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Keywords
Haplogroups, haplotypes, Hungary, Bulgaria, Population genetics, Romania, Y Chromosomes

Disciplines
Anthropology | Genetics and Genomics | Life Sciences | Social and Behavioral Sciences

Comments
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Abstract

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Introduction

Vlad III The Impaler, commonly known as Dracula, descended from the dynasty of Basarab, the first rulers of independent Wallachia, in current southern Romania. As a ruler, he fiercely resisted the Ottoman expansion. He infamously applied cruel punishments to his enemies and to traitors, including massive impalements that gave rise to his dark legend. His dynasty, the Basarab, took its name from the first ruler of Wallachia, Basarab I, who rebelled against Charles I of Hungary and in 1330 gained the independence of the country from the Kingdom of Hungary. The dynasty ruled until the assassination of Michael the Brave in 1601 [1].

The name Basarab most probably means father ruler in the Turkic Cuman language. Cumans were a confederation of two different people: the Cuman people that came from the east of the Yangtze River, and the Kipchak people, a Turkic tribal confederation, which occupied a vast territory in the Eurasian steppe, from north of the Aral Sea to the north region of the Black Sea. They expanded into Moldavia, Wallachia and Transylvania by the 11th century, influencing the politics of the region and establishing several royal dynasties, one of which may have been the Basarab of Wallachia [3]. Otherwise, contemporaries identified Basarab I as a local Romanian or Vlach (the local Romanic-speaking population), as Charles of Hungary referred to him as “Basarab our unfaithful Vlach” [3]. Whether the dynasty appeared...
was of Cuman or Romanian/Vlach origin is a subject of intense debate among historians [1,2,3,4].

Patrilineal surnames mirror the inheritance of the non-recombinant part of the Y-chromosome (NRY), making surnames as markers of male ancestry useful to help answering questions on the history and structure of populations in combination with genetic studies. Additionally, the study of the NRY lineage in males with the same surname could shed light on the history of lineages bearing this name. Males with identical patrilineally inherited surname descending from a common male ancestor will carry the same Y-chromosome lineage, and share higher levels of co-ancestry among them than with the rest of individuals carrying the same NRY lineage in the population [5]. However, there are confounding factors including multiple male founders for the same surname, extra-marital paternity, drift, and surname change from one generation to the next [5]. Another limiting factor of using surnames as genetic markers is the time depth of inherited surnames, which is highly variable across countries and populations [5].

The study of present day Romanian Basarab genetics has the potential to answer interesting questions about the history of the dynasty of the famous Romanian prince. Here we present a study on the Y chromosome of 29 individuals carrying the Basarab surname in present Romania and in 484 individuals from four Romanian and three neighbour populations (Bulgaria, Ukraine, and Hungary) by typing 131 SNPs and 17 STR markers in the NRY. Under the hypothesis that carriers of the Basarab name at present could be the direct descendants of the first ruler of Wallachia, we wanted to ask the following questions: are individuals presently bearing the Basarab surname actually the direct descents of Basarab I? If so, which was the Y-chromosome lineage of the dynasty? Are the Basarab individuals of Cuman or Romanian/Vlach origin?

Table 1. Descriptive statistics of genetic diversity in the Basarab and seven different populations studied.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>k</th>
<th>H</th>
<th>π</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basarab</td>
<td>29</td>
<td>15</td>
<td>0.9286 ± 0.0276</td>
<td>17.66 ± 7.81</td>
<td>0.7759 ± 0.0472</td>
</tr>
<tr>
<td>Romania Cluj</td>
<td>48</td>
<td>45</td>
<td>0.9973 ± 0.0049</td>
<td>20.23 ± 6.31</td>
<td>0.9086 ± 0.0214</td>
</tr>
<tr>
<td>Romania Brasov</td>
<td>50</td>
<td>31</td>
<td>0.9796 ± 0.0076</td>
<td>18.53 ± 6.61</td>
<td>0.8849 ± 0.0255</td>
</tr>
<tr>
<td>Romania Dolj</td>
<td>37</td>
<td>28</td>
<td>0.9775 ± 0.0135</td>
<td>20.99 ± 7.92</td>
<td>0.8962 ± 0.0227</td>
</tr>
<tr>
<td>Romania Mehedinti</td>
<td>11</td>
<td>10</td>
<td>0.9818 ± 0.0463</td>
<td>18.55 ± 7.50</td>
<td>0.9091 ± 0.0656</td>
</tr>
<tr>
<td>Bulgarian</td>
<td>98</td>
<td>95</td>
<td>0.9994 ± 0.0016</td>
<td>19.69 ± 6.58</td>
<td>0.8824 ± 0.0198</td>
</tr>
<tr>
<td>Hungarian</td>
<td>189</td>
<td>182</td>
<td>0.9994 ± 0.0007</td>
<td>20.29 ± 6.84</td>
<td>0.9258 ± 0.0087</td>
</tr>
<tr>
<td>Ukrainian</td>
<td>43</td>
<td>43</td>
<td>1.0000 ± 0.0050</td>
<td>18.67 ± 6.80</td>
<td>0.8527 ± 0.0328</td>
</tr>
</tbody>
</table>

Abbreviations: N, sample number; k, number of different STR haplotypes; H, haplotype diversity; π, average number of pairwise differences in absolute number of repeats; D, haplogroup diversity.

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Figure 1. Map with the geographical location of the populations sampled. County distribution of Basarab individuals is shown in circles and Romanian populations in blue squares. Neighboring samples used for comparison are shown in green squares.
doi:10.1371/journal.pone.0041803.g001
Results

Descriptive statistics for the studied populations (Figure 1) are shown in Table 1, and haplogroups and haplotypes for the Basarab and the other Romanian populations are given in Table S1. Haplogroups and haplotypes for the surrounding populations of Bulgaria, Hungary and Ukraine are given in Table S2. In general, the Basarab show lower levels of haplotype diversity compared to neighboring samples. Haplotype and haplogroup diversity is especially low in Basarab, and this may be due to founder effects within bearers of the same name. Otherwise, haplogroup diversity in the Romanian populations in this study is higher than previously reported in other Romanian populations (0.7828 in Constanta, 0.8048 in Ploiesti, [6]) but this may be due to the lower level of phylogenetic definition in the previous study.

PCA based on haplogroup frequencies grouped the Basarab with the southern Romanian populations of Dolj, Brasov and Mehedinti, and the Ukrainian population. The Romanians from Cluj, the Hungarians and the Bulgarians were more scattered in the plot (Figure 2). The Basarab individuals appear just in the middle of Romanian populations, indicating a common genetic background.

The Basarab sample clusters into 11 lineages (Table 2), with six main lineages comprising 82.8% of the samples. Some lineages such as J-M241 and E-V13 are over-represented in the Basarab compared to the general Romanians. The age of each cluster was computed with the method, as in [7]. All the lineages in the Basarab show clear geographical clustering but, with one possible exception, none of them reaches the Middle Ages. It is worth noting that these lineages include a few non-Basarab individuals, but they are all from Hungary or Bulgaria. None is an exact haplotype match, and these coincidences may be caused either by sharing a recent common ancestor with the Basarab, or by the homoplastic nature of STR mutation. Three Basarab founding lineages are found in haplogroup E1b1b1a2-V13; if they were considered together, their joint age would be 1740±615 years, well beyond the establishment of the Romanian nobility. Similarly, if the two lineages in haplogroup I2a-P37.2 were pooled, their joint age would be 960±480 years, or 250 years before the actual founding of the Basarab dynasty. Only Basarab individuals were considered in the age estimations.

Discussion

The presence of different Y-chromosome lineages among the individuals that currently carry the name Basarab indicates that not all of them could be direct descendents of the dynasty. Extra-pair paternity could explain the existence of highly different male lineages in a dynasty, but only a very high rate could explain the diversity found in the Basarab population studied. Otherwise, descendants of the Craiovești boyars/noblemen, a family that claimed direct descent from the Basarab House, may have kept the Basarab name, adding diversity to the Y lineage. Indeed, the genetic evidence indicates that Basarab is a polyphyletic name, with multiple male founders that would explain the pattern of diversity.

The use of nicknames to distinguish among individuals with the same given name was common in Romania in the past centuries [8]. Although the most common nickname was the patronymical, others designated the place of origin. Later on, these nicknames...
became family names. Basarab may thus also indicate a demonym
descendants of the dynasty of the first rulers of Wallachia. It seems
people in Romania that bear the name Basarab are direct
origin than in passing down its genes.

The time depth estimated for most (although not all) of the
common lineages in the Basarab is in agreement with the time of
establishment of surnames in Romania, as seen before in other
populations [10]. Only one of these lineages within Romania dates
back to medieval times. Interestingly, two Hungarian individuals
share this haplotype, and it is well known that a major migration of
Cumans took place from the actual territory of Romania to
Hungary in the 13th century, where they asked protection from
the Hungarian kings against the advancing Mongol invasion [4].
Although tempting, it is impossible to clearly link this particular Y
haplotype to a Cuman origin. Nonetheless, we cannot rule out the
possibility that one of the Y-chromosome lineages found in the
Basarab was indeed the lineage carried by the dynasty. Unfortu-
nately, given the results obtained in this study, only the analysis of
the remains of Basarab I or any of his known descendants could
confirm or not this hypothesis.

Although Cumans came from East Asia, other authors have
reported that they also showed Caucasoid features [3]. Historians
agree that Cumans mingled with the populations they encountered
[3]. West Eurasian Y-chromosome haplogroup R1a1 has been
found in admixed East Asian populations as early as in the early
Bronze Age [11]. The single study on the genetics of Cumans [12]
was based on mitochondrial DNA (mtDNA) and showed that just
one individual out of 11 in a medieval burial in Hungary did not
carry a Western Eurasian but an East Asian haplogroup
(haplogroup D). Otherwise, D is also one of the most frequent
mtDNA haplogroups in southern Siberia [13]. However, one can
calculate that, given the political dominance of the Cuman,
asymmetrical admixture would preserve the Eastern lineages more
readily in the NRY than in mtDNA. Thus, we could attribute a
Cuman origin to a Basarab lineage if it belonged to an East Asian
haplogroup, but a European haplogroup could be carried both by
the Cumans and by the native Romanians/Vlachs. As shown in
the PCA, the haplogroup composition of the Basarab is very
similar to that of the general Romanian population, and none of
the haplogroups they carry are particular of Central or East Asia.
Therefore, our results are consistent both with an ethnic Cuman
and/or a Romanian/Vlach origin. On the other hand, the extensive
presence of Western Eurasian haplotypes in both known medieval
Cuman burials and in individuals bearing the Basarab name
suggests a significant probability that Basarab I may also have
been carrying a Western Eurasian haplotype.

To the best of our knowledge, this is the first genetic study on
the surname of a royal dynasty. We have shown that not all the
people in Romania that bear the name Basarab are direct
descendants of the dynasty of the first rulers of Wallachia. It seems
that the House of Basarab was rather more successful in extending
its name than in passing down its genes.

Materials and Methods

Samples

We sampled a total of 29 unrelated adult males bearing the
Basarab name and 149 non-Basarab males from different counties
in Romania: 38 from Dolj, 11 from Mehedinți (both counties
located in the ancient territory of Wallachia), 50 form Cluj, and 50
from Brașov. For the purpose of this study, we treat the Basarab
individuals as a population. All individuals were interviewed in
order to assess the geographical and ethnical origin of their
grandparents. None of the individuals knew to be related to other
volunteers bearing the Basarab name from the present cohort. The
number of bearers of Basarab surname (1:120,000 individuals) has
been estimated based on identification through telephone registry; 169
individuals with the Basarab have been indentified in

Table 2. Lineages found in the Basarab sample.

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Haplogroup</th>
<th>individuals</th>
<th>origin</th>
<th>age±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 E1b1b1a2-V13 RU226*</td>
<td>Sibiul Basarab</td>
<td>150±150 ya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 E1b1b1a2-V13 RU239*</td>
<td>Gorj Basarab</td>
<td>200±200 ya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 E1b1b1a2-V13 RU231*</td>
<td>Sibiul Basarab</td>
<td>240±120 ya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 I2a-P37.2 RU227*</td>
<td>Sibiul Basarab</td>
<td>150±150 ya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 I2a-P37.2 RU246*</td>
<td>Caras-Severin Basarab</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 J2b2-M241 RU219*</td>
<td>Sibiul Basarab</td>
<td>200±115 ya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 G2a-P15 RU237</td>
<td>Bacau Basarab</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 J1e-P58 RU242</td>
<td>Gorj Basarab</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 J2a2-M67 RU238</td>
<td>Bacau Basarab</td>
<td>300±300 ya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 R1a1a-M17 RU243</td>
<td>Ilfov Basarab</td>
<td>600±283 ya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 R1a1a7-M458 RU244</td>
<td>Ilfov Basarab</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*) individuals within the same lineage carrying the same haplotype.
doi:10.1371/journal.pone.0041803.t002

The time depth of inherited surnames is highly variable across
countries and populations [5]. In Romania, it was not until 1895
(Law on the name, nr. 18/March 1895) that the first law obliging
people to have a first name and a surname was passed [9].
However, in the rural areas this regulation was not effectively
applied until two or three decades after. Therefore, the expected
time depth of inherited surnames in Romania should be around
100–150 years.
Romania (from a population of 19.8 million individuals). This can be an understimation considering the number of individuals in a family and the Basarab not present in the telephone registry. Among this group, 150 individuals were contacted telephonically and 29 individuals who fulfilled all the inclusion criteria agreed to participate in the study.

DNA was extracted from fresh blood by standard phenol-chloroform methods. In order to investigate how the Basarab relate not only to the Romanian but to the surrounding populations, general populations from the Ukraine (N = 43), Hungary (N = 192), and Bulgaria (N = 100) were also used for comparison (Figure 1, map of the sampling area).

Ethic Statement
Written informed consent was obtained from the participants and analyses were performed anonymously. The project obtained the ethics approval from the Institutional Review Board of the Comitè Étic d’Investigació Clínica – Institut Municipal d’Assistència Sanitària (CEIC-IMAS) in Barcelona, Spain.

Y-chromosome Typing
We genotyped 121 SNPs in the non-recombining region of the Y chromosome as described previously [14]. In addition, six SNPs were genotyped in a single multiplex including M91, M139, M60, M186, M175 and M17, and four single SNPs were typed with individual TaqMan assays (L48, M458, L2, and L20). Nomenclature of the haplogroups is in accordance with the Y-Chromosome Consortium [15]. Detailed phylogeny may be found at Y-DNA SNP Index - 2009 [http://isogg.org/tree/ISOGG_YDNA_SNP_Index09.html].

All the individuals were typed for a set of 19 STRs: 17 with the Yfiler kit (Applied Biosystems) (DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, GATA H4) plus DYS388 and DYS426. As the Yfiler kit amplifies DYS385a/b simultaneously avoiding the determination of each of the two alleles (a or b), DYS385a/b were excluded from all the analyses performed. Individual data is provided in Tables S1 and S2.

Statistical Analyses
Descriptive statistics (number of different haplotypes k, haplotype diversity H, average number of pairwise differences in absolute number of repeats π and haplogroup diversity D) were calculated with Arlequin 3.4 [16]. Principal component analyses (PCA) based on the haplogroup frequencies were calculated using STATISTICA 7 package [http://www.statsoft.com]. Since the patrilinear inheritance of surname was established in Romania in the second half of the 19th century, then the expected time depth for NRY variation within a Romanian surname should be around 150 years. The exceptions were the noble families, such as the descendants of a recent common ancestor. Such groups were then dated with the ρ method [17,18], using a joint mutation rate of 1.667 x 10−3 per year, or one mutation per 600 years [7]. Given its complex nature, DYS309II and DYS335 were not used in networks or lineage inferences.

Supporting Information
Table S1 Haplogroups and haplotypes of the Romanian individuals in this study. Bas, Basarab; Bra, Romanians from Brassov; Dol, Romanians from Dolj; Chu, Romanians from Chuj; Meh, Romanians from Mehedinți.

(XLSX)

Table S2 Haplogroups and haplotypes of the individuals from the populations surrounding Romania used in this study. hun, Hungary; bul, Bulgaria; ukr, Ukraine.

(XLSX)

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Author Contributions
Conceived and designed the experiments: DC MGN. Performed the experiments: BMC PS TP. Analyzed the data: BMC FC LRA. Contributed reagents/materials/analysis tools: MI RI SB LK HP HM. Wrote the paper: BMC FC MI JvdM DC MGN.

References