

# The Y-SNP Z-381 Is a Patrilineal DNA Marker of the Royal Bourbon Family of France

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**How to cite this paper:** Lucotte, G., & Dieterlen, F. (2019). The Y-SNP Z-381 Is a Patrilineal DNA Marker of the Royal Bourbon Family of France. *Advances in Anthropology*, 9, 70-79.

<https://doi.org/10.4236/aa.2019.91005>

**Received:** October 26, 2018

**Accepted:** January 29, 2019

**Published:** February 1, 2019

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## Abstract

More than 1100 unrelated males from West-Europe were analysed by molecular hybridization experiments for the p49, f *Taq* I polymorphisms, and by polymerase chain reaction for the R-S21 and Z381 Y-SNP markers. The corresponding two SNP haplogroups showed similar packty frequency distribution patterns centred on Belgium, the Netherlands, Denmark and the north of Germany. Louis XVI, the last French King, is Z381<sup>+</sup>. A complete corrected Y-STRs profile is established for Prince Sixte-Henri of Bourbon-Parma, a living representative descendant of the French Bourbon family; exploration of further Y-SNP markers located under Z381 shows that he is of the DF98 haplogroup.

## Keywords

Y-Chromosome DNA Markers, Y-STRs Profile, Y-SNPs, French Bourbons, Prince Sixte-Henri of Bourbon-Parma

## 1. Introduction

Although discovered before, the Y-SNP (Single Nucleotide Polymorphism of the Y-chromosome) Z-381 is a useful Y-DNA polymorphism that was taken into consideration in the 1000 Genomes Project (Rocca et al., 2012). It was intensively studied for the frequencies by the Larmuseau group in West-European populations (Larmuseau et al., 2012, 2013a).

Furthermore, it was shown (Larmuseau et al., 2013b), studying three living male members of the Bourbon family (Axel of Bourbon-Parma, Sixte-Henri of Bourbon-Parma, and Joao Henrique of Orléans-Braganza), that the Z-381 DNA marker is the Y-SNP characteristic of the French Bourbon patrilineal lineage.

The goals of the present study are: 1) To construct an accurate Z-381 allele map frequency (Lucotte, 2015a) of West-Europe; 2) To show that Louis XVI, the

last French King, is Z-381<sup>+</sup>; 3) To verify that Sixte-Henri Bourbon-Parma (**S-HB-P**) is well Z381<sup>+</sup> and to give a corrected version of his complete Y-STRs (Short Tandem Repeats of the Y-chromosome) profile; taking **S-HB-P** as the living representative individual of the French Bourbon patrilineal lineage, we have explored other Y-SNP DNA markers located under Z-381.

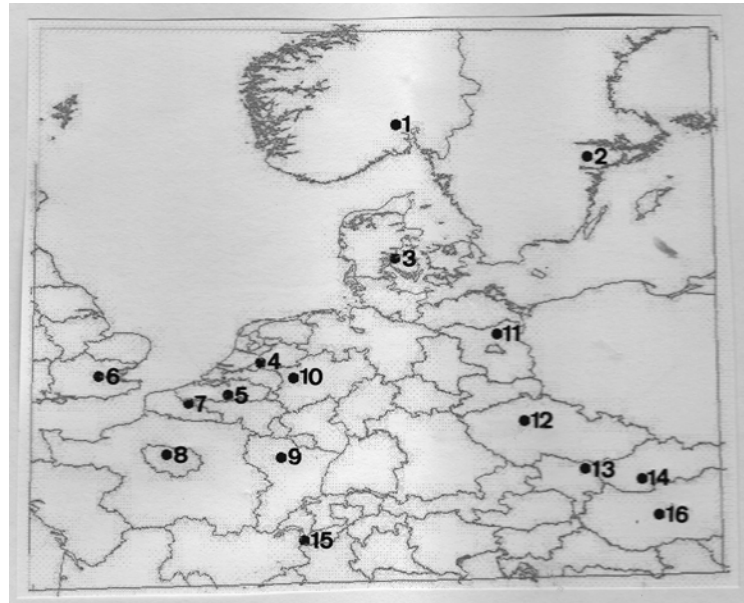
## 2. Subjects and Methods Used

The population sample (16 populations) consisted of 1118 unrelated adult males from West-Europe (**Table 1**); they originate from 13 countries. All samples of blood were collected from volunteer donors, with informed consent; their classifications were based on their grandfather's birthplaces. The geographic locations of the populations analysed are shown in **Figure 1**.

Genomic DNA of volunteer donors was extracted from whole blood by a classic method (Gautreau et al., 1983). At least 5 µg of genomic DNA were restricted with *Taq* I enzyme, separated by electrophoresis on a 1.5% agarose gel, transferred to Hybond N<sup>+</sup> membranes by the Southern blot (SB) method, and hybridized with two probes (p49f and p49a) according to Lucotte et al. (1994); the *Taq* I fragments (most of which being male-specific) permit to detect those of the volunteers who are of the haplotype XV (=haplogroup M269).

**Table 1.** Geographic characterization of the sixteen populations studied (N: numbers of subjects in each population).

Population numbers	Countries	Towns	Coordinates on the map		N
			x	y	
1	Norway	Oslo	12.5	60	40
2	Sweden	Stockholm	18	59	52
3	Denmark	Copenhagen	12	55.5	51
4	The Netherlands	Leiden	5.2	52	57
5	Belgium	Brussels	4.4	50.9	53
6	England	London	0	51.5	56
7	France	Lille	3.1	50.6	64
8		Paris	2.3	48.9	196
9		Nancy	6.2	48.8	52
10	Germany	Mulheim	6.6	51.5	59
11		Berlin	13.5	53	129
12	Czech Republic	Prague	14.4	50.1	61
13	Austria	Vienna	16.5	48.5	93
14	Slovakia	Bratislava	18.4	48.2	50
15	Switzerland	Basel	7	46	51
16	Hungary	Budapest	19	47	54
<b>Total</b>					<b>1118</b>



**Figure 1.** Sampled populations (population numbers refer to table 1) and their locations in West-Europe in this map, country (or main region) outlines are drawn approximately.

By PCR (Polymerase Chain Reaction) it is possible, using the Family Tree DNA (FT-DNA) procedure

(<https://www.geni.com/projects/R-U106-Y-DNA/12031>) to detect those of them that are of the R-S21 = U106 haplogroup. Genotypes at Z-381 (Z381<sup>-</sup> or Z381<sup>+</sup>) were also determined (**Figure 2**) using the method of FTDNA, according to the instructions given by the Company.

Y haplotype and SNPs maps were realized with the Spatial Analyst program (Arcview software) using the classical Kriging procedure (Dieterlen & Lucotte, 2005). We used the inverse distance weighting (IDW), which performs well with scarce data. The IDW method was computed for the five nearest neighbours (the grid has 250 rows and 355 columns) and we used a power of 2 (so that the influence is greater at large distance than with a high power).

The Z-381 genotype was tested on genomic DNA extracted from Louis XVI hairs (Lucotte et al., 2016). Z-381 genotype and the complete Y-STRs profile of S-HB-P were obtained, after his informed consent, according to the FT-DNA procedure.

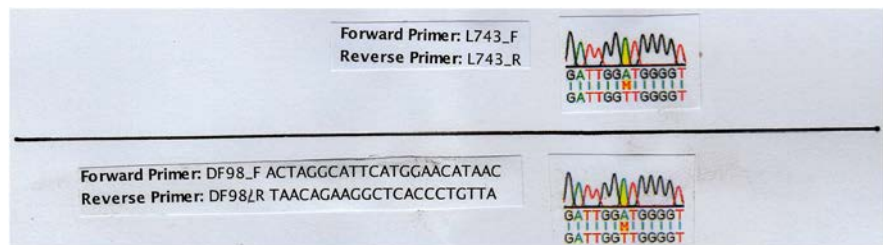
In the determination of Y-SNP markers of S-HB-P located under Z-381, the terminal Y-SNP detected that we obtained is DF-98 (**Figure 2** shows also the PCR method we used for genotyping DF98).

### 3. Haplotype XV, Haplogroup R-S21 and Z-381 Isfrequency Maps in West-Europe

**Table 2** gives numbers of donors being of haplotype XV, of haplogroup S21 (=U106) and who are Z381<sup>+</sup> (who are positive in the PCR reaction for the Z381 target indicated on the above **Figure 2**) in each population.

**Table 2.** Numbers (and %) of donors being of haplotype XV, of haplogroup S21 and Z381<sup>+</sup> in each population.

Population numbers	N	Haplotype XV (%)	Haplogroup S21 (%)	Z381 <sup>+</sup> (%)
1	40	21 (52.5)	17 (42.5)	5 (12.5)
2	52	18 (34.6)	12 (23.1)	3 (5.8)
3	51	23 (45.1)	17 (33.3)	5 (9.8)
4	57	28 (49.1)	22 (38.6)	8 (14)
5	53	22 (41.5)	16 (30.2)	6 (11.3)
6	56	26 (46.4)	9 (16.1)	6 (10.7)
7	64	30 (46.9)	9 (14.1)	5 (7.8)
8	196	76 (38.8)	7 (3.6)	5 (2.6)
9	52	24 (46.2)	14 (26.9)	3 (5.8)
10	59	26 (44.1)	20 (33.9)	4 (6.8)
11	129	42 (32.6)	30 (23.3)	6 (4.6)
12	61	17 (27.9)	9 (14.8)	2 (3.5)
13	93	37 (39.8)	26 (27.9)	4 (4.3)
14	50	2 (0.1)	0	0
15	51	20 (39.2)	12 (23.5)	4 (7.8)
16	54	5 (9.3)	1 (1.9)	1 (1.9)

**Figure 2.** Above: genotyping (primers and DNA sequence) for R-381. Below: genotyping (primers and DNA sequences) for DF98 (according to FT-DNA).

**Figure 3** shows the isofrequency map of haplotype XV in West-Europe; there is a slight cline of decreasing haplotype XV frequencies from West to East; that corresponds to the general cline of decreasing haplotype XV European frequencies already described (Lucotte & Hazout, 1996; Lucotte & Loirat, 1999). This figure shows also the isofrequency map of haplogroup S21 that is, corresponding to that reported previously (Lucotte, 2015a), more packty in distribution: S21 frequencies are more elevated in the northern continental part of Europe (corresponding to Belgium, the Netherlands, Denmark and the North of Germany) than that in the peripheral southern countries.

The Z381<sup>+</sup> isofrequency map (**Figure 4**) accentuates this last distribution,

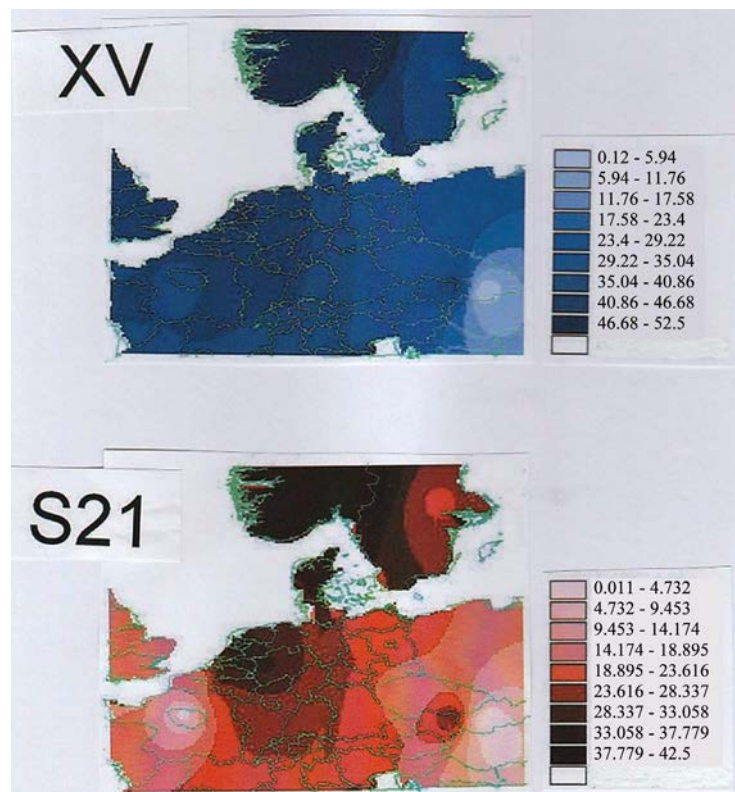
Z381<sup>+</sup> frequencies being slightly lower in the French Belgian side than in that of the Netherland side of the country. So we confirm, on a more rational basis, results previously obtained (Larmuseau et al., 2013a) concerning the general pattern of Z381<sup>+</sup> frequencies already described for six Belgium selected regions.

#### 4. Louis XVI Is Z381<sup>+</sup>

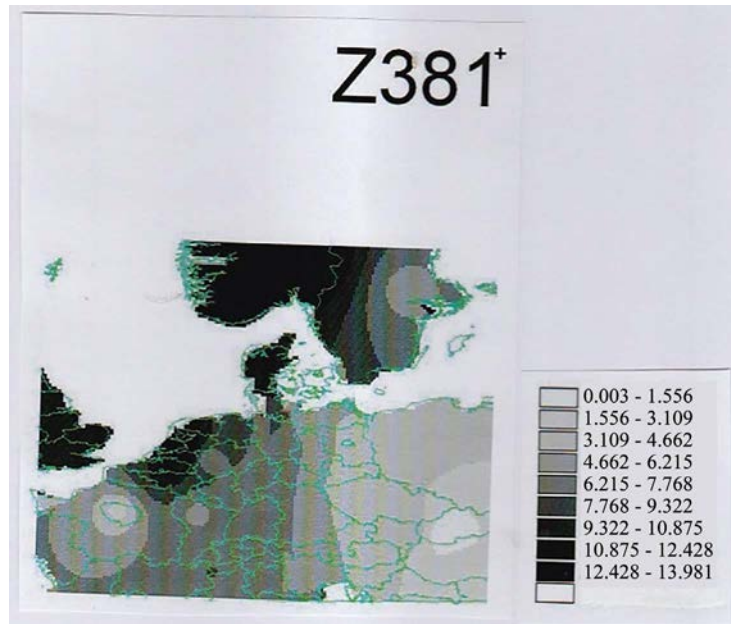
We have obtained previously the genomic DNA of the King Louis XVI (Lucotte et al., 2016). Testing for Z-381 on his DNA shows that Louis XVI is Z381<sup>+</sup>. **Figure 5** represents that on the simplified masculine genealogy of the French Bourbons, together with the Z381<sup>+</sup> status of three living Bourbons already tested (Larmuseau et al., 2013b).

#### 5. The Y-STRs Profile of Sixte-Henri of Bourbon-Parma

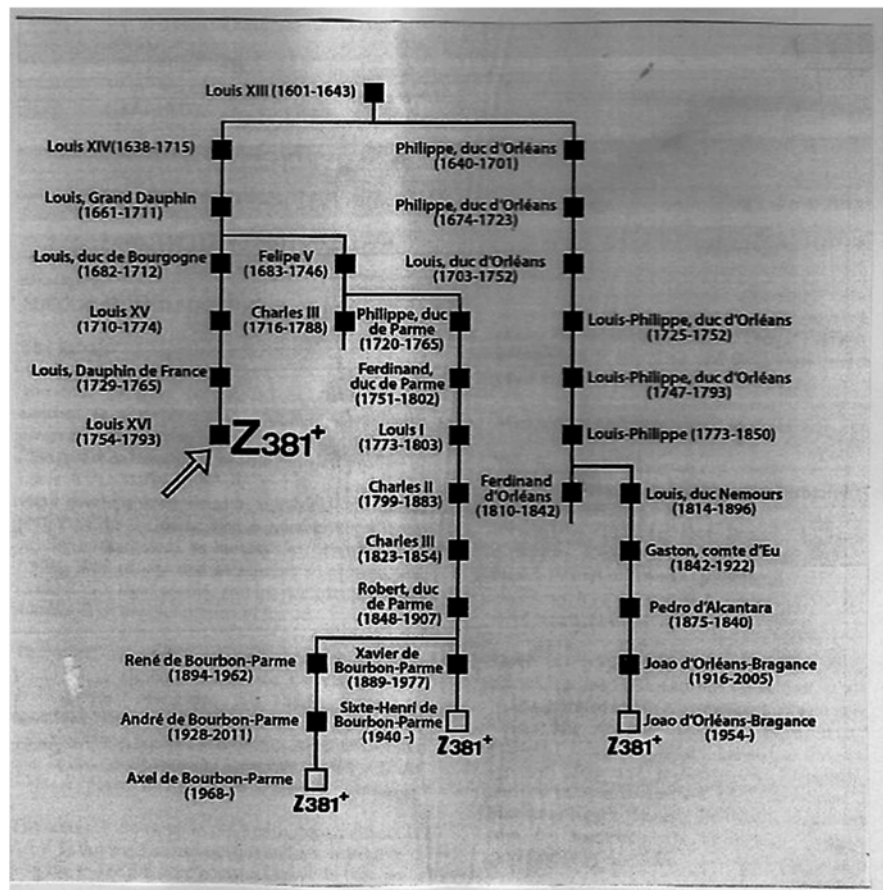
We confirm that Sixte-Henri of Bourbon-Parma (**S-HB-P**) is Z381<sup>+</sup>. **Table 3** shows the Y-STRs profile of **S-HB-P**, compared to that already obtained (Larmuseau et al., 2013b). Our own study concerns 58 Y-STR markers, for a total of 67 allele values; it represents a complete Y-STRs profile. We consider that this profile is the characteristic of one of that we name “the living French Bourbon of reference”.



**Figure 3.** *Above:* isofrequency map of haplotype XV. Isofrequency lines indicate the artificial limits of the areas with various nuances of blue (white rectangle: no data available). *Below:* isofrequency map of haplogroup S21. Isofrequency lines indicate the artificial limits of the areas with various nuances of brown to pink.



**Figure 4.** Isofrequency map of Z381<sup>+</sup>. Isofrequency lines indicate the artificial limits of the areas with various nuances of black to grey.



**Figure 5.** Masculine genealogy of the French Bourbons and relatives, descending from the common male ancestor Louis XIII. The *propositus* Louis XVI is arrowed (the three living Bourbons already tested are represented as open squares).

**Table 3.** The complete Y-STRs profile (allele values are determined by the use of the FT-DNA kit Y-DNA-37) of Sixte-Henri of Bourbon-Parma (ND: allele value not determined). The three allele values in italics do not correspond to those reported previously in Larmuseau *et al.* (2013b).

DYS markers	Allele values		Supplementary (not studied previously) <b>DYS markers</b>	Allele values
	Larmuseau <i>et al.</i> , 2013b	Present study		
DYS393	13	13	DYS531	11
DYS390	23	23	DYS578	9
DYS19	14	14	DYF395S1	16
DYS391	10	10		16
DYS385	11	11	DYS590	8
	14	14	DYS537	10
DYS426	12	12	DYS641	10
DYS388	12	12	DYS472	8
DYS439	12	12	DYF406S1	10
DYS389I	13	13	DYS511	10
DYS389b	16	16	DYS425	12
DYS392	13	13	DYS413	23
DYS4458	18	18		23
DYS459	9	9	DYS557	15
	10	10	DYS594	10
DYS455	11	11	DYS436	12
DYS454	11	11	DYS490	12
DYS447	25	25	DYS534	15
DYS437	15	15	DYS450	8
DYS448	19	19	DYS444	12
DYS449	28	28	DYS481	22
			DYS520	20
DYS464	15	15	DYS446	13
	15	15	DYS617	12
	16	16	DYS568	11
	16	16	DYS487	13
DYS460	12	12	DYS572	11
Y-GATA-H4	12	<i>11</i>	DYS640	11
YCAII	19	19	DYS492	13
	23	23	DYS565	12
DYS456	17	17		
DYS607	19	<i>15</i>		
DYS576	16	16		
DYS570	17	17		
CDY	35	35		
	39	39		
DYS442	18	<i>13</i>		
DYS438	12	12		
DYS635	23	ND		

Comparison of 37 allele values (for 30 Y-STR markers) so obtained to those previously published shows that there are three mistakes in the initial study: allele value = 12 (instead of 11) at Y-GATA-H4, allele value = 19 (instead of 15) at DYS607, and allele value = 18 (instead of 13) at DYS442. The two last of them (differing from 4 and 5 units, respectively) cannot be considered as simple technical errors.

## 6. Y-SNPs of Sixte-Henri of Bourbon-Parma Located under Z-381

With a first set of detection for **S-HB-P** genomic DNA Y-SNP markers located under Z-381, we found that it is U198<sup>-</sup>, L47<sup>-</sup> and L48<sup>-</sup>; this excludes that it be part of this corresponding Z-301 sub-lineage of Z-381. On the other hand, it is Z156<sup>+</sup>; this establishes that it is part of this corresponding Z-381 sub-lineage.

With a second set of detection, we found that it is L1/S26<sup>-</sup>; but it is Z306<sup>+</sup>, Z305<sup>+</sup> and Z304<sup>+</sup>. With a third set, it is DF96<sup>-</sup> and DF98<sup>+</sup>. This last result is of some importance: Sixte-Henri of Bourbon-Parma, being R-DF98, is so identified through DNA as being of the “King Cluster” (Little, 2012).

**Table 4** summarizes what we know concerning the main successive Y-SNP markers of the DNA Sixte-Henri of Bourbon-Parma; their corresponding haplogroups, and their approximate ages (according to Iain Mc Donald) are also given in the table.

## 7. Discussion

Prince Sixte-Henri of Bourbon-Parma is a highly representative living descendant of the royal French family of Bourbons. We establish here his complete (based on fifty-eight different Y-STR markers and sixty-seven corresponding allele values) Y-STRs profile.

We also show in the present study that the main successive SNP markers of his Y-chromosome are, in order: M269 (haplotype XVI) > U106 > Z381 > Z156 > (Z306, Z305 and Z304) and > DF98. This last SNP marker confers to **S-HB-P** the status of being presently of the *R1b1a1a2a1a1c1a1* haplogroup.

Being R-DF98, **S-HB-P** belongs to the King Cluster, becoming to the House of Wettin. The house of Wettin belongs to a clade defined by the following Y-SNP mutations: U106 > Z381 > Z156 > Z306 > Z304 > DF98, and other mutations downstream.

This Y-DNA lineage comes from Prince Albert of Saxe-Coburg and Gotha (1819-1861), who was the husband of Queen Victoria (1819-1901). The paternal Winsor DNA line continues back to Franz Josias (Germany, 1697-1764), John (Elector of Saxony, 1468-1532) and further to Dietrich I of Wettin (Germany, 916-976). Numerous royal lineages ([https://www.the-kings-son.com/royal\\_wettin\\_haplogroup](https://www.the-kings-son.com/royal_wettin_haplogroup)) are from the House of Wettin. The Y-DNA signature from the House of Wettin is characterized as haplogroup R1b-U106, with terminal Y-SNPs Z305 and DF98 (<https://www.surnamed.com/?articles=y-dna-of-the-british-monarch>).



**Table 4.** Successive main Y-SNPs of **S-HB-P**'DNA, their corresponding haplogroups (from ISOGG-R chart, 2018) and ages (in years BC: Before Christ).

Y-SNPs	Haplogroups	Ages
M269 (haplotype XV)	<b>R1b1a2</b>	5350 - 3150 BC
↓		
S21/U106	<b>R1b1a1b1a1a1</b>	2800 - 2050 BC
↓		
Z381	<b>R1b1a1b1a1a1c</b>	≈2400 BC
↓		
Z156	<b>R1b1a1b1a1c1</b>	≈2300 BC
↓		
DF98	<b>R1b1a1a2a1a1c1a1</b>	≈1500 BC

Detection of further Y-SNPs downstream (beyond DF98) of **S-HB-P**'DNA necessitates to obtain the full-length sequence of his Y-chromosome. So the Big Y test procedure, previously used by us in French dynasty to a living descendant of the Emperor Napoléon the First (Lucotte et al., 2015b), is now in progress to establish the Y-chromosome sequence of Sixte-Henri de Bourbon-Parma.

## 8. Conclusion

In conclusion, we have obtained a complete Y-STRs profile of Sixte-Henri of Bourbon-Parma. Like the French King Louis XVI, Prince Sixte-Henri of Bourbon-Parma is Z381<sup>+</sup>, an Y-SNP that is found at appreciable frequencies in the North of Germany, in Belgium, the Netherlands and Denmark. Exploration of the Sixte-Henri of Bourbon-Parma 'Y-SNPs located under Z-381 shows that DF98, the main DNA marker of the "King cluster", is the terminal Y-SNP of his differentiation.

## Acknowledgements

We thank Sixte-Henri of Bourbon-Parma for the donation of his DNA sample. This study is a part of the genetic investigations developed in the Institute of Molecular Anthropology concerning royal families in France. The authors thank very much Camila Wasserbach and Dr. Daniel Edgeworth in making our genetic investigations possible and for their competent advice.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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