An Y-chromosome STR Profile of Karl Wilhelm Naundorff (1785?-1845)

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Abstract: We complete in the present paper our previous work (Lucotte *et al.* 2014, *Int J Sciences*, 3 (7): 28-32) on DNA marker of Karl Wilhelm Naundorff by the study of his Y-chromosome profile. For fourteen Y-STRs studied allelic values are identical to those of Hugues de Bourbon, the living 4th generation descendant of the Naundorff' elder branch. These datas establish that H. de Bourbon is patrilinealy related to K.W. Naundorff.

Keywords: Y-chromosome markers; short tandem repeats (STRs); allelic values; Hugues de Bourbon; members of the Naundorff' family

Introduction

The king of France Louis XVI and the Queen Marie-Antoinette were beheaded in 1793, during the french Revolution. Their two children Marie-Thérèse Charlotte (1778-1851) and Louis-Charles (1785-1795?) remained imprisoned in the Temple (in Paris) where they survived to the death of their parents. According to the official records, Louis-Charles (Louis XVII) died of tuberculosis in the Temple on 8 June 1795. But this version of his death has been repeatedly questioned; one of the most persistent theories claims that it was a substitute who died on 8 June.

Subsequently some individuals claimed to be Louis XVII. The most famous of them, Karl Wilhelm Naundorff (1785?-1845), came in Paris in 1833 where he could apparently provide sufficient circumstantial evidence to convince ex-members of the courts of Versailles and Tuileries of his descent. In 1836 Naundorff was deported to England by the French authorities; he died on the 10 August 1845 in Delft (The Netherlands). Recently (Lucotte *et al.*, 2014) based on reproducible results obtained on DNA extracted from authentic Naundorff' hairs, we have determined his mtDNA sub-haplogroup; this haplogroup is HV0, according to modern mtDNA haplogroup nomenclature.

In the present study, we compare the Naundorff' Y-chromosome STR profile to that, already published (Lucotte and Roy-Henry, 2014), of Hugues de Bourbon. Hugues, born in 1974, is the living 4th generation descendant of Naundorff. The goal of this study was to verify the patrilineal descendance of Hugues from Karl Naundorff.

MATERIAL AND METHODS

Naundorff' genomic DNA used is that which was extracted from his hair number 3 (Lucotte *et al.*, 2014), that had a bulb and was covered by numerous dandruff.

From this DNA, we amplified 15 Y-STRs by using the AmpFirst Identifiler PCR amplification kit (Amp FIRSTY filer™, Applied Biosystems), according to the instructions given by the Company; this amplification kit is specially adapted to the study of a–DNA.

Results about amplification of the amelogenin gene were already published (Lucotte *et al.*, 2014), showing that Naundorff is XY. The fifteen STRs studied are the following: DYS19 (= DYS394), DYS385, DYS 389I and II, DYS390 (= DYS708), DYS391, DYS392, DYS393 (= DYS395), DYS438, DYS439 (= GATA-A4), DYS448, DYS456, DYS458 and DYS635 (= GATA-C4). To detect the longest STR alleles, we proceeded to three successive assays with progressive degrees of stringency.

RESULTS

Table 1 shows results obtained comparing 15 Y-STR allelic values of Naundorff, compared to those corresponding to Hugues de Bourbon.

At the first passage allele value for Naundorff are identical to those already published (Lucotte and Roy-Henry, 2014) for Hugues, concerning: DYS19 = 14, DYS385.a = 11, DYS389.I = 13, DYS391 = 12, DYS392 = 13, DYS393 = 13, DYS438 = 12, Y-GATA-A4 = 12, DYS456 = 15 and DYS458 = 18.

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For the second passage Naundorff' allele value for DYS385.b = 14, identical to that of Hugues. DYS389.b value is calculated as DYS389II *minus* DYS389I; for the second passage, Naundorff' allele value for DYS389.b = 16, identical to that of Hugues. At the second passage also Naundorff' allele for Y-GATA-C4 = 23, that is identical to that of Hugues.

It is at the third passage only that the longest value for DYS390 = 24, identical to that of Hugues, was determined. At the third passage also, Naundorff' allele value for DYS448 = 18. This value is slightly different (= 19) to that of Hugues; but we can reasonably admit that the real Naundorff' allele value for DYS448 is in fact 19, because of the artefactual phenomena of "allele drop in" (an occasional experimental error arising in a-DNA typing).

We don't obtain results for Naundorff' STR Y-GATA-H4; so this marker was not explored further.

DISCUSSION
Hugues de Bourbon is a true patrilineal descendant from Naundorff
As shown in Table I, the Naundorff' Y-STR profile determinate here (Table 1) is then identical (but for DYS448) to that of Hugues de Bourbon for almost 15 sites. These datas demonstrate that Hugues de Bourbon, the living 4th generation descendant of the elder branch from K.W. Naundorff (Figure 1), is related to him by a non-interrupted chain (from II2: Charles-Edmond de Bourbon, III2: Charles-Louis-Mathieu de Bourbon, IV1: Charles-Louis-Edmond de Bourbon) of Y-chromosome identical DNA markers.

Non-accordance to Naundorff patrilineage in the younger branch
Table 1 gives results previously obtained concerning the nine first STRs for Charles-Louis and Henri-Emmanuel de Bourbon V2 and V3, respectively). They are identical to those of Hugues de Bourbon for two on nine markers only: DYS389.I = 13 and DYS390 = 24; moreover DYS389.I is variable between the three Bourbons published (Larmuseau *et al.*, 2013), and DYS390 is also variable between Hugues de Bourbon and these three (Lucotte and Roy-Henry, 2004).

For the seven other STR allelic values, Charles-Louis and Henri-Emmanuel differ from those of Hugues: DYS19 = 15, *versus* 14; DYS385.a = 14, *versus* 11; DYS385.b = 15, *versus* 14; DYS389.b = 17, *versus* 16; DYS391 = 10, *versus* 12; DYS392 = 12, *versus* 113 and DYS393 = 15, *versus* 13. These datas establish clearly that Hugues de Bourbon on one side, and Charles-Louis and Henri-Emmanuel on the other side, are patrilinely (by the way of Y-chromosome DNA markers, at least) unrelated.

Louis-Jean-Charles-Aldeberth (IV2 in Figure 1) had probably the same Y-STR profile that those of his two sons. Consequently, it is above him that the non-biological paternity event occurred.

Datation of the common ancestor between Hugues de Bourbon and other Bourbons

Larmuseau et al. (2013) collected three living relatives (Axel de Bourbon-Parme, Sixte-Henri de Bourbon-Parme and João-Henrique d'Orléans-Bragance) men of the House of Bourbon and established their common Y-profile for thirty-one different STRs. Table 1 gives the list of the fifteen firsts of them; compared to those of Hugues de Bourbon, allelic values of Hugues differ from those commons for the three Bourbons (Lucotte and Roy-Henry, 2014) for only three STRs: DYS390 = 24 (a one-step plus mutation) versus 23, DYS391 = 12 (a two—step plus mutation) versus 10, and DYS456 = 15 (a two-step minus mutation) versus 17. Naundorff had identical variable allelic values (Table 1) to those of Hugues.

We estimate the TMRCA (Walsh, 2001) between Hugues de Bourbon and other Bourbons for such a 15-marker test: *12-1-2* (12 exact matches, 1 one-step mismatch, 2 two-step mismatches). Because the mean generation time = 25 years in the series of Henri IV to Louis XVI and Karl Naundorff to Hugues de Bourbon, we choose that value for the calculation of the time estimation.

In our own calculation of the TMRCA between Hugues de Bourbon and the three Bourbons, we adopted the following peculiar principles (with n = number of generations, and μ = mutation rates) for sites where is no difference between the two, $0.5P = (n^2\mu^2/2) + (1-n\mu)$; for the site where is one difference (one-step mismatch mutation) between the two, $0.5P = (1-n\mu)(2n\mu)$; and for the sites where there is two differences (two-steps mismatches mutations) between the two, $0.5P = n^2\mu^2/2$. Table 2 gives the calculation for the three variable STRs DYS390, DYS391 and DYS456, with the known mutation rates (Burgarella and Navascues, 2011).

Our global estimate with 50% probability is that Hugues de Bourbon and the three Bourbons studied by Larmuseau *et al.* (2013) have a common ancestor towards 1300.

This date corresponds approximatively to those of the first Bourbons in the genealogical House of Bourbon' tree: Jacques I de Bourbon (1319-1361), Jean I de Bourbon (1344-1393); the Bourbons first became an important family in 1268, with the marriage of Robert, Count of Clermont (the sixth son of the king Louis IX of France) to Béatrice de Bourgogne, heiress to the Lordship of Bourbon. Their son Louis

(1280-1342) was made duke of Bourbon in 1327, and takes the name of Louis I de Bourbon. The Condé branch separates from the Bourbon' one since Louis the First (1530-1569), the first prince of Condé.

CONCLUSION

Results reported in the present study concerning comparisons of genetic markers of the Y-chromosome between Karl Wilhelm Naundorff and Hugues de Bourbon establish that K.W. Naundorff is identical to Hugues for 14 STR allelic values on 15 studied. Consequently Hugues de Bourbon is well the biological 4th generation of the elder branch descendant from Karl Wilhelm Naundorff by patrilineage.

List of abbreviations

a-DNA: ancient DNA; mtDNA: mitochondrial DNA; μ : mutation rate; n: number of generations; P: probability; PCR: Polymerase Chain Reaction; STR: Short Tandem Repeat; TMRCA: Time of the Most Recent Common Ancestor; X: X-chromosome; Y: Y-chromosome.

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Table 1. Y-STR allelic values for Karl Naundorff, Hugues de Bourbon, and the three Bourbons already published by Larmuseau et al. (2013). The variable allele values between Hugues de Bourbon and the three Bourbons are in italics.

| Nbrs | Y-STRs | Naundorff —————> | | | Hugues de Bourbon | The three Bourbons [2] | V_2 , V_3 | Mutation rates |
		1	**2**	**3** (s[1])				
1	DYS19	14			14	14	15	
2	DYS385.a	11			11 [3]	11	14	
3	.b	-	14		14[3]	14	15	
4	DYS389.I	13			13	13-14	13	
5	.b	-	16		16	16	17	
6	DYS390	-	-	24	*24*	23	24	4.7×10^{-3}
7	DYS391	12			*12*	10	10	2×10^{-3}
8	DYS392	13			13	13	12	
9	DYS393	13			13	13	15	
10	DYS438	12			12	12		
11	Y-GATA-A4	12			12	12		
12	DYS448	-	-	18	19	19		
13	DYS456	15			*15*	17		3.3×10^{-3}
14	DYS458	18			18	18		
15	Y-GATA-C4	-	23		23 [3]	23		

[1]. Three sucessive assays: 1, 2 and 3, with progressive degrees of stringency.

[2]. From Larmuseau *et al.,* 2013.

[3]. From lucotte and Roy-Henry, 2014: Three new allelic values (for DYS385.a and .b, and Y-GATA-C4) are added here.

Table 2. Modes of the TMRCA calculation between Hugues de Bourbon and the three Bourbons (Table 1) for variable sites; n = number of generations.

Differences	STRs	Calculations
One step mutation difference	DYS390	$0.5P \text{ (1step diff.)} = (1-n\ 4.7 \times 10^{-3})(2n\ 4.7 \times 10^{-3})$
Two steps mutation difference	DYS391	$0.5P \text{ (2 steps diff.)} = n^2\ 2 \times 10^{-3} /2$
	DYS456	$0.5P \text{ (2 steps diff.)} = n^2\ 3.3 \times 10^{-3} /2$

Figure 1. Paternal descendance from I1 (Karl-Wilhelm Naundorff, 1785?-1845) in the Naundorff family. II1: Auguste-Jean-Charles-Emmanuel (1872-1914); II2: Charles-Edmond (1833-1883); II3: Louis-Charles (1876); II4: Abel-Louis-Charles (1877); II5: Louis-Charles-Edmond (1878-1940); II6: Aldeberth (1840-1887); II7: Ange-Emmanuel (1843-1878). III1: Henri-Charles-Louis (1899-1960); III2: Charles-Louis-Mathieu (1875-1944); III3: René-Louis-Charles (1896-1980); III4: Louis-Charles-Jean-Philippe (1865-1940); III5: Henri-Jean-Edouard (1867-1937); III6: Ange-Emmanuel-Gilles-Alphonse (1869-1938); III7: Jean-Louis-Marie (1870); III8: Charles-Ferdinand (1871-1873). IV1: Charles-Louis-Edmond (1929-2008); IV2: Louis-Jean-Charles-Aldeberth (1908-1975). V1: Hugues-Charles-Guy (1974-...), arrowed; V2: Charles-Louis (1933-…), arrowed; V3: Henri-Emmanuel (1935-2004), arrowed.