The case of the unreliable SNP: recurrent back-mutation of Y-chromosomal marker P25 through gene conversion

Susan M. Adams, Turi E. King, Elena Bosch* and Mark A. Jobling

Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK

* Current address: Unitat de Biologia Evolutiva, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Doctor Aiguader 80, 08003 Barcelona, Catalonia, Spain

Address for correspondence and reprints: Prof Mark A. Jobling, Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK
Tel.: +44 (0)116 252 3427. Fax: +44 (0)116 252 3378. Email: maj4@leicester.ac.uk

Keywords: Y chromosome; Paralog; PSV; SNP; Gene Conversion; Phylogeny

Abstract

The Y-chromosomal binary marker P25 is a paralogous sequence variant, rather than a SNP: three copies of the P25 sequence lie within the giant palindromic repeats on Yq, and one copy has undergone a C to A transversion to define haplogroup R1b (designated C/C/A). Since gene conversion is known to be active in the palindromic repeats, we reasoned that P25 might be liable to back mutation by gene conversion, yielding the ancestral state C/C/C. Through analysis of a set of binary markers in Y chromosomes in two large samples from Great Britain and the Iberian Peninsula we show that such conversion events have occurred at least twice, and provide preliminary evidence that the reverse conversion event (yielding C/A/A) has also occurred. Because of its inherent instability, we suggest that P25 be used with caution in forensic studies, and perhaps replaced with the more reliable binary marker M269.

1. Introduction

Y-chromosomal binary markers, mostly single nucleotide polymorphisms (SNPs) are widely used in population studies [1], and are of increasing interest in forensic analysis [2,3]. Sets of binary markers on the non-recombining region of the Y chromosome define compound haplotypes known as haplogroups that show a high degree of geographical specificity, making them potentially useful in prediction of population of origin of a DNA sample. Haplogroups are considered to be stable and robust definitions of Y chromosome lineages, due to the very low mutation rate of most binary markers – for SNPs, around 10^{-9} per base per generation [4]. This stability allows haplogroups to be arranged into a unique maximum parsimony tree [1,5,6], showing evidence of recurrent mutation at only 6 of 240 SNPs [6].

About 5% of the human genome sequence is now known to be in the form of paralogous segments [7] – duplicated sequences that can be tens of kilobases in length, displaying extremely high (>95%) sequence similarity. On the Y chromosome the proportion of such paralogous sequence is much higher than the genomic average – 30-45%, depending on how it is defined [8]. An important property of these paralogs is their propensity to gene conversion – the non-reciprocal transfer of sequence information from one paralog copy to another [9,10]. Y-chromosomal binary markers located within paralogs might therefore be expected to display unusual mutational properties reflecting the action of gene conversion as well as conventional base mutation.

Most of the commonly analysed Y-chromosomal DNA sequence variants were ascertained from material thought to be single-copy, and are therefore underrepresented in paralogous segments of the chromosome.

However, one important marker, P25 [11], defining the most frequent haplogroup in western European populations (hg R1b), lies in the triplicated paralogous 315-kb 'g'-type repeats [12] of the *AZFc* region [13] on Yq (*Figure 1*). It is thus a paralogous sequence variant (PSV), rather than a SNP [14]. In the reference database sequence, which derives from a hg R1b* chromosome, the most distal repeat copy, g3, contains the derived P25 A-allele, while the more proximal g1 and g2 repeats both contain the ancestral C-allele (designated: C/C/A).

We hypothesised that P25 might be subject to gene conversion because of its paralogous location, and therefore might display evidence of apparent back mutation, in which the A-allele-carrying copy is converted by a copy carrying the C-allele (C/C/C). We sought such conversions by phylogenetic analysis in population studies, and here we demonstrate that conversions have indeed occurred at least twice in two western European populations; these unusual mutational characteristics must be taken into account in both population studies and forensic investigations.

2. Materials and Methods

2.1 DNA samples

Samples from two western European regions were studied: 421 males originating from Great Britain (King et al., manuscript in preparation) and 1000 males from the Iberian peninsula [15]. All samples were obtained with appropriate informed consent.

2.2 Binary marker typing

Binary markers (M18, M37, M65, M73, M126, M153, M160, M167, M173, M222, P25 and SRY-1532 [5,11,16]) were typed using the SNaPshot minisequencing procedure (Applied Biosystems), using either published primer sequences [17] or sequences derived from published information [6]. Extension products were analysed on an ABI3100 genetic analyser (Applied Biosystems). For assessment of P25 allele copy number variation, areas under peaks were estimated using GeneScan 3.7 software (Applied Biosystems).

2.3 Y-STR typing

19 Y-STRs were typed, and alleles designated, as described [15], using an ABI3100 genetic analyser (Applied Biosystems). DYS385 was not typed in the British samples.

2.4 Data analysis

Weighted median-joining networks of Y-STR haplotypes were constructed using Network 4.0 [18], applying the reduced median and median joining algorithms successively [19]. The weighting scheme was as described [19], and weights assigned to Y-STR loci according to variance in a

random sample of 50 British and 50 Iberian Peninsula hg R1b3* chromosomes (unpublished data). The significance of population differences in conversion chromosome frequencies was assessed using a population differentiation test in Arlequin [20].

3. Results

As part of a population study, we typed the three binary markers M173, SRY-1532b (also known as SRY10831.2) and P25 in 421 males originating from Great Britain and 1000 males from the Iberian peninsula. Chromosomes that are derived for SRY-1532b or P25 belong to haplogroups (hg) R1a or R1b respectively, while chromosomes derived for M173 but ancestral for both P25 and SRY-1532b are classified as belonging to hg R1* ([6]; *Figure 2a*). We found 22 such chromosomes in the British sample (5.2%), and 10 (1.0%) in the Iberian Peninsula sample (*Table 1*). To ask whether any of these 32 chromosomes in fact represent back-mutations of P25 (C/C/A to C/C/C) through gene conversion we typed further SNPs defining sub-haplogroups of R1b in the same samples.

We found that 31 of the 32 apparent hg R1* chromosomes (P25: C/C/C) were in fact derived for the 'downstream' SNP M269, and that one of these, IP285, was derived both for M269 and for M167 (*Table 1*). These 31 chromosomes therefore carry recurrent mutations (homoplasies). The most likely explanation for the homoplasies is recurrent back-mutation at P25 within hgR1b (*Figure 2b*). We reject the alternative explanation, recurrent mutation at M269 and M167 within hgR1*, since, while P25 is a PSV, there is no evidence to suggest that M269 and M167 are anything other than ordinary SNPs with ordinary (and very low) mutation rates; in particular, independent and ordered recurrence of both SNPs within the same lineage (in chromosome IP285) is extremely unlikely. Our observations are therefore best explained by back-mutation, through gene conversion, at P25.

Thirty of the apparent hg R1* chromosomes therefore in fact belong to hg R1b3 (designated R1b3-P25b in *Figure 2b*), and one to hg R1b3f (R1b3f-P25c; *Figure 2b*). There are thus at least two independent conversions of the P25 PSV in our sample. In an attempt to ask if the hg R1b3-P25b chromosomes owe their origin to a single event or to multiple events, we compared their Y-STR haplotypes by constructing a weighted median joining haplotype network (*Figure 3*). The 30 chromosomes possess 29 different haplotypes, with one pair of British chromosomes sharing an identical haplotype; there is no clear geographical partitioning. The network lacks the compact, star-like structure that might be indicative of a recent common origin for the conversion chromosomes. Also, the mean pairwise difference between haplotypes is statistically indistinguishable from those of equally sized and population-matched samples of non-conversion chromosomes from hg R1b3* (data not shown). A network like this could represent either an ancient founding conversion event followed by drift, or a number of independent conversions, and we cannot distinguish between these possibilities.

P25 C/C/C chromosomes that are underived for the all of the three markers M18, M73 and M269 remain ambiguous, since they could either genuinely be members of hg R1*, or members of hg R1b* that have undergone reversion from P25 C/C/A (designated R1b-P25d in *Figure 2b*). There is one chromosome in our set in this category, IP481, and its Y-STR haplotype can be compared with those of the two genuine hg R1b* chromosomes (*Figure 3*). The hg R1* chromosome lies distant in the network from the two hg R1b* chromosomes, which does not support the idea that it really belongs to hg

R1b-P25d. However, this remains a possibility, since the poorly sampled hg R1b* may be a highly diverse lineage.

As well as conversions of the P25 A-allele by the C-allele, we expect the reciprocal conversion event to occur, and probably at a similar rate. In this case, the resulting chromosome would have two P25 sequences carrying the A-allele and only one carrying the C (C/A/A). Detection of such chromosomes requires a quantitative test, and is therefore less simple than that of the A-to-C conversion chromosomes, but SNaPshot minisequencing analysis reveals candidates in our dataset in which the signal strength of the A-peak is higher than that of the C (*Figure 4*), a phenomenon that is reproducible in blind tests. Of the 665 Iberian Peninsula chromosomes that show a green (A-allele) peak, 626 (94%) have a green: black (A: C) ratio lying between 0.8 and 1.8. However, for 20 chromosomes (3%) this ratio is >3, and for 13 (2%) it is >5. These may be instances of C-to-A conversion, but would require formal Q-PCR analysis to assess allele copy number accurately. Notably, we (in agreement with Sanchez et al. [14]) observe no chromosomes in which there is complete absence of a C-signal (double conversions: A/A/A). While conversion may be relatively frequent between the P25 sequences within the g2 and g3 repeats, which form part of the large palindrome P1 (*Figure 5a*; [12]), they may be relatively rare between either of these repeat copies and the more proximal g1 repeat copy (*Figure 5b, c*), which does not form part of the palindrome itself.

4. Discussion

We have identified chromosomes carrying back-mutations at the Y-chromosomal binary marker P25, which are the result of at least two independent gene conversion events within the large palindromic repeats (P1) on Yq (C/C/A to C/C/C). We have also found chromosomes with probable conversions in the opposite sense (C/C/A to C/A/A). Our findings are consistent with those of Rozen et al. [9], who present evidence of recurrent conversion at three PSVs in the *AZFc* region. Using an indirect method, a rate of 2.2×10^{-4} conversions per duplicated nucleotide per generation – some five orders of magnitude higher than the base mutation rate - has been estimated [9]. Because of the uncertainty pertaining to the allelic state of P25, we recommend that, in both forensic and population genetic applications, it be supplemented or replaced with the straightforward SNP M269.

The C/C/A to C/C/C conversion is effectively unidirectional, since the C/C/A genotype can be regenerated only by the extremely improbable event of a recurrent base substitution. Assuming no influence of natural selection on P25 allelic states, the rate at which C/C/C conversion chromosomes arise in populations will depend on the frequency of hg R1b (C/C/A), and subsequently be determined by demographic history and drift. As a proportion of all hg R1b chromosomes, the frequency of conversion chromosomes in the British population is significantly higher ($p<0.05$) than that in the Iberian sample - 8.2% vs. 1.5%. This may suggest that the British population has a history of more marked population expansion, or a larger effective population size, than that of the Iberian Peninsula.

P25 is not the only widely used marker within the palindromic repeats of Yq: the Y-STR DYS464 [21-24] lies in the 'r' type repeats of the *AZFc* region [13], and is present in four copies in the database sequence. It may owe its high diversity to gene conversion between copies, as well as to conventional slippage mutation processes. The location of these markers in the evolutionarily labile *AZFc* region lends them another unusual and potentially undesirable property in forensic and genealogical studies – the ability to signal infertility in a DNA donor by their absence through *AZFc* deletions [12,13].

Acknowledgements

We thank all DNA donors. M.A.J. was supported by a Wellcome Trust Senior Fellowship in Basic Biomedical Science (grant no. 057559), S.M.A. and E.B. by the Wellcome Trust, and T.E.K. by a Wellcome Prize Studentship (grant no. 061129).

Table 1: Haplotypes of P25 conversion chromosomes, and chromosomes belonging to hgs R1b* and R1*

[a] 'YCC nomenclature [1]; [b] '0' – underived allele; '1' – derived allele; [c] nd – not done. The six markers M37, M65, M126, M153, M160 and M222 within hg R1b3 were also typed (not shown) – all were underived in all chromosomes.

			Binary markers[b]							**Y-STRs**																	
sample	**mutation-based hg name**	**hg[a]**	M173	SRY-1532b	P25	M18	M73	M269	M167	DYS19	DYS385[c]	DYS388	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS434	DYS435	DYS436	DYS437	DYS438	DYS439	DYS460	DYS461	DYS462
Great Britain																											
GB143	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	23	12	13	13	11	11	12	15	12	12	11	12	11
GB160	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	14	30	25	11	13	13	11	11	12	15	12	12	11	12	11
GB184	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	25	11	13	13	11	11	12	15	12	12	10	12	11
GB354	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	14	30	24	12	13	13	11	11	12	14	12	11	11	12	11
GB526	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	15	nd	12	13	29	24	11	13	13	11	11	12	15	12	13	10	12	11
GB982	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	25	12	13	13	11	11	12	15	12	12	10	12	11
GB1005	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	24	11	13	13	12	11	12	14	12	12	11	12	11
GB1091	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	15	nd	12	13	29	24	11	13	13	12	11	12	14	12	12	11	13	11
GB1257	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	23	11	13	14	11	11	12	15	12	13	11	11	11
GB1261	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	28	24	11	13	13	12	11	12	14	12	12	11	13	11
GB1436	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	24	11	13	13	12	11	12	14	12	12	11	13	11
GB1458	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	28	24	10	13	13	11	11	12	15	12	12	11	12	11
GB1555	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	13	nd	12	13	29	24	11	13	13	12	11	12	14	12	12	11	13	11
GB1674	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	24	11	13	13	11	11	12	15	11	12	11	12	11
GB1981	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	24	11	13	13	11	11	12	15	12	11	11	12	11
GB2008	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	24	11	13	13	11	11	12	15	12	12	11	12	11
GB2160	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	30	24	10	13	13	11	11	12	15	12	13	11	12	11
GB2219	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	24	10	13	13	11	11	12	15	11	11	11	12	11
GB2311	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	24	11	13	13	11	11	12	15	12	13	11	12	11
GB2351	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	12	29	25	11	13	13	11	11	12	15	12	11	11	12	11
GB2432	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	30	24	10	13	13	11	11	13	15	12	12	11	12	11
GB2462	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	nd	12	13	29	24	11	13	13	11	11	12	15	11	12	11	12	11
Iberian Peninsula																											
IP85	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	12-14	12	13	29	25	11	13	13	11	11	12	15	12	12	11	12	11
IP132	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	11-14	12	13	29	23	11	13	13	11	11	12	15	13	12	11	11	11
IP137	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	11-14	12	13	29	25	11	13	12	11	11	12	15	12	14	10	12	11
IP485	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	11-14	12	14	30	25	11	13	13	11	11	12	15	12	12	11	13	11
IP695	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	11-14	12	13	29	24	10	13	13	10	11	12	15	11	12	11	12	11
IP805	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	12-14	12	13	29	24	11	13	13	11	11	12	16	12	11	11	12	11
IP823	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	11-14	12	13	29	23	11	13	13	11	11	12	14	12	11	11	12	12
IP1011	R1b3-P25b	R1b3*	1	0	0	0	0	1	0	14	11-14	12	13	29	24	10	13	13	11	11	12	14	12	12	11	12	11
IP285	R1b3f-P25c	R1b3f	1	0	0	0	0	1	1	15	11-14	12	13	29	24	10	13	13	11	11	12	15	12	12	11	13	11
IP481	R1* / R1b-P25d	R1*	1	0	0	0	0	0	0	15	11-13	12	14	30	24	10	13	13	11	11	12	14	11	11	11	12	11
IP637	R1b*	R1b*	1	0	1	0	0	0	0	17	13-14	12	13	28	22	11	13	13	11	11	12	14	11	12	11	12	11
IP456	R1b*	R1b*	1	0	1	0	0	0	0	16	13-14	12	13	29	25	11	13	13	11	11	12	14	12	12	11	12	11

Figure Legends

Figure 1: **Position of P25 sequences in the g-type repeat units of the *AZFc* region.**

The Y chromosome idiogram (top) shows the approximate position of the *AZFc* region. The schematic representation below shows *AZFc*-region paralogous repeat units as arrows [12], and includes the position and sequence states of the P25 PSV in Y chromosomes belonging to hg R1b and other haplogroups [hg Y(xR1b)] [6].

Figure 2: Phylogenetic relationships of lineages within haplogroup R1.

a) Phylogeny of haplogroups defined by M173, SRY-1532b, and P25 [1,6].
b) Recurrent back-mutation at P25. Phylogeny from (a), expanded to include sub-haplogroups within hg R1b defined by M18, M73, M269 and M167 [1,6]. The two observed homoplasic instances of the P25 C-allele are indicated by hgs R1b3-P25b and R1b3f-P25c. A hypothetical haplogroup defined by back-mutation at P25 on a chromosome ancestral for M18, M73 and M269 is shown in grey (hg R1b-P25d); note that this would be indistinguishable from hg R1*. Examples of SNaPshot electropherograms are shown to the right.

Figure 3: Median joining network of haplotypes in P25-conversion chromosomes.

Circles represent haplotypes, with area proportional to frequency. Lines between circles are mutational steps, with the shortest line representing a single step. Chromosomes belonging to hgs R1*, R1b*, and R1b3f are indicated.

Figure 4: SNaPshot electropherograms showing possible C/A/A conversion.
Chromosome IP9 shows a normal A:C ratio, while IP807 has a reproducibly higher ratio.

Figure 5: Possible configurations of the *AZFc* region leading to gene conversion of P25 PSVs.
Repeat units of the *AZFc* region [12] are shown paired intrahelically (parts a and b) or interhelically (part c). Within paired regions, gene conversion could lead to transfer of sequence information from one copy of P25 to another (in dotted ovals).

a) Giant hairpin formation by the P1 palindrome allowing conversion between g2 and g3 copies of P25. This would lead to the conversions observed in this study, and may be more frequent than the conversions shown in (b) and (c).
b) Stem-loop formation allowing conversion between g1 and g3 copies of P25.
c) Unequal pairing of sister-chromatids allowing conversion between g1 and g2 copies of P25.

References

[1] M.A. Jobling, C. Tyler-Smith, The human Y chromosome: an evolutionary marker comes of age, Nat. Rev. Genet. 4 (2003) 598-612.

[2] M.A. Jobling, Y-chromosomal SNP haplotype diversity in forensic analysis, Forensic Sci. Int 118 (2001) 158-162.

[3] J.M. Butler, Recent developments in Y-Short Tandem Repeat and Y-Single Nucleotide Polymorphism analysis, Forensic Sci. Rev. 15 (2003) 91-111.

[4] R. Thomson, J.K. Pritchard, P. Shen, P.J. Oefner, M.W. Feldman, Recent common ancestry of human Y chromosomes: Evidence from DNA sequence data, Proc. Natl. Acad. Sci. USA 97 (2000) 7360-7365.

[5] P.A. Underhill, P. Shen, A.A. Lin, L. Jin, G. Passarino, W.H. Yang, E. Kauffman, B. Bonné-Tamir, J. Bertranpetit, P. Francalacci, M. Ibrahim, T. Jenkins, J.R. Kidd, Q. Mehdi, M.T. Seielstad, R.S. Wells, A. Piazza, R.W. Davis, M.W. Feldman, L.L. Cavalli-Sforza, P.J. Oefner, Y chromosome sequence variation and the history of human populations, Nat. Genet. 26 (2000) 358-361.

[6] Y Chromosome Consortium, A nomenclature system for the tree of human Y-chromosomal binary haplogroups, Genome Res. 12 (2002) 339-348.

[7] J.A. Bailey, Z. Gu, R.A. Clark, K. Reinert, R.V. Samonte, S. Schwartz, M.D. Adams, E.W. Myers, P.W. Li, E.E. Eichler, Recent segmental duplications in the human genome, Science 297 (2002) 1003-1007.

[8] H. Skaletsky, T. Kuroda-Kawaguchi, P.J. Minx, H.S. Cordum, L. Hillier, L.G. Brown, S. Repping, R. Pyntikova, J. Ali, T. Bieri, A. Chinwalla, A. Delehaunty, K. Delehaunty, H. Du, G. Fewell, L. Fulton, R. Fulton, T. Graves, S.-F. Hou, P. Latrielle, S. Leonard, E. Mardis, R. Maupin, J. McPherson, T. Miner, W. Nash, C. Nguyen, P. Ozersky, K. Pepin, S. Rock, T. Rohlfing, K. Scott, B. Schultz, C. Strong, A. Tin-Wollam, S.-P. Yang, R.H. Waterston, R.K. Wilson, S. Rozen, D.C. Page, The male-specific region of the human Y chromosome: a mosaic of discrete sequence classes, Nature 423 (2003) 825-837.

[9] S. Rozen, H. Skaletsky, J.D. Marszalek, P.J. Minx, H.S. Cordum, R.H. Waterston, R.K. Wilson, D.C. Page, Abundant gene conversion

between arms of massive palindromes in human and ape Y chromosomes, Nature 423 (2003) 873-876.

[10] E. Bosch, M.E. Hurles, A. Navarro, M.A. Jobling, Dynamics of a human interparalog gene conversion hotspot, Genome Res. 14 (2004) 835-844.

[11] M.F. Hammer, A.J. Redd, E.T. Wood, M.R. Bonner, H. Jarjanazi, T. Karafet, S. Santachiara-Benerecetti, A. Oppenheim, M.A. Jobling, T. Jenkins, H. Ostrer, B. Bonné-Tamir, Jewish and Middle Eastern non-Jewish populations share a common pool of Y-chromosome biallelic haplotypes, Proc. Natl. Acad. Sci. USA 97 (2000) 6769-6774.

[12] T. Kuroda-Kawaguchi, H. Skaletsky, L.G. Brown, P.J. Minx, H.S. Cordum, R.H. Waterston, R.K. Wilson, S. Silber, R. Oates, S. Rozen, D.C. Page, The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men, Nat. Genet. 29 (2001) 279-286.

[13] T.E. King, E. Bosch, S.M. Adams, E.J. Parkin, Z.H. Rosser, M.A. Jobling, Inadvertent diagnosis of male infertility through genealogical DNA testing, J. Med. Genet. 42 (2005) 366-368.

[14] J.J. Sanchez, M. Brion, W. Parson, A.J. Blanco-Verea, C. Borsting, M. Lareu, H. Niederstatter, H. Oberacher, N. Morling, A. Carracedo, Duplications of the Y-chromosome specific loci P25 and 92R7 and forensic implications, Forensic Sci. Int. 140 (2004) 241-50.

[15] E. Bosch, A.C. Lee, F. Calafell, E. Arroyo, P. Henneman, P. de Knijff, M.A. Jobling, High resolution Y chromosome typing: 19 STRs amplified in three multiplex reactions, Forensic Sci. Int. 125 (2002) 42-51.

[16] L.S. Whitfield, J.E. Sulston, P.N. Goodfellow, Sequence variation of the human Y chromosome, Nature 378 (1995) 379-380.

[17] S. Paracchini, B. Arredi, R. Chalk, C. Tyler-Smith, Hierarchical high-throughput SNP genotyping of the human Y chromosome using MALDI-TOF mass spectrometry, Nucleic Acids Res. 30 (2002) e27.

[18] H.-J. Bandelt, P. Forster, A. Röhl, Median-joining networks for inferring intraspecific phylogenies, Mol. Biol. Evol. 16 (1999) 37-48.

[19] R. Qamar, Q. Ayub, A. Mohyuddin, A. Helgason, K. Mazhar, A. Mansoor, T. Zerjal, C. Tyler-Smith, S.Q. Mehdi, Y-chromosomal DNA variation in Pakistan, Am. J. Hum. Genet. 70 (2002) 1107-24.

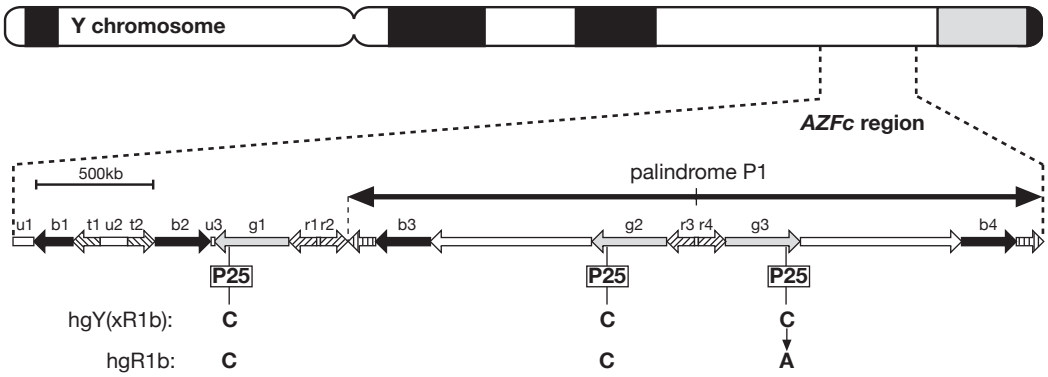
[20] S. Schneider, D. Roessli, L. Excoffier (2000) Arlequin ver. 2.0: A software for population genetics data analysis. 2.0 ed. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

[21] A.J. Redd, A.B. Agellon, V.A. Kearney, V.A. Contreras, T. Karafet, H. Park, P. de Knijff, J.M. Butler, M.F. Hammer, Forensic value of 14 novel STRs on the human Y chromosome, Forensic Sci Int 130 (2002) 97-111.

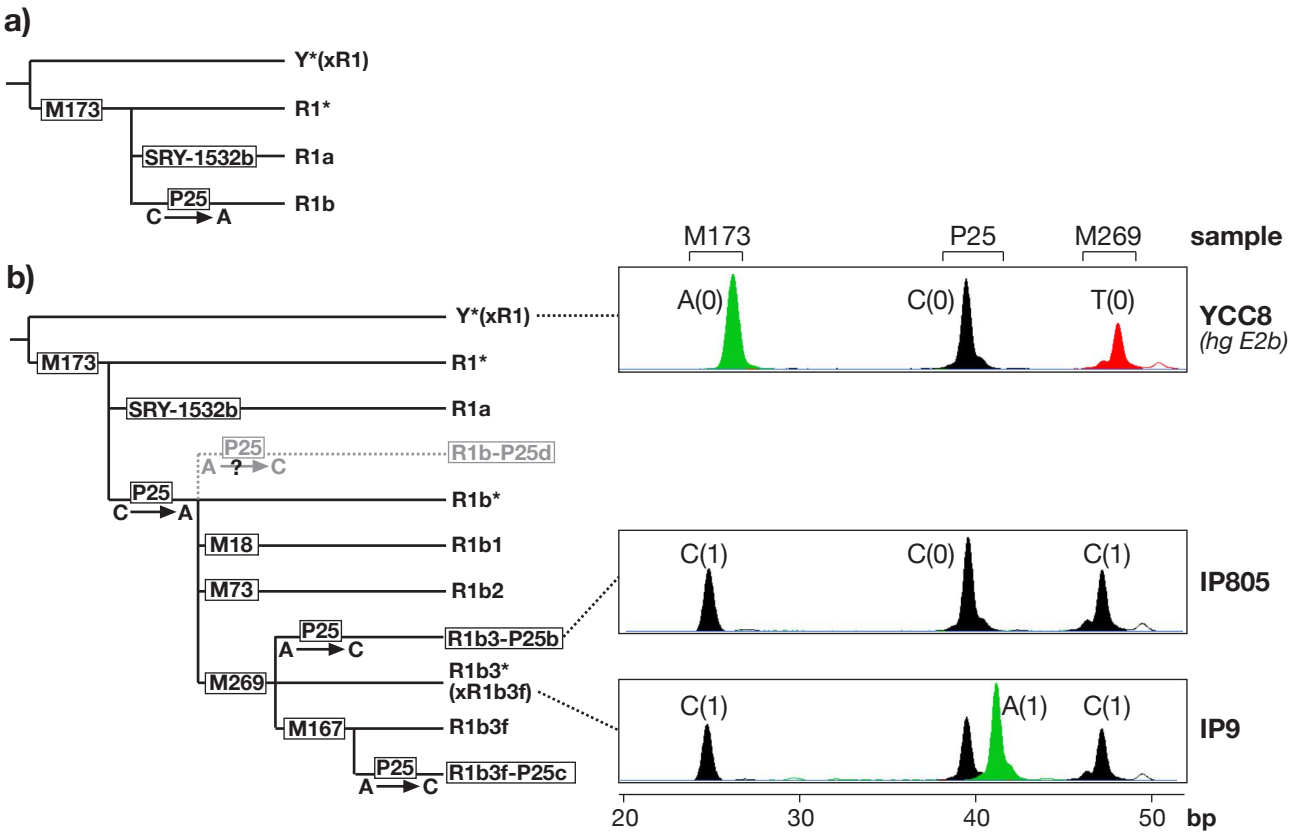
[22] J.M. Butler, R. Schoske, Forensic value of the multicopy Y-STR marker DYS464, Progr. Forensic Genet. 10 (2003) 278-280.

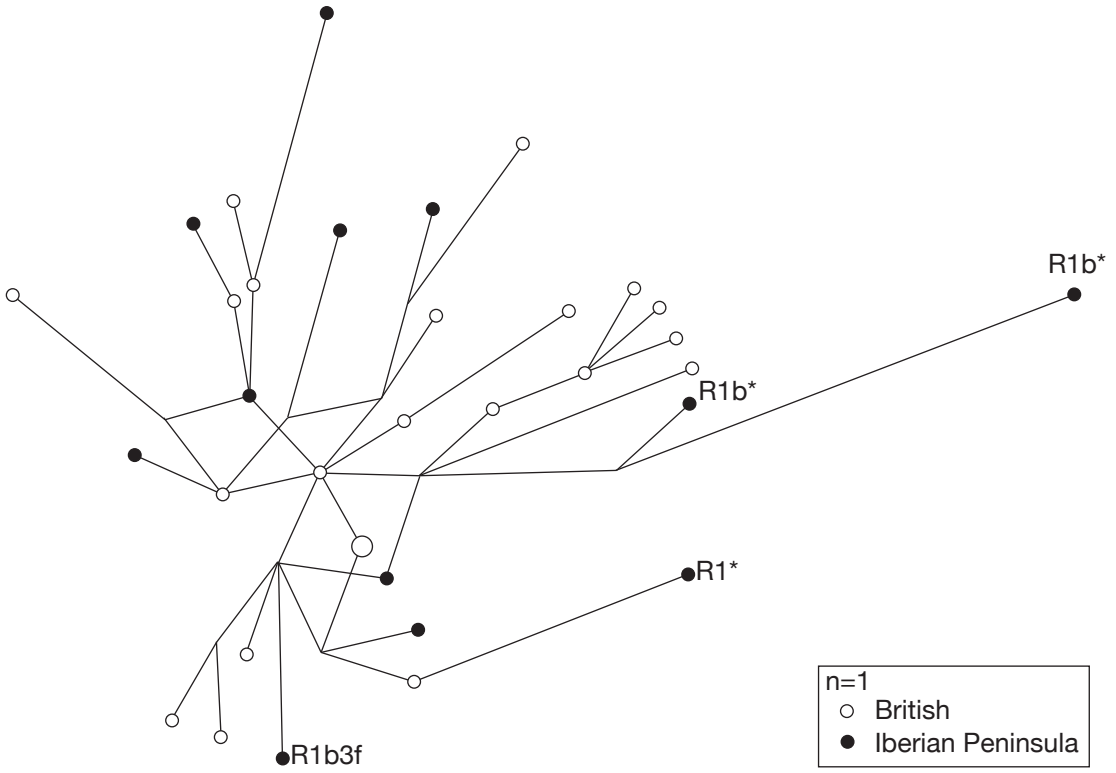
[23] B. Berger, H. Niederstatter, A. Brandstatter, W. Parson, Molecular characterization and Austrian Caucasian population data of the multi-copy Y-chromosomal STR DYS464, Forensic Sci. Int. 137 (2003) 221-30.

[24] R. Schoske, P.M. Vallone, M.C. Kline, J.W. Redman, J.M. Butler, High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays, Forensic Sci. Int. 139 (2004) 107-21.

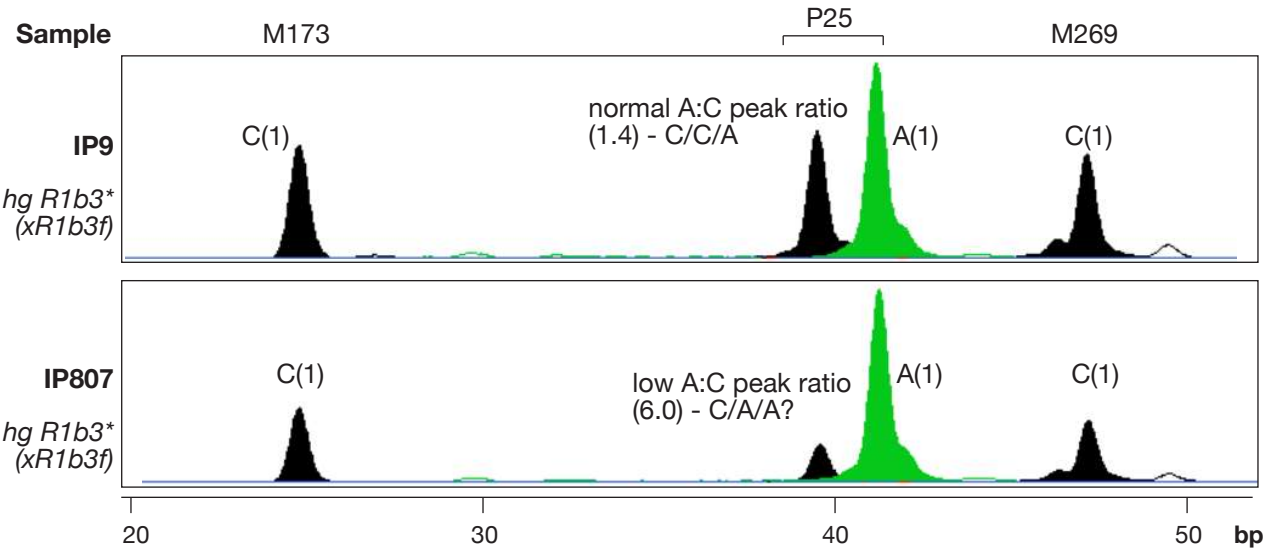


Adams et al., Figure 1

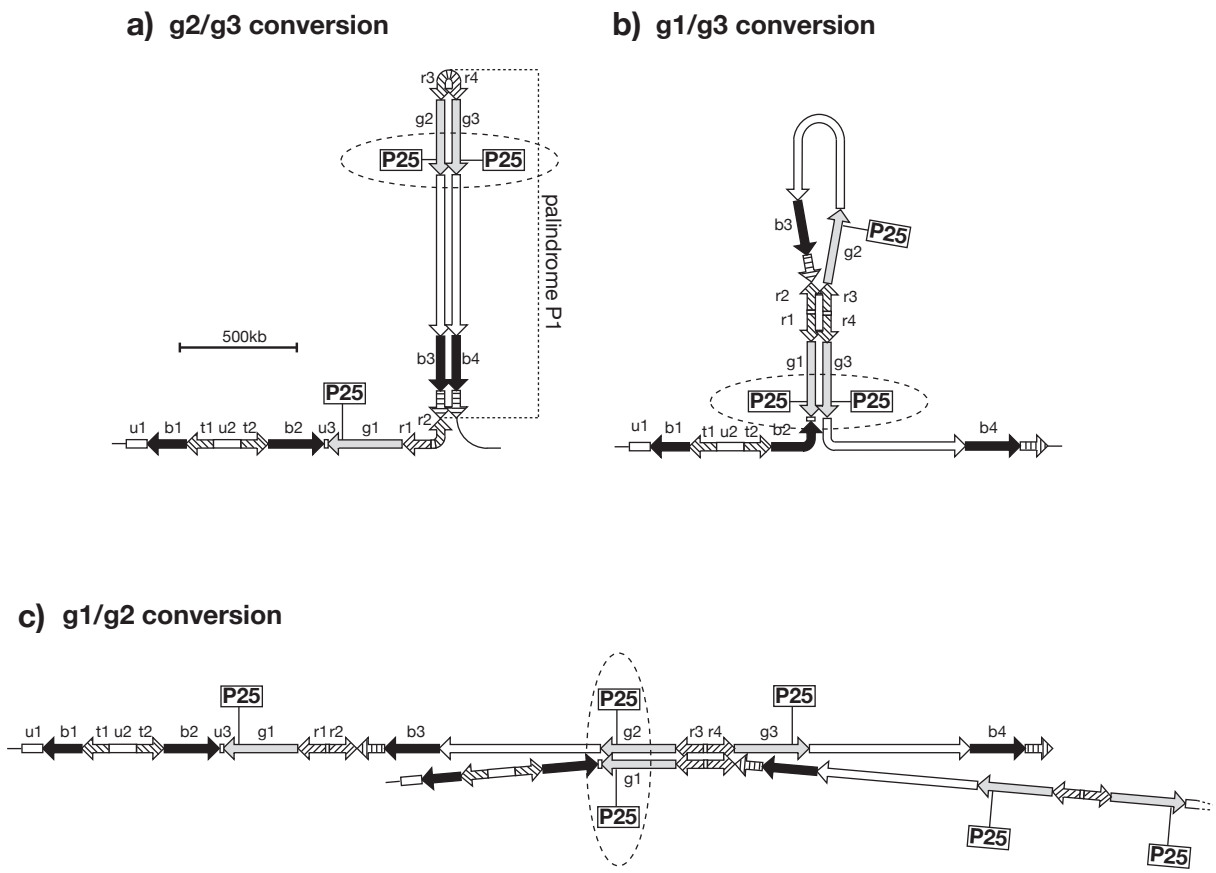

a)
Y*(xR1)
M173
R1*
SRY-1532b
R1a
P25
C → A
R1b
b)
Y*(xR1)
M173
R1*
SRY-1532b
R1a
P25
A ? C
R1b-P25d
P25
C → A
R1b*
M18
R1b1
M73
R1b2
P25
A → C
R1b3-P25b
M269
R1b3*
(xR1b3f)
M167
R1b3f
P25
A → C
R1b3f-P25c
M173
P25
M269
sample
A(0)
C(0)
T(0)
YCC8
(hg E2b)
C(1)
C(0)
C(1)
IP805
C(1)
A(1)
C(1)
IP9
20
30
40
50
bp



Adams et al., Figure 3



Adams et al., Figure 4



Adams et al., Figure 5