromosome markers.

Y chromosome STR multiplexes

Two manufacturers produce the main Y-STR multiplex kits used in forensic casework today employing up to 23 and 27 loci, respectively. Table 2 shows the loci included.

Y-STRs may or may not be tested routinely by forensic providers worldwide and the ability to compare with reference profiles will depend on the retention policies of individual jurisdictions. While their usefulness will be discussed below, because the inheritance of these loci is not independent, the product rule used for autosomal STRs cannot be employed and haplotype frequencies must be used instead. Because of the high variability of haplotypes, so the reference haplotypes used to provide reliable frequencies need to be extremely large and representative of the different populations, and that has relied on the collaboration of many scientists across the world.

YHRD

YHRD (www.yhrd.org) is an online Y chromosome haplotype reference database built from an initial collaboration with 31 forensic and anthropological institutions across Europe who contributed almost 5000 male minimal haplotypes to the collection initiated by academics at the Charité University in Berlin; the database went live in 2000 [25]. Since then, it has expanded enormously with additional contributors, worldwide, and is now in release 63 (September 2020). A similar database, set up in the US, was to be decommissioned in 2019 and all US haplotypes transferred to YHRD with appropriate guidance for SWGDAM compliant searches being provided for US subpopulations.

The collection of populations is classified into metapopulations based on both geographical and linguistic determinants and now consists of 33 different metapopulations, with about 50% being Eurasian (mainly Western and Eastern European), 25% Asian (mainly from China) and the other composed of mainly admixed populations and people from African, Native American, Aborigine and Inuit populations [26]. The database is essentially five separate databases, each with a defined set of Y-STR markers. Table 3 shows the current position of the database which now also includes a collection of SNPs.

For whatever purpose a Y-haplotype is defined, it is likely that an estimate of its frequency in a relevant population will be needed. The YHRD database provides frequency estimates based on different statistical models.

Y-STR guidelines

Y-STR loci exhibit the same characteristics as autosomal loci in terms of stutter and repeat unit types, and interpretation rules need to be defined locally. The rules more obviously differ from autosomal guidance because Y-STRs are not observed in females and normally only present with a single allele unless the locus is

Table 2. Y STR markers currently used in forensic DNA analysis from multiplex kit manufacturers — Promega (blue), ThermoFisher (pink) and a non-commercial set (grey). Loci in bold are 'rapidly mutating'.

Locus	Minimal haplotype[a]	PowerPlex Y	AmpF/STR Yfiler	PowerPlex Y23	Yfiler Plus
DYS19	●	●	●	●	●
DYS385a/b	●	●	●	●	●
DYS389 I	●	●	●	●	●
DYS389 II	●	●	●	●	●
DYS390	●	●	●	●	●
DYS391	●	●	●	●	●
DYS392	●	●	●	●	●
DYS393	●	●	●	●	●
DYS437		●	●	●	●
DYS438		●	●	●	●
DYS439		●	●	●	●
DYS448			●	●	●
DYS456			●	●	●
DYS458			●	●	●
DYS635			●	●	●
Y-GATA-H4			●	●	●
DYS481				●	●
DYS533				●	●
DYS549/				●	
DYS570				●	●
DYS576				●	●
DYS643				●	
DYS449					●
DYS460					●
DYS518					●
DYS627					●
DYF387S1a/b					●

[a]Kayser, M., Caglia, A., Copach, D. et al. (1997). Evaluation of Y-chromosomal STRs: a mulicenter study. Int. J. Leg. Med. 110, 141–149.

duplicated. Because of its familial nature consideration must also be given to any relevant legal requirements of the jurisdiction and the prevailing population substructure.

Y chromosome STR loci variations are more common than seen in autosomal STRs, with multicopy loci such as DYS385 and DYF387S1 being observed routinely. DYS385 is a constitutively duplicated locus comprised of two linked STR sub-loci with a GAAA repeat structure in different locations [27]; the two alleles are frequently different sizes and when one primer pair is used both are amplified and these are considered together as a single haplotype. The parts are called 'a' and 'b', and by convention the one with the smallest number of repeats is defined as 'a', but this does not imply assignation to one or other of the two chromosomal locations.

SWGDAM guidance
The Scientific Working Group on DNA Analysis Methods (SWGDAM) published Y chromosome interpretation guidelines in 2014 [28], which superseded their 2009 guidance, outlining the principle of using Y-STR analysis as an additional tool to autosomal DNA, or instead of autosomal DNA when wanting to detect males

Table 3. Composition of the Y-HRD database release 64 (May 2021) giving the number of haplotypes in each set. The minimal haplotype includes alleles at STRs DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385. The maximal haplotype includes alleles at the 27 STRs typed across the different commercial multiplexes, including Yfiler Plus.

Dataset	Number of haplotypes
Minimal	337 449
PowerPlex Y	296 791
Yfiler	227 004
PowerPlex Y23	93 998
Maximal	40 201
Y-SNPs	24 429

in a mixture with an abundance of female DNA. The guidance also recommends the retention of samples for possible future Y-STR analysis.

The use of the counting method is suggested for reporting frequencies, preferably also with a confidence interval. This is calculated from $n/(N+1)$, where n is the number of times the haplotype is seen in a database of size N. They recommend reporting the match probability as:

$$\Pr(A|A) = \theta + (1 - \theta)pA$$

where A is the haplotype of interest, $\Pr(A|A)$ is the probability of observing haplotype A given that is has already been seen once. pA is the upper bound of the confidence interval for the profile probability estimated by counting. Theta (θ), representing population substructure and broadly similar to F_{ST} [29], is likely to be very low (at a maximum of around 0.0007 for a 12-locus haplotype in all populations) and decreases as the number of loci typed increases and so will have little effect on the estimation.

Because the Y chromosome is inherited independently of other chromosomes, they remark that the haplotype frequency can be multiplied, applying the product rule, with frequencies of autosomal loci.

Finally, the guidance recommends that mixtures in which it has not been possible to determine a major or conditioned haplotype should only be used for exclusionary purposes.

ISFG guidance

The DNA Commission of the International Society of Forensic Genetics (ISFG) has also provided recommendations on the interpretation of forensic Y-STRs [30]. While they recognise that comparisons of haplotypes can provide a simple qualitative statement in forensic reports: inconclusive; exclusion; or non-exclusion, they regard that as a minimum requirement and state that a quantitative statement is desirable. They have set out a conditional decision tree which is illustrated in Figure 4.

The use of either the counting method or the Discrete Laplace formula [31] to estimate population frequencies is recommended. The former may be over conservative, especially when profiles are not represented in the database, which is commonly seen. The DNA Commission prefers the use of the Discrete Laplace model which ensures that rare haplotypes retain evidential power, providing that ancestral haplotype clusters are represented in the database. Some jurisdictions use the augmented counting method, estimating frequencies as $(n+1)/(N+1)$ in contrast with the simpler $n/(N+1)$ used by SWGDAM.

When a quantitative inference is made, the quality of the data supporting it is relevant. The ISFG has set down fundamental requirements for reference Y-STR databases: full specific kit-haplotypes from randomly selected individuals; meta- and sub-population data; population datasets of sufficient size to ensure representation of the population and its extended lineage clusters; submitters of data to have engaged in national or international proficiency tests. Instead of using a match probability which will not account for extended lineage matches, Anderson and Balding [32] propose reporting the median and 95 percentile number of males
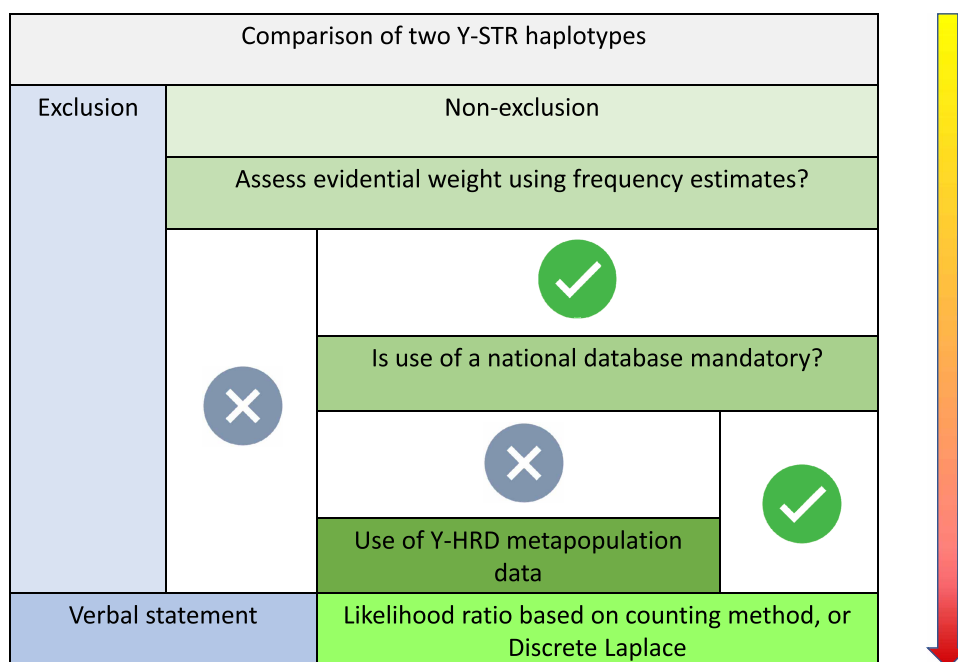
Figure 4. ISFG Y chromosome guidelines, illustrated here in a flow chart with successive decisions in Y-STR haplotype analysis [30].

expected to match others in a database of a particular size. Guidance also suggests that reports should use a standard format stating competing hypotheses:

Hypothesis 1: Person X is the source of the DNA

Hypothesis 2: A random man Y from the reference population Z is the source of the DNA, and Y is another man than X

To be followed by a statement akin to: *The Y-STR profile detected in the crime stain is LR* (where LR is the likelihood ratio) *times more probable to observe under hypothesis 1 than under hypothesis 2. This notwithstanding, paternal relatives have a high probability to have the same Y-STR profile and will in that case have the same likelihood ratio.*

To put this into context, paternally related individuals of 20 generations distant are 18% more likely to share the same Y23 haplotype and even if 27 markers are used, 5.5% will still share the same haplotype [32].

Uses of Y-STRs in the criminal justice system

Y-STRs have been used in criminal casework for almost 30 years [33], highlighting its main advantage in cases of alleged sexual assault in which the contribution of a male can be detected when present in a small amount in a mixture with female DNA. It may sometimes also reveal that more than one male has contributed to a mixture, which might not have been revealed in the standard autosomal analysis.

Routine analysis of sexual assault cases will normally employ chemical tests to suggests the presence of semen, confirmed with microscopic visualisation of sperm cells. This will often be followed by a differential extraction of epithelial and sperm cells to enable standard autosomal DNA profiling of the male specific-enriched contribution to a male–female mixture. Differential extraction, or differential lysis, was first described by Peter Gill in 1985 [34], at a time when DNA analysis was in its infancy and the polymerase chain reaction (PCR) that has transformed DNA typing had not yet been published [35], only just having been invented. Using a process that continues today, albeit with refinements, Gill described incubation of extracted DNA with an SDS/proteinase K/dithiothreitol mixture which tends to lyse epithelial cells, whereas sperm nuclei have a highly developed proteome, rich in protamine to allow the DNA to be tightly packed in the head,

offering more protection in a lytic environment. The robust structure of sperm nuclei serves to protect these cells from damage which might otherwise significantly impact on the health of a fertilised oocyte [16].

Use of Y-STRs in routine casework is limited, however, particularly because of the inability to identify a person rather than a lineage, and a separate analysis for these markers could consume valuable case material. Massively parallel sequencing (MPS) techniques allow analysis of large numbers of DNA markers, including Y-STRs, in a single analysis, but until these techniques are introduced into routine forensic casework, the decision to undertake a Y-STR analysis will remain with the individual laboratory and associated guidance. Without an enrichment process for the male material, analysis of the male contribution in MPS multiplexes may still be compromised if present at low levels because of competitive amplification with more abundant DNA. In some cases, such as if semen has originated from an azoospermic male, or there has been contact or penetration of a female in which non-sperm DNA has been deposited at a low concentration, a separate analysis of Y-STRs can provide key evidence in a case.

Ethical issues associated with Y chromosome testing in criminal justice

There are several important cases involving Y-STR evidence, described more fully in the online supplementary material, that raise important ethical issues.

Albert DeSalva (*the Boston Strangler*)'s identity was confirmed after he had died years later through Y chromosome matching. DeSalva had confessed to the murder, yet no evidence had been found linking him to the crime. Investigators, determined to resolve the issue, violated the privacy of his nephew by testing a discarded water bottle. Although there are various laws in different US states, almost all allow for the non-consensual collection of human tissue for DNA [36]. This approach has been used in many cases, such as in the identification of the Golden State Killer. Clearly envisaged as a way to identify the potential guilty party, rather than a known innocent, the practice also raises issues about the identification of (untested) relatives of the individual and the potential for revealing unintentional secrets. In contrast, in 2006, the U.K. made DNA theft a criminal offence without consent [37] unless for law enforcement purposes, which would not fit these circumstances. Deliberate familial searching in the US is limited to using an offender DNA database in many states [38] and so there may have been an additional violation in this particular case.

After others were accused, the 1996 murderer of *Angie Dodge* was solved in 2019, but not without problems involving a very close Y-STR match impacting on an innocent that include a clear example of confirmation bias. Michael Usry Jnr, was required to provide a DNA sample because a close match was identified between the crime scene material and Usry's father. Usry had been in the state at the time of the murder. Although he was cleared of involvement after a month of waiting, nevertheless questions continue to be raised about his innocence in social media [39].

The murder of *Marianne Vaastra* in 1999 in The Netherlands raises additional ethical concerns. The racial prejudice exhibited by the local population led to the biased investigation of asylum seekers in the early stages of the investigation, unethical also because of wasting of time in catching the perpetrator, but also for the associated costs. The later illegal, albeit potentially justifiable, inference from analysis of the biogeographic ancestry (BGA) of the Y chromosome carried by the perpetrator, suggesting a male of Northwest European origin, has led to changes in the law to allow the process in future.

The prediction of BGA remains a highly sensitive issue, however, particularly in Germany, which continues to disallow its use; inference through the Y chromosome is, nevertheless, allowed [40]. Concerns have not diminished, however, as there was suspicion that haplotypes submitted to YHRD from minority groups, such as Uyghurs and Roma people, were collected without proper informed consent [41]. While there is a consensus that such profiles should be removed, this is contrasted with the possible negative impact on these minority groups when estimating the frequency of Y chromosome match.

Biogeographic ancestry

While a person's ancestry is rooted in concepts of language and culture associated with social constructs around the concepts of race and ethnicity, inference of a person's biogeographical ancestry is assessed through an understanding of genetic diversity in different population groups with respect to their distribution across world regions.

The distribution of polymorphisms that are not impacted by natural selection reflects the history of mobility of our species as populations have dispersed from Africa over many thousands of years. The influence of assortive mating, in which individuals are more likely to mate with each other, and genetic drift, in which the effect of random variability is more marked in smaller populations, plus the increase in population size over time, has produced genetic differentiation across the world [42]. Analysis of genetic markers, whether STRs, SNPs or other polymorphisms has shown that individuals living on the same continent are more similar in comparison with those in different continents.

Direct-to-consumer testing has led to a lay expectation of certainty in BGA but a broad continental ancestry from a broad view across an individual's genome is likely the best that can be done [43].

Examination of the Y chromosome can, however, only inform us about the BGA of our paternal ancestors, and only if we are male. While Y-STRs haplotypes can infer geographic ancestry, population origin and human migration routes can be best examined through examination of SNPs because of their low mutation rates. Phylogenetic trees of evolutionary relationships are modelled from characteristic SNPs in different population groups, defining core haplogroups [44,45]. Named alphabetically, the deepest branches in the phylogeny are haplogroups A and B that are essentially restricted to Africa, supporting a likely African ancestry for humans. Figure 5 illustrates the rooted phylogenetic tree with haplogroups being defined by characteristic SNPs and Figure 6 shows the core geographic locations.

BGA made from the point of view of the Y chromosome may be very different from autosomal inference and its usefulness will depend on the ancestral groups within the relevant population. In the Vaastra case an inference was made of a north-western European origin for the perpetrator; this presumably was because more, or only, people from that region were represented within the YHRD database. That might have been a reasonable inference because the crime took place in a northern province that has not been impacted by immigration from other parts of the world, but that confidence may not be as strong in other parts of Europe. Such an inference may in truth be very deep-rooted as Figure 1 makes clear and the British Isles has a long history of the presence of Africans in its population which is not evident on a general population view. Haplogroup A is regarded as being African-specific, yet a population study of the British Isles revealed a collection of 'indigenous' Northern England residents with this same haplogroup, all, in this study, with the same surname, that was likely to have been introduced into the country some time before the late eighteen century [46]. This will, of
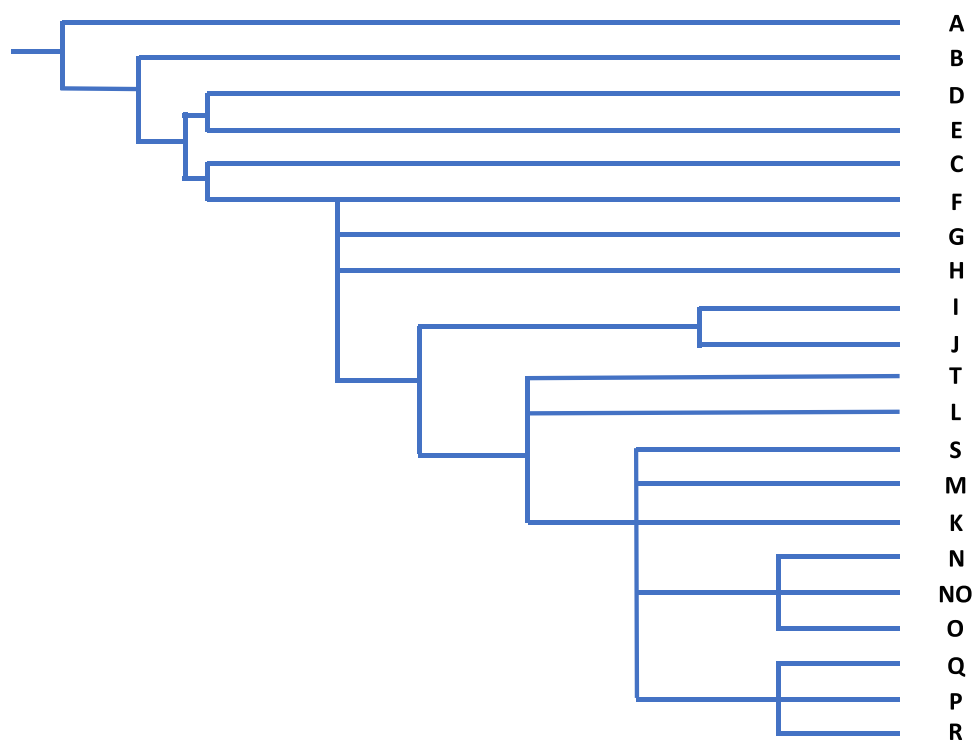

Figure 5. Y chromosome core haplogroup phylogeny [46].

Figure 6. Geographic representation of likely origin of dominant Y-DNA haplogroups (data from [46]).

course, not be the only example and highlights the potential danger of relying on BGA inference from the Y chromosome in populations with differing demographic histories.

Kinship

Y-STRs are frequently used in the examination of a paternal lineage, whether for criminal of civil investigations. The Y-STRs selected for including in forensic multiplexes are generally chosen for their population differentiation although early multiplex collection use, such as the STRs from the minimal haplotype collection, showed that this could be population dependent [47] and subsequent multiplexes have included markers that can offer better cross-population differentiation.

While it is expected that the Y chromosome passed from father to son will provide the same Y-STR haplotype, mutations are not infrequent. This is because male germ cells undergo more multiplication events than in female egg cells leading to such mutational events being more likely to be revealed in investigations of paternity than in maternity.

Rapidly mutating Y-STRs

Analysis of a large number of Y-STRs in father-son pairs undertaken by Ballantyne et al. [48] added a further set of Y-STRs with high mutation rates that can be particularly useful in some instances, such as the increased potential for discriminating between people that otherwise share a Y-haplotype. Figure 7 illustrates the power of these 13 rapidly mutating (RM) YSTRs (DYF387S1, DYF399S1, DYF403S1, DYF404S1, DYS449, DYS518, DYS526, DYS547, DYS570, DYS576, DYS612, DYS626 and DYS627) to separate individuals living in different continents, or different counties, that otherwise share the same Yfiler haplotype. About half of these RM loci are now included in the Yfiler Plus multiplex. Potentially the set has the power to differentiate close relatives such as siblings, or even father and son. These markers also improve the ability to discriminate between closely
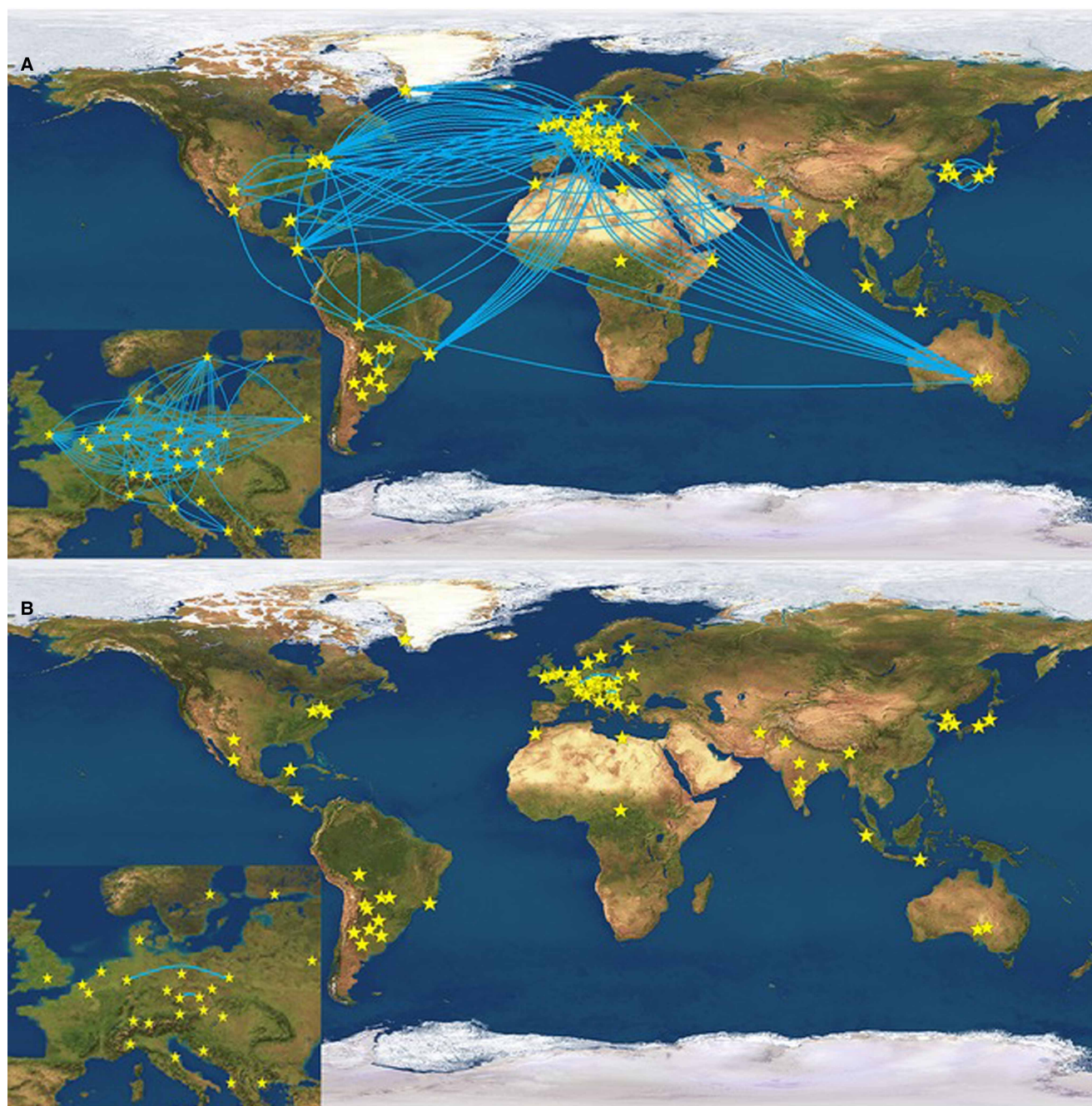
Figure 7. Illustration of the power of rapidly mutating (RM) Y-STRs [48].
Blue lines link pairs that share the same Y-haplotype. A — testing using 17 Yfiler STRs; B-testing using 13 RM YSTRs. Links across Europe are magnified in the inset.

related individuals with 26.9% of father–son relationships having at least one mutational difference, in comparison with Yfiler, and brothers being distinguished from each other in 56.3% of cases in comparison with 10% when using Yfiler. This distinction can be of particular importance in certain forensic cases.

Civil uses of Y-STRs

Because of their paternal lineage, Y-STRs, while not being able to prove paternity, can be of assistance in certain cases. For example, when if the putative father is deceased or missing, a matching Y chromosome

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haplotype with another paternally related family member can help support the relationship. Y-STRs are frequently used in cases of inheritance where individuals may want to claim on an estate where the link may be disputed. In such circumstances, it is helpful to analyse as large many Y-STRs as possible, while avoiding the more rapidly mutating STRs.

Y-STRs can also be helpful in the triage of disaster victim identification, or in the identification of bodies in mass graves.

Use of Y-STRs in cases of historical interest

Y-STRs have been used in many cases of historical interest and but can raise other non-genetic matters that may need to be considered by living participants to these studies. In addition, it must always be foremost in the minds of investigators that these are lineage markers and there may be other explanations for their findings. Four examples are detailed in the supplementary material. These, far from providing definitive answers to the truth in some cases, have also raised other disappointments and unexpected findings, Three are historic, and one more recent.

The genetic investigation of the claims that *Thomas Jefferson*, one of the American Founding Fathers, fathered a child with one of his African slaves, has revealed several undesired consequences. Furthermore, the disproving of a theory that an American nineteenth-century mass murderer (*The American Ripper*) did not escape punishment and went on to commit similar crimes in the U.K. as Jack the Ripper, has disappointed the conspiracy theorists.

In the U.K., the discovery and identification of the skeleton of *Richard III* using mitochondrial DNA has, through analysis of the Y chromosome, revealed genetic inconsistencies in the line of succession of some members of the peerage and a more recent investigation in the *Baronetcy of Stichill* has forced transfer of a peerage from one side of a family to another which has raised issues of 'informed consent' as set down in data protection law.

Summary

- Analysis of Y-STRs can be of high importance, particularly in sexual assault cases where female material in a female–male mixture may otherwise overwhelm the genetic evidence.

- There are key circumstances where analysis of the Y chromosome can provide investigators with additional intelligence, such as ancestry, but this should always be viewed in the context of other available genetic and non-genetic information because it can only inform on lineages, rather than being a tool for identification.

- As a lineage marker, the Y chromosome can provide a useful tool to triage remains in a mass disaster and can also provide historic evidence linking families over many generations.

- Analysis of the Y chromosome can, however, raise important ethical issues that may adversely affect many people and should never be undertaken without that consideration.

Competing Interests

The author declares that there are no competing interests associated with this manuscript.

Abbreviations

CODIS, combined DNA index system; MPS, massively parallel sequencing; PARs, pseudoautosomal regions; SNPs, Y-single nucleotide polymorph; STR, short-tandem repeat.

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Supplementary case details for the Y Chromosome and its use in Forensic DNA Analysis

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Important case examples of Y-STRs in criminal justice system

The Boston Strangler

Eleven women were murdered in Boston between 1962 and 1964 and many were sexually assaulted. The nineteen-year old Mary Sullivan appears to be the last victim and Albert DeSalva apparently confessed to the crime, yet there was no other evidence and DNA from another male had later been linked to her body [1], placing doubts on his confession. DeSalva was murdered while in prison serving time for a number of rapes. Slides with DNA taken from Sullivan had not provided any useful DNA when first examined but had been retained and in 2013 a Y chromosome haplotype was produced, matching a paternally related nephew of DeSalva by testing DNA from a bottle he had discarded [2]. Matching DNA (presumably from the Y chromosome) was later recovered from DeSalva at exhumation.

Angie Dodge

In 1996 the eighteen-year old Angie Dodge was raped and murdered. Christopher Tapp was a suspect and, although his DNA did not match with that found at the crime scene, he was convicted in 1997 in the belief that he was one of several involved. Tapp also 'confessed' although allegedly that was coerced. In 2014, investigators had submitted a 35 locus Y-STR haplotype to the Sorensen Y-chromosome database [3], which revealed a match at 34 loci. A court order was issued revealing the identity of that person, Michael Usry Snr. In the belief that the donor of the semen was likely to be a close relative (within three or four generations), police focused on family members before identifying Michael Usry Jnr as a likely candidate, because of his age, links to the area, and also possibly because he made 'dark' films involving murder.   The accused Michael Usry was forced to provide a DNA sample and had to wait about one month before he was exonerated. Investigations continued into the provider of the DNA sample from the scene and in 2019, Brian Leigh Dripps was arrested and confessed, saying that he acted alone.

Marianne Vaatstra

In 1999 sixteen-year-old Marianne Vaatstra was raped, and her throat cut in a brutal murder. In the area was an asylum seekers' centre and the local population believed that the murder was one more typical of a non-European, leading to local tension [4]. As part of the investigation, two foreign males raised suspicions, but both provided non-matching DNA, as did 162 others who also provided their DNA. Y-STR analysis, and a search of the YHRD database (albeit illegal in Dutch legislation), revealed that the DNA was most likely from a white male, of likely north-west European ancestry [5].

In 2012 the law in The Netherlands was changed to allow familial searching and an investigation of 802 individuals, selected for genetic, residence, or family name reasons was also unsuccessful. A large-scale dragnet was authorised involving over 7000 males invited to provide their DNA for DNA testing. Y-chromosome STR testing was undertaken of the volunteers, revealing a Y chromosome and subsequent autosomal match with a Dutch European who was found guilty of her murder in 2013.

Y-STRs in cases of historical interest

Thomas Jefferson (1743-1826)

Thomas Jefferson was the third president of the United States of America. In 1802 a disaffected political journalist published an accusation that Jefferson had fathered a child (Tom) with one of his slaves, Sally Hemings [6]. Sally Hemings had several more children in her lifetime who, it has been suggested, could also have been fathered by Jefferson or by a male child of Jefferson's sister.

In 1998 a group of geneticists [7] reported their findings of a study of Y chromosomes from living descendants of: Jefferson's paternal uncle (Field Jefferson); the alleged son of Thomas Jefferson and Sally (Thomas Woodson); another son of Sally's sons (Eston Hemings); and the paternal grandfather of the Jefferson's nephews (Carr family) through his sister. They made use of a series of eleven STRs, seven biallelic markers and a minisatellite MSY1 and concluded that the most likely explanation for their results was that Thomas Jefferson was the father of Eston Hemings, but not Tom's father; no links were found with the Carr's grandfather's family descendants. Although other potential explanations were aired, they viewed them unlikely.

In 2011 Jobling [8] pointed out that the 'Field' haplogroup, T1a, used as the Thomas Jefferson reference, was very rare and therefore this match was unlikely to be a coincidence, but there was also a possibility that any other male in the same patrilineal line as Field, alive at the time, such as Thomas Jefferson's brother, could be an alternative father. While the question of paternity cannot be resolved genetically, historically it has been documented that Thomas Jefferson and Sally Hemings were both present in the same place at the relevant time before the birth of several of her children.

As highlighted above, this investigation raised other issues: in addition to the disappointment by descendants of Thomas Woodson that they were not related to Jefferson, a more recent issue of non-paternity was also revealed.

Richard III (1483-1485)

The investigation that took place of a skeleton excavated from a car park in Leicester, indicating that this was most likely the skeletal remains of Richard III, revealed through archaeological, osteological, radiocarbon and genetic analysis of mitochondrial DNA, also uncovered another surprise when his Y chromosome was analysed [9]. Y chromosome analysis of presumptive male descendants of Edward III (great-great-grandfather of Richard III) were shown not to match with that found in the skeleton. Some nineteen generations separated the individuals according to genealogy. With an estimated 16% possibility of a non-paternity event occurring in the intervening time (assuming an average 0.9% non-paternity rate for Western societies [10] this provides a reasonable explanation for the findings, but where this false paternity occurred has not been discovered and a more recent investigation of another male claiming to be a direct descendant of Edward III has not resolved the matter [11].

HH Holmes (1861-1896)

Born Herman Webster Mudgett, HH Holmes is probably one of America's most prolific killers, confessing to 27 murders but suspected of many more; some believe that he was also the person responsible for the Whitechapel murders. He was convicted and hanged in 1896, requesting that his coffin be buried deep in concrete for fear of grave robbers [12]. Rumours abounded however that Mudgett had escaped the gallows and his great-great grandson commissioned an investigation with the aim of proving that. Historical dental examination, however, matched with medical examination reports from Holmes and the Y23 chromosome haplotype from the skeleton also matched that of his descendant [13].   While the rumours of Holmes escaping his execution are now proven to be unfounded, this analysis has not resolved other conspiracy theories.

Baronetcy of Stichill

The baronetcy of Stichill was granted to Robert Pringle (and male heirs of his body) by Charles II in 1683. The 8th Baron was Sir Norman Robert Pringle (1871-1919) and he had three sons Norman Hamilton Pringle 9th Baron and Ronald Steuart Pringle being the first two registered. Norman Hamilton Pringle's son, Sir Steuart Robert Pringle became the 10th Baron (figure 1S). In 2009 Ronald Steuart Pringle's son, Norman Murray (Murray), the then current Baron's first cousin, initiated a 'Pringle' surname study with the stated aim of discovering which of any living Pringles might be entitled to be the Clan Chief, the last claimant to this Scottish clan having died in 1738.

Whether or not that would ever have been a realistic approach to answer the question, Murray approached Pringles to give their DNA for a Y chromosome study with that aim. Sir Steuart, although not interested in being the Clan Chief because of the associated responsibilities, was finally persuaded. The Y chromosome study revealed that there must have been an issue of non-paternity such that the 9th Baron could not have been the biological son of the 8th Baron and that he had wrongly inherited the title, as therefore had the 10th Baron, Sir Steuart himself. As a result of the revelation Murray Pringle issued a claim to the baronetcy and in 2016 Murray Pringle became Sir Norman Murray Archibald MacGregor Pringle, 10th Baronet as the result of a Privy Council Judgement [14].

The claim was fought by the son of the then 10th Baronet on several grounds, which were dismissed, but it does raise important issues:

Consent is an important consideration in any genetic testing. The Human Genetics Commission publication on Direct-to-Consumer Genetic Testing [15] states:
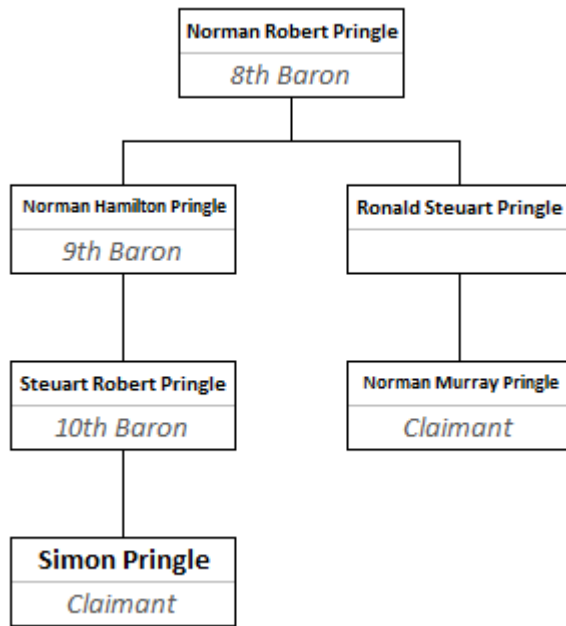
A genetic test should be carried out only after the person concerned has given free and informed consent. Informed consent can only be provided when a consumer has received sufficient relevant information about the genetic test to enable them to understand the risks, benefits, limitations and implications (including the implications for purchasing insurance) of the genetic test.

While consent was given for the purposes of determination of who might be the most senior person in the clan of Pringles, it was argued by Simon Pringle (son of Sir Stuart who had recently died) that this did not extend to consent to question his baronetcy. The court, however, stated that while the processes might have breached the Data Protection Law in some respects, Sir Steuart should have been aware "that if his DNA excluded him from a claim to be the clan chief, it might also form the basis of a challenge to his entitlement to the Baronetcy".

A wider issue has been opened by this judgement allowing anyone to challenge the holder of a hereditary title by providing Y chromosome lineage evidence that questions the veracity of a historic family tree.

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                    ┌─────────────────────────┐
                    │ Norman Robert Pringle   │
                    │      8th Baron          │
                    └─────────────────────────┘
                               │
              ┌────────────────┴────────────────┐
    ┌─────────────────────────┐      ┌─────────────────────────┐
    │ Norman Hamilton Pringle │      │ Ronald Steuart Pringle  │
    │      9th Baron          │      │                         │
    └─────────────────────────┘      └─────────────────────────┘
               │                                │
    ┌─────────────────────────┐      ┌─────────────────────────┐
    │ Steuart Robert Pringle  │      │ Norman Murray Pringle   │
    │      10th Baron         │      │      Claimant           │
    └─────────────────────────┘      └─────────────────────────┘
               │
    ┌─────────────────────────┐
    │     Simon Pringle       │
    │      Claimant           │
    └─────────────────────────┘
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Figure 1S: Baronetcy of Stichill – family tree illustrating position of claimants to the 11th Baronetcy (Simon Pringle) and 10th Baronetcy (Norman Murray Pringle)