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Comparison between the Y-chromosome STR Profiles of Hughes de Bourbon and other Members of the House of Bourbon

Gérard Lucotte[1] ✎, Bruno Roy-Henry[1]

[1]Institute of Molecular Anthropology, 75005 Paris, France

Abstract : We have obtained a high resolution Y-chromosome STR profile of Hughes de Bourbon, the French living male descendant of the Naundorff 's family. This STR profile was compared to those of three members of the House of Bourbon recently published (Larmuseau *et al, Eur J Hum Genet* 2013 ; oct.9). In our own system of comparison based on 27 STRs, the Hughes de Bourbon profile is very similar to those of the other de Bourbon published, differing from them by six mutations only. Hughes de Bourbon had also the same STR of sub-terminal differentiation (S21), and belongs to the Y-subhaplogroup R1b1a2a1a1. These datas are sufficient to include Hughes de Bourbon as a member of the House of Bourbon.

Keywords : Y-chromosome haplotype ; members of the de Bourbon family ; single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs)

INTRODUCTION

Karl Wilhelm Naundorff (initially written Naundorf) died in 1845 in Delft (the Netherlands), where he was buried under the name of Louis-Charles, Duc de Normandie, « Louis XVII » ; on his tomb one can read : « *Ici repose Louis XVII, Roi de France et de Navarre, né à Versailles le 27 mars 1785, décédé le 10 août 1845,* » Louis XVII was the name of Louis-Charles (1785-1795 ?), the second son of the French royal family : Louis XVI (1754-1793) and Marie-Antoinette (1755-1793). According to the official records, Louis-Charles died of tuberculosis in the Temple (in Paris) on 8 June 1795.

At the beginning of the 19th Century, about several ten of individuals claimed to be the son of Louis XVII. The most famous of them is Naundorff, who came in Paris on May 1833. He could apparently provide sufficient circumstantial evidence to convince ex-members of the court of Versailles (and specially Mme de Rambaud, one previous camerist of the Dolphin) of his descent. Naundorff was deported to England by the French authorities, after twenty six days of prison, on july 1836.

About fifteen years ago, Jehaes et al [1] excluded Naundorff as the son of Marie-Antoinette on the basis of mitochondrial DNA (mtDNA) sequences of his remains compared with the sequences obtained from the hairs of two sisters of Marie-Antoinette, Marie-Antoinette herself, and with the sequences obtained from DNA samples of two living maternal relatives. Very recently [2] the same Laboratory produced the Y-chromosome STR profiles of three different members of the House of Bourbon. The goal of the present article is to compare these profiles to that of Hughes de Bourbon, a direct French Naundorff's descendant, in order to elucidate patrilinear relationships between him and these members of the Bourbon's family.

Hughes de Bourbon (born in 1974) is the 4th generation living descendant (Figure 1) of Karl Naundorff. The Naundorff's legacy bears today legally (confirmatory judgement of the Tribunal de la Seine, 26 November 1913) in France the « de Bourbon » name ; initially, the judgments of Bois-le-Duc (12 march 1888) and of Maëstricht (20 may 1891) Tribunals accorded the use of the « de Bourbon » name to the Naundorff's family members. Hughes de Bourbon is the unique son of Charles-Louis-Edmond de Bourbon (Charles XII *de jure*) the last dynast of the elder branch ; so Hughes could be the present King *de jure*, since 2008.

Hughes de Bourbon himself, interested by his own patrineal inheritance, contacted us in June 2011.

MATERIAL AND METHODS

Hughes de Bourbon (**H**) is the propositus for this study. Buccal swab samples from this DNA donor were collected with informed consent. DNA extraction was done by using the manual classical method already described [3].

The southern blot method of analysis using p49f, a *TaqI* probes [4] was first used to determine the low-resolution Y-chromosome haplotype of **H.** The Y-SNPs M343, P25, P297, M269, L23, L51, L11 and

✎ **Gérard Lucotte (Correspondence)**
✉ lucotte@hotmail.com
☎ 06 98 82 92 61

S21 / U106 were then successively genotyped for the sample by real-time PCR, in order to establish his initial haplogroup and the subsequent sub-haplogroups ; successive R1b sub-clades were characterized according to the rules (last update 2013) of the International Society of Genetic Genealogy (ISOGG)[5]. High resolution profiles for Y-STRs were obtained using the Family Tree DNA (FTDNA) Y-DNA 67 and the Genetrack-Biolabs (GB) Y-DNA 66 kits, according to the instructions given by these two Companies. The composite haplotype obtained was submitted to the Whit Athey's Haplogroup Predictor [6], to obtain probabilities for the inferred haplogroup.

RESULTS

By southern blot analysis we showed that **H** belongs to the Y-chromosome haplotype XV (*A3, C1, D2, F1, I1*). According to our previous publications [7-9], haplotype XV is the main haplotype in Western Europe, reaching up to more than 70% of French Basques males.

Haplotype XV corresponds [10] to the large Y-haplogroup R1b, determined by the SNP M343 and described as such in the first published haplogroup tree [11].

Successive SNPs tested

Table 1 shows the eight successive SNPs (after M343) genotyped as positives in **H**. The terminal SNP of this list (S21 / U106) corresponds to clade R1b1a2a1a1. The published frequency maps of Western Europe [12,13] for S21 frequencies show relatively high concentrations (around 35%) of these genetic markers in the Netherlands, in Denmark and in Northern Germany ; in France the frequency declines from 5-10% in the East to 1-5% in the rest of the Country, falling down to less than 1% in the Bassin d'Aquitaine.

Bourbons studied by Larmuseau *et al* [2] were all three assigned by Z381 typing to the corresponding sub-haplogroup. We have genotyped **H** for this peculiar SNP : he is Z381^{-} ; consequently, contrary to the other Bourbons tested, **H** belongs to sub-haplogroup R1b1a2a1a1 (the ISOGG clade equivalent to that found by Larmuseau *et al* [2] for Z-281* is R1b1a2a1ac). The frequency of sub-haplogroup Z381* is not well known until now for most parts of West-Europe ; in Flanders [14], the Z381 estimated frequencies vary between 5.5% and 12.9 % among the various regions.

A complete Y-STR profile

We obtain a complete Y-STR profile of **H** by using the FTDNA Y-DNA 67 and the GB Y-DNA 66 kits. **H** belongs to the clade R1b1a2a1a1 (in the previous 2012 ISOGG nomenclature) by calculation with the Whit Athey's Haplogroup Predictor.

We have compared the Y-STR profiles (Table 2) between **H** and the three Bourbons already published [2]: Axel de Bourbon-Parme (**A**), Sixte-Henri de Bourbon-Parme (**SH**) and Joao-Henrique d'Orléans-Bragance (**JH**). A total number of 27 Y-STR genetic markers were compared.

The palindronic STR markers DYS385.a and b, DYS459.a and b, DYS464.a,b,c and d, and DYS724=CDY. a and b were excluded for the comparisons, because of our personal experience in the field of royal families in France, of Napoléon the First[15] : these palindromic Y-STR markers are not so easy to use for comparing « allele » values between lateral branches. In fact, the precise mechanisms of individual variation for these sorts of markers are poorly understood (K Norwed, personal communication). We have no data to compare for DYS365.

Table 2 shows, in our own system of comparison for 27 STR genetic markers, that there is one variable allele (DYS570= 16) out of 27 between **A** and **SH** (separated by 5 meioses). There are two variable alleles (DYS389. I = 14 and DYS449 = 29) between **JH** and both **A** and **SH** separated by, respectively, 23 and 22 meioses. Six variable alleles only (DYS390 = 24, DYS391 = 12, DYS447 = 24, DYS456 = 15, DYS607 = 16 and GATA-H4 = 10) distinguish **H** and **SH**, if we take **SH** as the Bourbon of reference : BOR (because he had no mutations, compared to others, in this system of comparison).

Among the variable alleles between **H** and **SH**, two (alleles 24 for DYS390 and for DYS447) correspond to a gain or loss of one repeat unit (1 step mutation) for these STRs, three (alleles 12 for DYS391, allele 15 for DYS456 and allele 10 for GATA-H4) correspond to a gain or loss of two repeat units (2 steps mutations) and one (allele 16 for DYS 607) corresponds to a loss of three repeat units (3 steps mutation). We ignore the number of meioses between **H** and the last common ancestor (LCA) Louis XIII, if any ; but, if Naudorff is Louis XVII, this number is 11.

Mutation rates [16] for the variable loci are always in the 10^{-3} range (Table 2) ; the variable allele = 29 for both **JH** and **H** (for DYS449) correspond to the highest mutation rate for Y-STRs observed so far [16]. We do not know the mutation rates of YCAII. a and b but, because of the observed fiability of these genetic markers for this palindromic STR in our previous investigations [15, 17], we have included its in the present study.

Uniqueness of this Y-STR profile

The 67 Y-STR profile of **H** is unique, being not found for any other individual in the FTDNA database. We found by eSearch that the most proximal individual found in this database (ID : HMTT2) differs from **H** for 12 alleles ; but his haplogroup is unknown for the moment. Table 3 gives the list of the four individuals in the database differing from **H** by 14, 15, 16 and 18 alleles only. The first two (one individual from Scotland and the other of unknown origin) differ from **H** by 14 and 15 alleles, respectively ; both are R1b1a2a1a. The third proximal individual (16 alleles of difference) from Italy, belongs to the R1b1a2a1a1b3c1 (L21) haplogroup ; and the fourth (differing by 18 alleles), from Italy also, belongs to the R1b1a2a1a1b (P312) haplogroup. So, an allelic difference value until [14-15] delineates the 67 Y-STR profiles corresponding to the R1b1a2a1a1 haplogroup.

DISCUSSION

Karl Wilhelm Naundorff was excluded from the Bourbon House in an initial genetic study [1], on the main basis of the non-correspondences between his mitochondrial DNA hypervariables HVR1 and HVR2 sequences detected in one of his presumed right humerus bone and those of Anne de Roumanie, a living woman related to Marie-Antoinette by seven steps of female transmission. Another argumentation in the same direction is the loss of an intermediate (located in mtDNA between HVR1 and HVR2) *Hae*III restriction site in the Naundorff's bone, compared to its gain in Anne de Roumanie and in hairs of Marie-Antoinette and her sisters.

But now, new informations about Y-chromosome DNA polymorphisms seems well to be telling us another story : the Y-chromosome DNA of Hughes de Bourbon, the living 4th generation descendant of Naundorff, shows very strong similarities to those of three members of the House of Bourbon (Axel de Bourbon-Parme, Sixte-Henri de Bourbon-Parme and Joao-Henrique d'Orléans-Bragance) recently publised [2] : his STR (short tandem repeats) profile differs, in our 27 STR genetic markers system of comparison, by only six mutations from those of the three other Bourbons.

Moreover, Hughes de Bourbon belongs to the same terminal sub-clade of SNP single nucleotide polymorphism differenciation (clade R1b1a2a1a1) than that of the other three Bourbons previously studied ; the R-Z381 sub-haplogroup is a subgroup of R-S21 (S21 being the antepenultian SNP genotyped in the lineage), an haplogroup found actually at relatively high frequencies in the Netherlands and in Northern Germany.

Six mutations of differences (present datas) for 27 STRs compared between Hughes de Bourbon and the other three de Bourbon previously genotyped [2] is a pertinent criterion to include (genetically) Hughes de Bourbon into the de Bourbon (surname) family : using their own panel of STRs tested [18], Larmuseau *et al* [19], in their pilot studies in Belgium on families of recent origins (mainly since the Industrial Revolution at the beginning of the 19th Century) retained a number of $\geq$ 6 differences (out of 38) as the borderline value to exclude some individuals from families identified by surnames (or closely variant surnames).

Another apparent aspect of some divergence between the STR profile of Hughes de Bourbon and those of the three other Bourbons studied is the observation that it differs from them by three « two-steps » mutations for DYS391, DYS456, and for GATA-H4 and even by another « three steps » mutation for DYS607, while – as well as for Hughes compared to others for DYS90 and for DYS447, as for Bourbons compared between them for DYS389. I, DYS449 and DYS510 – it is of the mode « one step » for the corresponding mutations. But the (known) mode of step-variation for STR is certainly not as simple as for SNPs. In our experience of the Napoléon family [15], for example, we have established that DYS454 (initially considered [20] as the most stable STR at that time) changes in a « four-step » fashion in only one generation, being like this a characteristic genetic marker of the « Jérôme (the youngest brother of Napoléon) lineage ».

But the most important bias in this sort of comparisons between the STR profile of Hughes de Bourbon and those of the three other de Bourbon recently published is due to the choice (P Delorme ; personal communication) of the Bourbons used for comparisons, and consequently that of the de Bourbon of reference (BOR). If, probably, the last common ancestor (LCA) in the tree (see Larmuseau et al. [2]) is Louis XIII, Sixte-Henri de Bourbon-Parme is in fact certainly not the BOR (he was artificially chosen here as such, because he had no mutations compared to all others) ; Sixte-Henri is separated from Axel de Bourbon-Parme by five meioses only, and it is not surprising that the two have pratically the same Y-STR profile. Axel derives lateraly from the main root at the level under Louis le Grand Dauphin, and is separated from the LCA by thirteen meioses. Joao-Henrique d'Orléans-Bragance, the most divergent of the three, is a descendant of Philippe, the Duc d'Orléans (laterally, the brother of Louis XIV) and is separated from the LCA by twelve meoises. If Naundorff is Louis XVII he is in the direct lineage, separated from the LCA by 11 meioses.

The practical consequence of these findings is that -in this hypothesis- the three Bourbons already published must be compared in relationship with Hughes de Bourbon, and not the opposite. For further comparisons it would be necessary to study other de Bourbon members, located more nearest to the main root. Another necessity for efficient comparisons is to genotype them with a complete set of STR genetic markers, as we do for Hughes de Bourbon.

The discovery of new SNP markers in the R1b1a2(M269) haplogroup is now a rapidly expanding field. As a consequence of the « 1000 Genomes Project », a set of new SNPs located above and below Z381 was recently described [21]. Their use could permit us to detect the precise banching point in the tree where Hughes de Bourbon separates from other de Bourbon members.

But the most important information to verificate is the initial assertion [1], based on mtDNA, that Naundorff is not Louis XVII. In fact, such an affirmation lies on the constatation by the authors of a non-concordance between mtDNA sequence variations between Anne de Roumanie (a sample of blood) and Naundorff (osseous samples) ; but the Naundorff's osseous samples originate from the coffin that was opened on the occasion of the restoration of his burial place in 1950 in Delft, and his hair samples – for which the authors were never successful to obtain any mtDNA sequences - were some hairs conserved in sealed enveloppes taken as samples at the same occasion (given by the archivist of the city of Delft).

If Naundorff is Louis XVII, the mtDNA haplogroup of his hairs must be of the « Habsburg » maternal type (P Germain ; personal communication). We are now involved in a study, based on authentificated Naundorff's hairs prealeved after his death, to clarify this important point.

CONCLUSION

Results reported in the present study concerning comparisons of genetic markers of the Y-chromosome between Hughes de Bourbon and three living males of the House of Bourbon recently published (Larmuseau *et al* [2]) establish that : 1/ Hughes de Bourbon belongs to the same terminal sub-clade of SNP patrilineage of current differenciation (clade R1b1a2a1a1, SNP sub-terminal marker S21) than the other Bourbons studied (these Bourbons were not tested for S21 but, being $Z381^+$, are obligatory $S21^+$). 2/ The high-resolution profile (in our own 27 – STRs system of comparison) of Hughes de Bourbon is greatly similar to those of the other Bourbons, differing from them by six mutations only. 3/ On the basis of this criterium of six mutations of difference Hughes de Bourbon can be considered as being a member of the de Bourbon family, according to the established rules of genealogical relationships in recent families with identical surnames. It must be noted that this six mutations threshold of differences concerns families of relatively recent origins [19]. So, because the Bourbon family is a very ancient one (that can be traced back at least since Henri IV, for the royal french dynasties), such a six mutations criterium of differences corresponds to a minimal value.

List of abbreviations

SNP : single nucleotide polymorphism ; STR : short tandem repeat ; mtDNA : mitochondrial DNA, ISOGG : International Society of Genetic Genealogy, FTDNA : Family Tree DNA ; GB : Genetrack-Biolabs.

Acknowledgments

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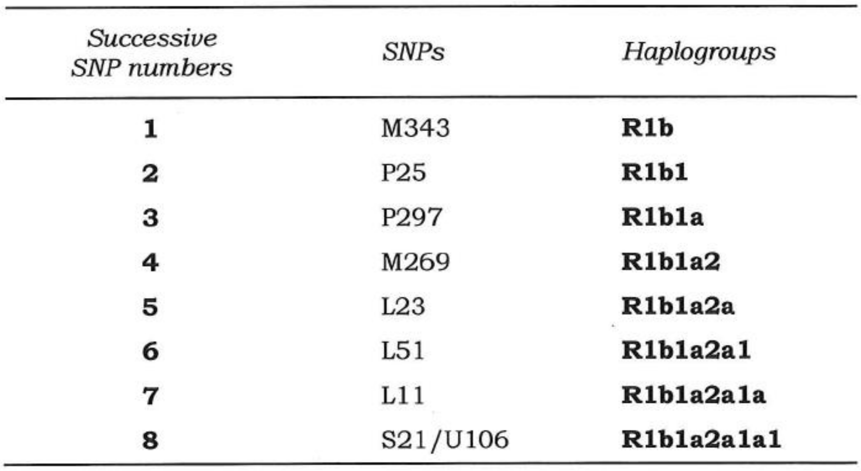
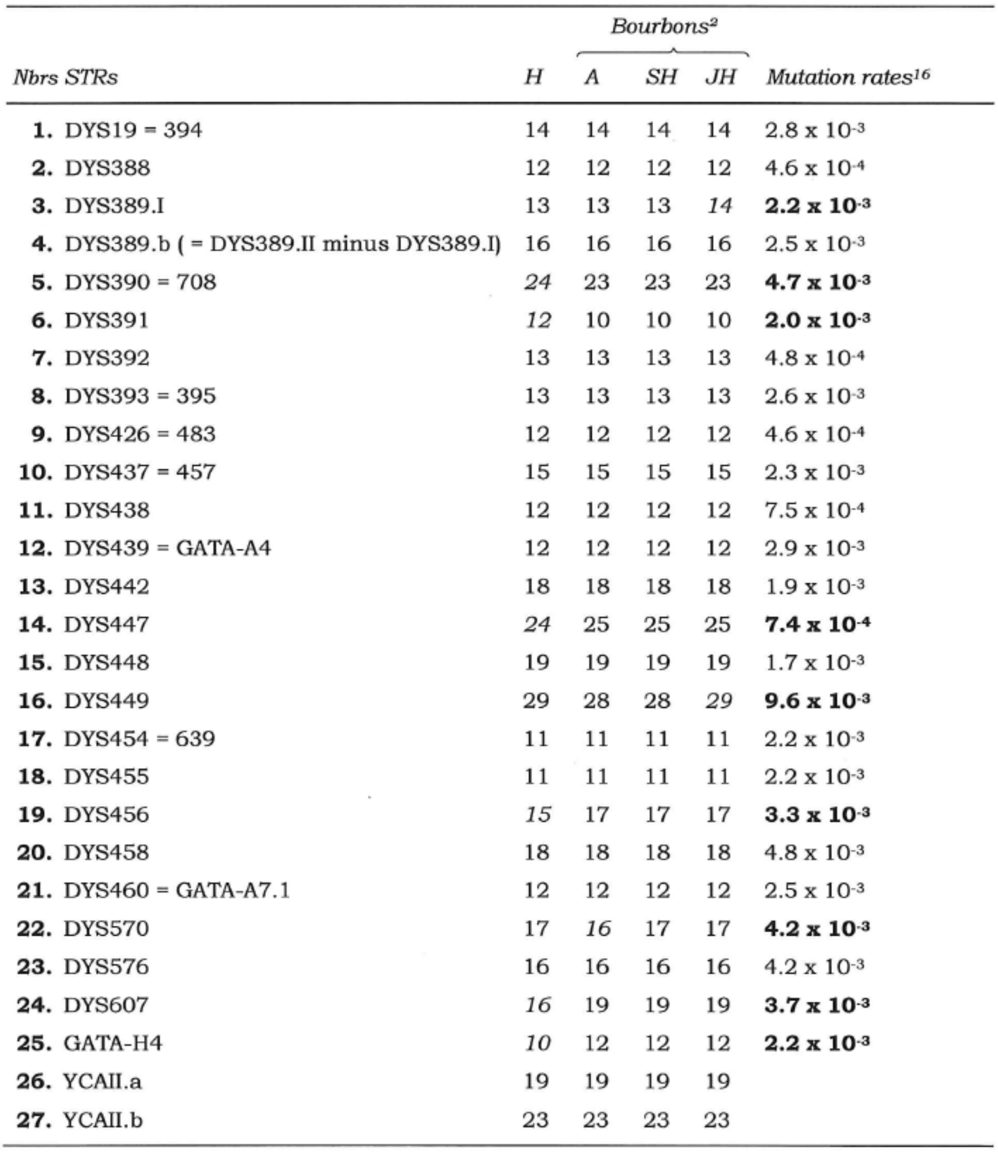
Table 1. Successive SNPs markers tested in Hughes de Bourbon and (in bold) the corresponding haplogroup and sub-haplogroups.



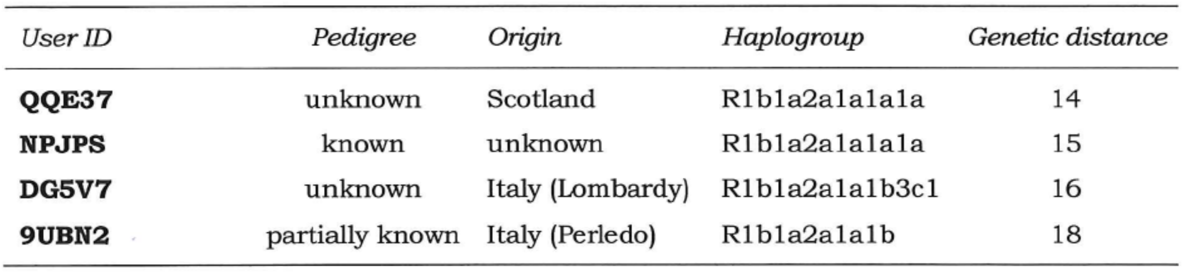
Successive SNP numbers	*SNPs*	*Haplogroups*
1	M343	**R1b**
2	P25	**R1b1**
3	P297	**R1b1a**
4	M269	**R1b1a2**
5	L23	**R1b1a2a**
6	L51	**R1b1a2a1**
7	L11	**R1b1a2a1a**
8	S21/U106	**R1b1a2a1a1**

Table 2. Compared 27 Y-STR profiles between Hughes de Bourbon and the three previously analyzed members of the Bourbon family; variable alleles are indicated in italics and the corresponding mutation rates in bold.



		Bourbons[2]			
Nbrs STRs	*H*	*A*	*SH*	*JH*	*Mutation rates*[16]
1. DYS19 = 394	14	14	14	14	2.8 x 10^{-3}
2. DYS388	12	12	12	12	4.6 x 10^{-4}
3. DYS389.I	13	13	13	*14*	**2.2 x 10^{-3}**
4. DYS389.b (= DYS389.II minus DYS389.I)	16	16	16	16	2.5 x 10^{-3}
5. DYS390 = 708	*24*	23	23	23	**4.7 x 10^{-3}**
6. DYS391	*12*	10	10	10	**2.0 x 10^{-3}**
7. DYS392	13	13	13	13	4.8 x 10^{-4}
8. DYS393 = 395	13	13	13	13	2.6 x 10^{-3}
9. DYS426 = 483	12	12	12	12	4.6 x 10^{-4}
10. DYS437 = 457	15	15	15	15	2.3 x 10^{-3}
11. DYS438	12	12	12	12	7.5 x 10^{-4}
12. DYS439 = GATA-A4	12	12	12	12	2.9 x 10^{-3}
13. DYS442	18	18	18	18	1.9 x 10^{-3}
14. DYS447	*24*	25	25	25	**7.4 x 10^{-4}**
15. DYS448	19	19	19	19	1.7 x 10^{-3}
16. DYS449	29	28	28	*29*	**9.6 x 10^{-3}**
17. DYS454 = 639	11	11	11	11	2.2 x 10^{-3}
18. DYS455	11	11	11	11	2.2 x 10^{-3}
19. DYS456	*15*	17	17	17	**3.3 x 10^{-3}**
20. DYS458	18	18	18	18	4.8 x 10^{-3}
21. DYS460 = GATA-A7.1	12	12	12	12	2.5 x 10^{-3}
22. DYS570	17	*16*	17	17	**4.2 x 10^{-3}**
23. DYS576	16	16	16	16	4.2 x 10^{-3}
24. DYS607	*16*	19	19	19	**3.7 x 10^{-3}**
25. GATA-H4	*10*	12	12	12	**2.2 x 10^{-3}**
26. YCAII.a	19	19	19	19	
27. YCAII.b	23	23	23	23	

Table 3. The four more proximal from **H** genotyped individuals found in the FTDNA database.



User ID	*Pedigree*	*Origin*	*Haplogroup*	*Genetic distance*
QQE37	unknown	Scotland	R1b1a2a1a1a1a	14
NPJPS	known	unknown	R1b1a2a1a1a1a	15
DG5V7	unknown	Italy (Lombardy)	R1b1a2a1a1b3c1	16
9UBN2	partially known	Italy (Perledo)	R1b1a2a1a1b	18

Figure 1. Overwiew of the patrilineal relationships – in five successive generations – of de Bourbon males (birth and death dates) since the paternal ancestor Karl Naundorff. The living Hughes de Bourbon, studed here, is given in grey.