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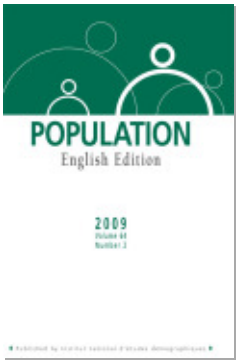

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Thirty Years of Research into Rendu-Osler-Weber Disease in France: Historical Demography, Population Genetics and Molecular Biology

After thirty years of research into Rendu-Osler-Weber disease, the authors review the contributions of the three successive approaches used to analyse this rare genetic disorder. First, historical demography sees patients as markers of past migration and population transfers. Starting out from a cluster of cases straddling the Ain and Jura départements*, the history of this population group was reconstituted on the basis of civil records, and their migration to other parts of France was studied by looking for family ties between the initial group and the cases observed elsewhere in the country. Second, epidemiology and population genetics give estimates of prevalence by* département *that are higher than those initially predicted in 1977, the start year of this study. They also provide statistical tools to test hypotheses of a single or multiple origin for the disease by looking for a common ancestor among affected families. Last, molecular biology provides a means to identify the genes responsible for the disease and their various mutations, thus confirming the existence of several different origins. Following the articles published in 1984 and 1989, (Plauchu and Bideau, 1984; Bideau et al., 1989), this article concludes a key phase in the study of Rendu-Osler-Weber disease in France, and opens the way for future international comparisons.*

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Rendu-Osler-Weber disease, also known as hereditary hemorrhagic telangiectasia (HHT) is characterized by small vascular malformations. It was first differentiated from haemophilia by the physicians Rendu (1896) and Osler (1901). Diagnosis is based on the simultaneous presence of three parameters: spontaneous nosebleeds (called epistaxis), telangiectasias (abnormal connections between venules and arterioles, with no intervening capillary bed) in specific regions such as the mouth or the fingertips, and a family history of the disease (Plauchu et al., 1992). An international conference in 1999 (Shovlin et al., 2000) confirmed the importance of these three diagnostic criteria, while adding a fourth: the presence of visceral lesions liable to cause life-threatening complications in the lungs, digestive tract, liver and brain (Plauchu et al., 1989). The symptoms of the disease develop gradually, and age at onset may vary. It is a genetic disorder with an autosomal dominant pattern of inheritance, transmitted directly from parents to children without skipping a generation. This means that the offspring of a carrier have a 50% risk of inheriting the mutation.

Demographic and genetic research into HHT in France began in the 1970s, when Professor Robert, head of the genetics department at Hôtel-Dieu in Lyon, identified a cluster of carriers in a region around hundred kilometres to the north-east of Lyon straddling the Ain and Jura *départements*. Headed by Henri Plauchu (geneticist) and Alain Bideau (historian-demographer), a research team was set up, bringing in students from a range of disciplines, including medicine, genetics, demography and history. The team published data that made it possible to analyse the frequency of the disease at regional and national levels (Bideau et al., 1979 and 1989), and to track its progression among the main affected population group (Bideau et al., 1992), while the study of genealogical networks provided clues about the history of HHT in the region (Heyer, 1991; Brunet, 1998). In association with a genealogical study of families recruited across the Rhône-Alpes region, the medical specialists of the team pursued their in-depth clinical study (Plauchu et al., 1989) to determine the chromosomal location of one of the two major genes whose mutations cause the disease (Vincent et al., 1995). Further key contributions by our team include the description of the gene mutations present in the affected French families (Lesca et al., 2004; Lesca et al., 2006), and the discovery of the mutation affecting the patient cluster in the Ain and Jura *départements*.

Armed with this new information, we have revised some of our provisional research findings and partially reinterpreted the data, notably regarding the genealogical relationships between affected families and the history of the observed mutations. We will begin by outlining the initial research questions and describing the resources deployed before presenting the key findings of three decades of investigation in this field.

I. Using epidemiology to identify a population

At the beginning of this research project, the frequency of HHT in the French population was unknown. The only available estimate, based on studies in specialized journals, was published by an American physician, Dr McKusick, in a catalogue of human hereditary diseases (McKusick, 1966, 1978 and 1983). According to this source, HHT was a rare pathology, with an estimated frequency of 1 case per 100,000 population.(1) The existence of 37 affected families in the Lyon region, ten of whom lived in a zone straddling the Ain and Jura *départements* (delimited by the towns of Saint-Claude, Oyonnax and Bellegarde-sur-Valserine), suggested that its frequency in France was higher (Manipoud, 1962). To verify this hypothesis, two epidemiological surveys were launched using different methodologies, one at national level and the other at regional level.

The national epidemiological survey

To identify the disease carriers at national level, we conducted a postal survey of physicians who were likely to have HHT carriers among their patients. Our goals were presented in a covering letter, which included a summary of the disease characteristics.

The physicians were invited to fill in a form, giving their identity and listing any HHT patients that they knew (subject to their prior consent), and to return the form in the stamped-addressed envelope provided.

The survey took place between 1982 and 1986, and excluded specialist physicians in fields unrelated to the symptoms of the disease. Out of more than 110,000 practicing physicians, 61,627 received our questionnaire and 8,144 (13.2%) replied. The replies received did not appear to come preferentially from the Lyon region, or from physicians with or without experience of HHT patients. For example, the highest response rates were observed in *départements* as disparate and as far apart as Haute-Savoie (25.8%), Loir-et-Cher (21.6%) and Dordogne (20.4%), and the lowest in Cantal (5.7%), Allier (8.0%) and Alpes-Maritimes (8.4%).

Among the physicians who returned the questionnaire, a large majority (87%) reported never having treated a carrier of the disease. A few (4.7%) said that they knew one or more carriers, or had treated them in the past, but for one reason or another, did not give their contact details. A total of 654 physicians, representing 8% of respondents, supplied the details of patients who had agreed to be contacted by our team. Of these physicians, 447 (68.3%) were general practitioners, most of whom knew only one family affected by HHT. The other physicians were mainly digestive disease specialists (66), ear nose and throat (ENT) specialists (58) and dermatologists (34), whose specialties are directly

(1) The same estimate was published in successive editions of McKusick's book from 1966 to 1983. The 1992 edition takes account of our own estimates.

linked to the main symptoms of the disease. Some knew of several affected families, notably those working in university hospitals with large catchment areas. One ENT specialist, for example, listed 8 patients, and one hospital-based digestive diseases specialist listed 15. Last came occupational health physicians (13), and small numbers of specialists in other fields (lung specialists, haematologists, cardiologists, geriatricians and ophthalmologists) most of whom knew only one HHT patient.

The contact details of 830 patients were thus obtained. However, when several closely related members of the same family were reported, we decided to select just one patient from among them.(2) Hence, after removing duplicates and close relatives, a total of 702 different family files were opened.

Estimating HHT frequency at national level

All the identified probands* were contacted by mail and invited to fill in a family questionnaire to detect relatives also affected by HHT. On the basis of their replies, we estimated that each proband had an average of four affected relatives. Using the known number of probands, the estimated number of relatives in the *département* and its total population, the extrapolated prevalence of the disease was established using the following formula:

$$\text{Prevalence in } \textit{département} = \frac{\text{number of probands in } \textit{département} \times 4}{\text{population of } \textit{département}}$$

Of course, this is only an approximation, given that all family members do not necessarily live in the same *département*. It nonetheless gives a plausible order of magnitude. Taken at national level, the mean prevalence of HHT in France is estimated at one case per 18,000 inhabitants, well above the level suggested by the American reference. Yet this mean prevalence is itself certainly an under-estimation since an empirical epidemiological survey cannot provide an exhaustive inventory of carriers. Moreover, the age structure of patients indicates that HHT is diagnosed more easily and more frequently among older adults who already present clear symptoms and who have been treated for the condition on more than one occasion. The number of young HHT carriers is very probably under-estimated.

This national average masks large disparities between *départements*, ranging from a maximum of 1 case per 3,375 inhabitants in Ain to a minimum of 1 per 126,000 in Sarthe (Table 1). Though no cases were detected in certain *départements*, carriers are present in all the French regions.

The *départements* with the highest numbers of probands are those with large populations (Paris, Nord, Rhône, etc.), but also more rural, less populous ones such as Ain (01), Deux-Sèvres (79) and Jura (39). Conversely, in terms of

(2) Close relatives comprise direct relatives up to and including first cousins. Grandchildren, uncles and nieces, for example, are close relatives under this definition.

* This term is defined in the glossary at the end of this article.

Table 1. Rendu-Osler-Weber Disease: ranking of *départements* based on the postal survey

By number of probands			By extrapolated prevalence			
Rank	*Département*[a]	Number of probands	Rank	*Département*[a]	Extrapolated prevalence	Population (1982)
1	Paris[b] (75, 91, 92, 93, 94, 95)	67	1	Ain (01)	1/3,375	418,500
2	Ain (01)	31	2	Deux-Sèvres (79)	1/4,287	343,000
3	Nord (59)	22	3	Jura (39)	1/5,062	243,000
4	Gironde (33)	21	4	Lozère (48)	1/6,192	298,500
5	Deux-Sèvres (79)	20	5	Loir-et-Cher (41)	1/6,700	296,200
6	Rhône (69)	20	6	Savoie (73)	1/6,739	323,500
7	Bouches-du-Rhône (13)	17	7	Ardèche (07)	1/7,444	268,000
8	Maine-et-Loire (49)	16	8	Tarn-et-Garonne (82)	1/7,938	190,500
9	Haut-Rhin (68)	15	9	Ariège (09)	1/8,481	135,700
10	Hérault (34)	14	10	Creuse (23)	1/8,750	140,000
11	Loire-Atlantique (44)	14	11	Hautes-Alpes (05)	1/8,750	105,000
12	Jura (39)	12	12	Mayenne (53)	1/9,707	271,800
13	Savoie (73)	12	13	Vosges (88)	1/9,900	393,000
14	Marne (51)	12	14	Alpes-de-Haute-Provence (04)	1/9,917	199,000

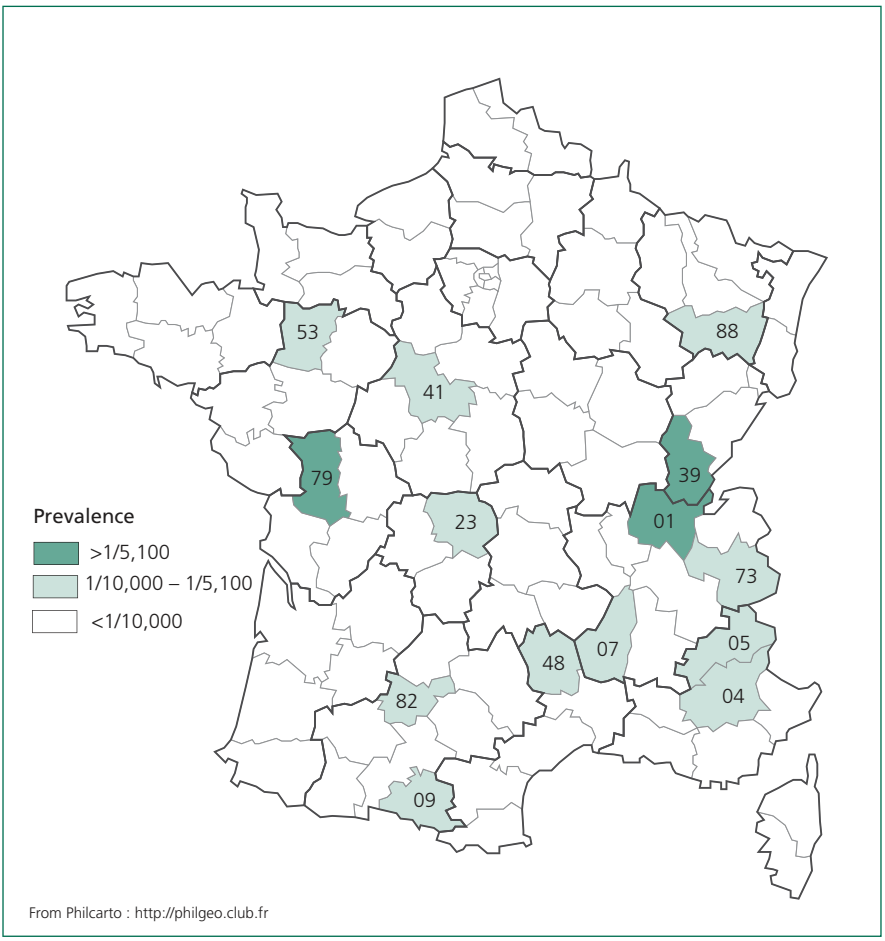
Source: Epidemiological survey, Plauchu, Bideau and Brunet, 1986.
[a] The numbers in brackets are the *département* numbers.
[b] It was not possible to distinguish between Paris *intra muros* and the inner suburbs: *départements* of Yvelines, Essonne, Hauts-de-Seine, Seine-Saint-Denis, Val-de-Marne, Val-d'Oise.

mean prevalence, the top-ranking *départements* are all rural and sparsely populated, with a mean age above the national average. This probably reflects the age-related bias in diagnosis and detection that we mentioned earlier. These *départements* are also scattered across the whole of France (Map 1).

The survey results confirm the higher prevalence of HHT in Ain (1 case per 3,375 inhabitants) and Jura (1/5,062), but they also reveal a new finding, namely a high frequency of HHT in other *départements*, notably Deux-Sèvres, very distant from the first two. They also demonstrate the absence of a dispersion gradient[(3)] around one or two clusters, while uncovering high prevalences in *départements* located very far apart. These findings are important as they appear to contradict the thesis of a recent (a few centuries) and unique founder effect accounting for all French carriers of the disease.

(3) Decrease in the number of disease carriers proportional to the distance from the disease epicentre.

Map 1. National dissemination of Rendu-Osler-Weber Disease.
Départements where extrapolated prevalence is above 1/10,000



Note: the numbers refer to the *départements* listed in Table 1.
Source: Epidemiological survey, Plauchu, Bideau and Brunet, 1986.

Local surveys and updated prevalence for several départements

The data collected systematically at national level served as a starting point for further investigations at local level. They were very costly, however, and remained limited in scope. Their aim was to focus attention on zones where the disease prevalence appeared to be higher. Given the wide range of different observation methods employed, the results cannot be used for direct comparison between *départements*. They nonetheless provide more accurate estimates of prevalence in specific areas.

For the Ain, Jura and Deux-Sèvres *départements*, the team physicians contacted all relevant hospital departments and a large number of private practitioners. These surveys provided material for several MD theses which focused on analysing the survey results and tracing the probands' pedigrees (Pétrequin, 1983; Venturini, 1983; Léhy, 1983; Plauchu, 1988; Maszelin, 1990). Last, in the zone of highest prevalence, straddling the Ain and Jura *départements*, we made direct contact with consenting families. We were thus able to estimate the exact number of HHT carriers in each one.

Thanks to these local surveys, we obtained a much more accurate estimate of the number of known cases. As a result, estimated prevalence rose from 1/3,375 to 1/996 in Ain, from 1/4,287 to 1/2,381 in Deux-Sèvres and from 1/5,062 to 1/1,380 in Jura (Table 2). The multiplier coefficient is at least 1.8 and is even above 3 in certain *départements*. Of course, these surveys and their results cannot be extrapolated to other *départements*, but they confirm that the national estimates given above represent a low minimum.

Table 2. Rendu-Osler-Weber disease. Extrapolated prevalence and updated prevalence in eight *départements*

Département	Number of carriers estimated from postal survey	Extrapolated prevalence (a)	Final number of known carriers	Updated prevalence (b)	Multiplier coefficient (b/a)
Ain (01)	124	1/3,375	420	1/996	3.4
Deux-Sèvres (79)	80	1/4,287	144	1/2,381	1.8
Jura (39)	48	1/5,062	176	1/1,380	3.7
Doubs (25)	24	1/9,875	44	1/5,386	1.8
Haute-Savoie (74)	32	1/15,453	84	1/5,886	2.6
Rhône (69)	80	1/18,062	288	1/5,017	3.6
Saône-et-Loire (71)	28	1/20,428	52	1/11,000	1.9
Isère (38)	32	1/29,281	88	1/10,647	2.8

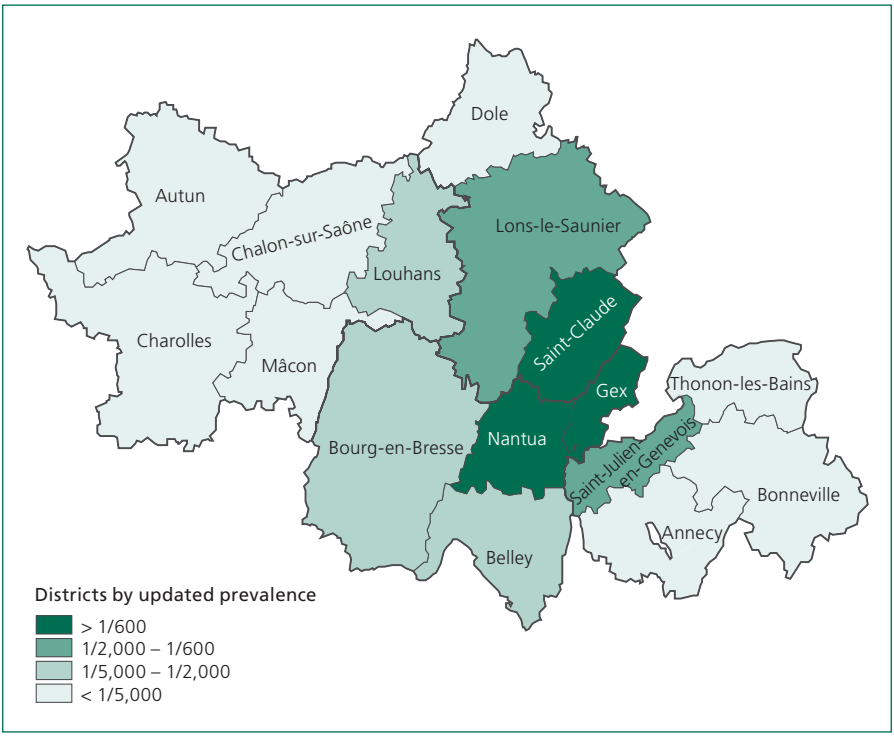
Source: Epidemiological survey, Plauchu, Bideau and Brunet, 1986.

Rendu-Osler-Weber disease is thus much less rare in France than we thought at the start of the study. We can confirm that local clusters exist, with a strong presence of the disease in the regions where they are located.

When the analysis is refined to district level, very high prevalences are observed. In Deux-Sèvres, the updated prevalence is around 1/1,193 in the Parthenay district, and in Ain, the highest levels are observed in the districts of Gex (1/300) and Nantua (1/346). This extreme concentration extends into the Jura *département*, with the Saint-Claude district (1/530) and, to a lesser extent, the Lons-le-Saunier district (1/1,526). The north of the *département*, by contrast, appears to be much less affected, with just 1/9,275 in the Dole

district (Map 2). These local surveys reveal very high and very localized concentrations of HHT carriers.[4] The concentration also extends east, to the Saint-Julien-en-Genevois district of Haute-Savoie, and west, to the Louhans district of Saône-et-Loire. Prevalences are low in the other districts of these *départements*, however, which are further from the central cluster straddling the Ain and Jura.

Map 2. Local concentration of Rendu-Osler-Weber Disease. Updated prevalence by district for 4 *départements*



Source: Epidemiological survey, Plauchu, Bideau and Brunet, 1986.

These surveys uncovered around one thousand probands, the vast majority of whom were over 50 years old. It is probable that the patients we identified were those with the most severe symptoms and that, given the broad range of clinical signs associated with the disease, the prevalence of HHT was again under-estimated.

Our final prevalence estimates were substantially higher than the levels commonly quoted in the literature, and showed very large geographical

(4) Our approach, which consisted of multiplying by four the number of carriers with a file in their name and assuming that they all lived in the same *département*, probably distorts the true geographical distribution of carriers and amplifies concentrations at *département* level.

disparities. Similar prevalence levels – in some cases locally higher – have since been found among other populations, notably in Denmark (Kjeldsen et al., 1999) and in the Netherlands Antilles (Westerman et al., 2003).

This work raised two new questions. Do certain affected families have a shared inheritance? Could HHT be the result of a single mutation, inherited by all French patients from a common ancestor? To answer these questions with the tools available at that time, we decided to retrace the family pedigrees of HHT carriers in a small region which seemed to be the epicentre of the main cluster.

II. Looking for a local founder effect: The contribution of genealogy and historical demography

Hundreds of families were reconstituted in order to retrace distant relationships between carriers of local origin. However, due to high levels of homonymy, the lack of reliable historical records and the vast quantity of documents involved, we soon abandoned our attempt to trace the ancestry of each carrier, family by family, in the most affected municipalities. For example, in the five villages of the Valserine valley (in the Ain *département,* bordering the Jura) we knew of 36 affected families living locally, and of 13 other families living elsewhere but with ancestors in the valley. For the Valserine valley, we systematically reconstituted the families mentioned in the parish registers and civil records from the late seventeenth century to the end of the twentieth century using the techniques of historical demography. For the other municipalities of the region, ancestor charts were established without family reconstitution.

Family reconstitution in the Valserine valley and analysis of genealogical networks

The analysis of parish registers and civil records was based on the principles laid down by M. Fleury and L. Henry (1965), with a few adjustments to take account of twentieth-century civil registration practices (marginal annotations in particular). Families were reconstituted using a semi-automated process pioneered by the Research Programme in Historical Demography of the University of Montreal (Beauchamp et al., 1977). Five municipalities in the Valserine valley were studied. Some 54,000 records drawn from parish and civil registers were analysed (27,000 christenings and births, 6,000 marriages, 21,000 burials and deaths), relating to more than 46,000 individuals. After the reconstitution process was complete, each individual mentioned in these documents was linked to all his or her near and distant relatives using software developed specifically for this purpose (Poulard et al., 1991).

It was thus possible to describe the demographic regimes prevailing in the valley and their changes over three centuries. We observed, for example, that choice of spouse and geographical mobility, key factors in the local concentration

of the disease, followed different patterns in the north and south of the valley. In the more remote and inacessible north, spouses were often brought in from the plateaus of the southern Jura (Bouchoux and Septmoncel plateaus) where many HHT carrier families are still found today. In the more open south, by contrast, with easier access to the major transport routes between Lyon and Geneva, spouses came from more diverse origins and mobility was higher than in the north (Bideau and Brunet, 2007).

The genealogical networks themselves were also subjected to statistical analysis, revealing interesting particularities of HTT transmission across successive generations. We drew up ancestor charts of all individuals born in the Valserine valley in the period 1950-1969, and ranked them by the maximum number of generations who lived locally, indicating for each one the generation of the oldest local ancestor (Brunet et al., 2006). Two very different groups emerged. The first minority group of ancestor charts (around one-quarter) included no more than four generations living locally, indicating that these families were recent arrivals in the valley (one century or less). The majority of charts (almost two-thirds) included one or more ancestors born in the valley at least eight generations earlier, i.e. in the late seventeenth or early eighteenth century. All the HHT carriers with family origins in the valley belong to this second group of family lineages marked by a certain geographical stability (permanent presence of at least one individual per generation) and a specific pattern of mating behaviour, notably involving cousin marriages (Bideau et al., 1994).

Genealogical matching, simulation of gene diffusion and founder effect

Though meticulous and exhaustive, this family reconstitution presents a major flaw common to many studies in historical demography: all the events registered in the municipalities concerned are known and interlinked, but as soon as a family or an individual leaves the study area, the events concerning them are lost to observation. To a certain extent, these gaps were filled for the twentieth century via the genealogical interviews conducted with the families concerned. These interviews also enabled us to identify family members with Rendu-Osler-Weber disease in previous generations.

The first task was to analyse the genealogical networks of living HHT patients and to identify any known points of overlap. These networks are very dense, since each family shares with other families several common ancestors who lived locally in the early eighteenth century. Genealogical overlap is expected for the entire village population of that time, but it is especially large in this case because of the high level of geographical endogamy. Genealogical convergence is not absolute, however, and we were able to identify 929 different early ancestors (late seventeenth century) who could theoretically have carried the disease (Heyer, 1991).

Among these ancestors, we focused our attention on those with the largest number of known HHT carriers among their descendants (the descendants of a single couple, for example, included 23 living carriers who were unaware of this distant family connection) and on those whose descendants were unrelated to the other carriers.[5] We then sought to account for all living patients with a local origin using these combinations involving the minimum number of ancestors. To account for all living carriers with ancestors in the Valserine valley, the optimal formula is obtained by grouping the descendants of 18 couples who lived at the end of the seventeenth century (Bideau et al., 1992).

The genealogical reconstitutions were combined with an experimental approach to simulate gene diffusion. On the basis of the reconstituted genealogical networks, it involved hypothesizing that one or other ancestor was a carrier of the disease. Taking account of the mode of HHT transmission, of this ancestor's descendants, of the mortality and mobility of his or her descendants over the generations, we then determined how many living patients are liable to have inherited the mutant gene thus transmitted. This method was developed by E. Heyer, who simulated tens of thousands of gene diffusion patterns (Heyer, 1991). We were thus able to find new optimal ancestor combinations which identify living patients of local origin with the highest probability. This experiment detected at least thirty ancestors living in the early eighteenth century who were certainly carriers of the disease. By extending this probabilistic reasoning, we estimated that the single founding ancestor, if he or she existed, lived in the sixteenth century or earlier, i.e. before the creation of the parish registers and notarial archives that we used as data sources.

Tracing family origins in the other départements

The ancestor charts of carriers with no origins in the Valserine valley were traced back using the standard technique of successive searches in the parish registers and civil records of the municipalities concerned. This was done notably for 63 carriers living within 30 kilometres of the Valserine valley, among whom a few common ancestors were found, although overlap was rare and limited. These ancestor charts, systematically traced back to the mid-eighteenth century (and beyond when the sources existed), did not reveal any overlap with the families mentioned above who had an ancestor in the Valserine valley.

The hypothesis of a recent founder effect in the region accounting for all identified patients thus became untenable. We have already seen, in the limited context of the Valserine valley, that the common ancestor, if any, lived in the sixteenth century at the latest. To include a unique founder among the ancestors of the families scattered around the edges of the valley, the founder effect would have to be pushed even further back in time. These findings led us to reject

(5) Of course, this reasoning is reliant upon the official filiations reported on the birth certificates.

the hypothesis of a single founder effect common to all carriers in this region, and to postulate that several different mutations are involved (Brunet, 1992).

III. Using molecular biology to search for the original mutation

The ancestor charts of most living patients were also established for all regions where carriers now live and for ancestors' places of origin. We thus obtained almost a thousand genealogical networks covering most of France upon which to base our reasoning and develop new hypotheses.

No evidence of a unique founder effect in France

Genealogical overlap was very rare among the hundred or so carriers living in *départements* close to the cluster of cases in the Valserine valley. Their ancestral origins are very diverse, scattered across the Rhône-Alpes region and beyond. The ancestor charts nonetheless revealed family links to the Valserine valley among 7 other patients (4 living in Ain, 2 in Haute-Savoie and 1 in Rhône).

Similar observations were made in other regions: nowhere is there any significant genealogical overlap from the eighteenth century up to today, and nowhere is there any apparent evidence of a recent founder effect. The only exception is Deux-Sèvres, one of the *départements* where prevalence is highest. Among the 35 carriers living in Deux-Sèvres who are not close relatives, around 15 instances of genealogical overlap were detected. The most substantial involves 6 carriers who all share a couple of common ancestors formed in the mid-nineteenth century. No kinship ties could be found with the 20 other carriers, however, and the identified ancestors who lived in the early eighteenth century were scattered over a very wide geographical area. So for the Deux-Sèvres *département*, likewise, a single recent founder effect cannot account for all the families studied. This implies either an early founder effect or the existence of several different mutations.

Note also that very little genealogical overlap was found between families of living carriers currently residing in different regions, and that no link was found between the carriers in the Valserine valley cluster and those in the Deux-Sèvres.

These findings pointed to the existence of several different mutations in France as a whole, some perhaps older than others and hence affecting a larger number of living carriers. Particular local demographic regimes may explain the greater impact of one or other of these mutations and, in some cases, its local concentration. Our research based on techniques of genealogy and historical demography concluded that at least four mutations probably exist at national level, and that an ancient founder effect dating back, very approximately, to the sixteenth century probably accounted for the cluster in the Valserine valley (Brunet, 1992, p. 69 and p. 179). Of course, at that time, we were far

from imagining the wide variety of mutations responsible for the disease or the very existence of several different genes. Proof that the same mutation was shared by several carriers had to come from molecular biology.

Identifying the different mutations

The identification of the mutations responsible for Rendu-Osler-Weber disease in France began in Lyon in 2000. Substantial progress has been made in recent months, shedding new light on earlier findings.

In 1993, the *ENG* gene coding for endoglin was localized on the distal portion of the long arm of chromosome 9, and this form of the disease was named HHT1 (Fernandez-Ruiz et al., 1993). Several teams then identified different mutations of the *ENG* gene, revealing the allele heterogeneity* of the disease and its gene heterogeneity*, since certain families did not carry mutations of this gene (Heutink et al., 1994; Shovlin et al., 1994). In 1995, our team found evidence of linkage with a locus of chromosome 12, and mutations of the gene *ACVRL1* were also identified (Vincent et al., 1995; Johnson et al., 1995). This second form of the disease, very similar in nature but due to a different gene, was named HHT2 to distinguish it from the first. In both cases, the same mutation is always transmitted from one generation to the next within a family. It is estimated that the mutations on the *ENG* and *ACVRL1* genes are responsible for about 90% of cases of the disease (Lesca et al., 2004, 2006).(6) Although the clinical expression of HHT is highly variable, both between and within families, there is a certain correlation between genotype and phenotype. Some complications, notably pulmonary, are more frequent in the HHT1 form of the disease, while others, such as those affecting the liver, are more frequent in the HHT2 form (Lesca et al., 2007).

The evidence of substantial gene and allele heterogeneity invalidates the hypothesis of a "national" founder effect affecting all French HHT carriers. Most mutations are "private", i.e. unique and specific to a given family. However, some have been found in several unrelated families, sometimes living on opposite sides of the world. We were able to study the molecular nature of the mutation among 96 patients with no known family relationship, of whom 89 lived in France and 7 in Italy. Among these 96 patients, we found 13 different mutations of the *ACVRL1* gene (Lesca et al., 2008), proving that despite the diversity of mutations, some are shared by several families. In this group, for example, the discovery of a specific mutation (p.Arg144X), which probably occurred more than five centuries ago, is common to several French and Italian families. Among these 96 patients, 36 (apparently unrelated) were identified in 2005 as carriers of the same c.1112dupG mutation of the *ACVRL1* gene, and

(6) The gene heterogeneity of the disease has been confirmed by recent data, with the detection of germinal mutations of the *MADH4* gene in certain families affected by a combined syndrome of HHT and juvenile polyposis (Gallione et al., 2004), and with the identification of two new loci, on chromosomes 5 and 7, respectively (Cole et al., 2005; Bayrak-Toydemir, 2006).

shared a common haplotype*. The uniqueness of this mutation and the identity of the markers located inside and adjacent to the *ACVRL1* gene indicate that these patients share a common ancestor, and hence prove the existence of a founder effect.(7) In fact, all carriers of this mutation live in the Rhône-Alpes region, or originate there, with a cluster in the east of the Ain *département*, in the precise area of the Valserine valley. The high prevalence of HHT in this region can thus be attributed to several mutations of two genes, but is explained in part by the founder effect associated with this specific c.1112dupG mutation.

The most plausible hypothesis is that this mutation occurred in a person who lived in the Valserine valley or nearby, that its local frequency increased gradually over the generations through the process of genetic drift*, and that it spread across the region from the early nineteenth century due to substantial out-migration from this isolated mountain valley. By observing the genotype of patients carrying the different mutation markers and applying a likelihood method (Génin et al., 2004), we estimated that this local mutation occurred between 250 and 425 years ago (Lesca et al., 2008). It is very interesting to compare this finding with the genealogical data presented above. Our first analysis suggested that the local mutation probably occurred in the sixteenth century. This second analysis places it somewhere between the mid-sixteenth and the seventeenth centuries. While the second date is refuted by the genealogical networks, the first date is perfectly plausible. However, this local founder effect, which quite probably occurred in the Valserine region in the sixteenth century, or at an earlier date, does not account for all the HHT carriers living in the Rhône-Alpes region. The disease shows considerable genetic heterogeneity in this region, where other mutations of the *ACVRL1* gene are found among carriers, along with various mutations of the *ENG* gene.

The situation is equally complex in the other regions, notably in Deux-Sèvres where the epidemiological survey revealed high prevalence of the disease. Several mutations are also present in this region, including mutation p.Arg374Gln on the *ACVRL1* gene which affects several families in France. Interestingly, it is associated with three different haplotypes, suggesting that it occurred independently on three occasions. One of these haplotypes is present in several patients in Deux-Sèvres and is probably the result of a more recent founder effect than that observed in the Ain *département,* but with a very wide confidence interval ranging from 50 to 375 years. A history of just 50 years is highly implausible, however, since no overlap has been identified in the families' genealogical networks over the last seven generations, spanning a period of two centuries (Lesca et al., 2008).

(7) Following further systematic exploration of patients recruited at the Centre national de référence in Lyon, 90 carriers of the mutation specific to this cluster have now been identified.

Conclusion

It is rare for a study to extend over such a long period (more than 30 years) and to involve researchers from such a broad range of disciplines. For these reasons, knowledge has been built up very progressively, with each stage adding new bricks to the edifice. Most of the initial hypotheses, first formulated in the 1970s, have been challenged or even totally invalidated. Rendu-Osler-Weber disease is much less rare than we believed at the time, and our epidemiological study, despite its imperfections, was the first to establish this fact. It became clear that a unique founder effect was an unlikely explanation for most carriers of the disease in France. The evidence uncovered by systematic genealogical research was subsequently confirmed by the exceptionally broad gene and allele heterogeneity of French HHT carriers. By contrast, the initial intuition that a large local cluster existed in a region straddling the Ain and Jura *départements* was gradually confirmed. Analysis of genealogical networks and demographic regimes suggested that HHT carriers from the Valserine valley shared a common ancestry. This local founder effect was confirmed by molecular biology and an approximate date of the local mutation was determined by combining the two approaches.

This study is exceptional in many respects and illustrates the advantages of a multi-disciplinary approach. Although France is the only country where HHT has been studied in such length and depth, and with such a large number of patients, researchers elsewhere have also focused on the disease in recent years. The diffusion of Rendu-Osler-Weber disease in other countries has recently been studied. Many Danish HHT carriers can probably be traced back to a local founder effect and a mutation that occurred more than three centuries ago (Brusgaard et al., 2004). In the Netherlands Antilles, one of the three mutations was probably introduced by a Dutch slave owner (Gallione et al., 2000). Thanks to the contribution of molecular genetics, we should now be able to reconstruct an international history of Rendu-Osler-Weber disease, leading to a better understanding of the diffusion and concentration of this and other genetic mutations.

GLOSSARY

- **Allele:** one of two or more alternate forms of a gene. Each allele is distinguished by one or more differences in the sequence of nucleotides. In our example, several different mutated alleles are responsible for HHT.
- **Allele heterogeneity:** a genetic disorder can be caused by different mutations of a single gene.
- **Founder effect:** large fluctuation in allele frequencies in a new population created by the migration of a small number of individuals away from a parental population. In our example, the mutation c.1112dupG may have been introduced by a migrant or have occurred in an inhabitant of the valley.
- **Gene heterogeneity:** a genetic disorder can be caused by mutations of different genes.
- **Genetic drift:** a process whereby allele frequency in small breeding populations changes due to random variations in allele transmission from one generation to the next. Within a few generations, this can lead to a much higher frequency of certain alleles or, conversely, their total disappearance.
- **Haplotype:** combination of different genes or genetic markers located on a single chromosome. In practice, the term often designates the genetic markers of a region of interest. These markers segregate together during meiosis, except in the case of genetic recombination.
- **Proband** first affected family member who seeks medical attention for a genetic disorder and who is the starting point of the pedigree chart. There is only one proband per family (also called the index case).

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Guy Brunet, Gaëtan Lesca, Emmanuelle Génin, Sophie Dupuis-Girod, Alain Bideau, Henri Plauchu • **Thirty Years of Research into Rendu-Osler-Weber Disease in France: Historical Demography, Population Genetics and Molecular Biology**

Rendu-Osler-Weber disease, also known as hereditary hemorrhagic telangiectasia (HHT), has been the focus of several interdisciplinary studies over the last thirty years. An initial epidemiological survey conducted in the 1980s revealed that the prevalence of this hereditary genetic disorder in France is much higher than previously thought, and brought to light several geographical clusters of HHT carriers. The subsequent analysis of the family genealogies of carriers in the main cluster and of the demographic history of the region did not confirm the thesis of a unique founder effect at national level, but provided clues for dating the occurrence of a local mutation. Last, thanks to the recent identification of the genes responsible for HHT, the considerable genetic heterogeneity of the disease has been confirmed and a large number of different mutations existing in France have been identified. Nonetheless, many carriers living in the main geographical cluster studied previously share a single mutation associated with a specific haplotype. At the end of this study, marked by its multi-disciplinary approach and its exceptional duration, the various converging strands confirm the existence of a local founder effect and provide consistent evidence for dating this mutation.

Guy Brunet, Gaëtan Lesca, Emmanuelle Génin, Sophie Dupuis-Girod, Alain Bideau, Henri Plauchu • **Trente ans d'étude de la maladie de Rendu-Osler en France : démographie historique, génétique des populations et biologie moléculaire**

La maladie de Rendu-Osler est une génopathie héréditaire qui a fait l'objet de plusieurs études interdisciplinaires au fil des trois dernières décennies. Une enquête épidémiologique menée dans les années 1980 a d'abord permis d'en établir la prévalence en France à un niveau nettement plus élevé que ce qui était alors admis, et de pointer l'existence de zones de forte concentration. L'étude généalogique et l'étude de démographie historique qui ont suivi, centrées sur le principal pôle de concentration, n'ont pas permis de prouver la théorie d'un effet fondateur unique à l'échelle nationale, mais d'émettre des hypothèses quant à la date d'apparition d'une mutation locale. Enfin, l'identification, au cours des dernières années, des gènes responsables de cette maladie a permis d'en établir la grande hétérogénéité génétique et d'identifier un grand nombre de mutations différentes présentes sur le territoire français. Toutefois, de nombreux malades résidant dans le principal pôle de concentration étudié précédemment partagent une mutation unique associée à un haplotype spécifique. Au terme de cette étude, exceptionnelle par son aspect interdisciplinaire et sa durée, les différentes démarches convergent pour affirmer l'existence d'un effet fondateur local, et s'accordent sur une datation approximative de cette mutation.

Guy Brunet, Gaëtan Lesca, Emmanuelle Génin, Sophie Dupuis-Girod, Alain Bideau, Henri Plauchu • **Treinta años de estudios sobre la enfermedad de Rendu-Osler en Francia: demografía histórica, genética de poblaciones y biología molecular.**

La enfermedad de Rendu-Osler es una enfermedad genética, estudiada bajo un enfoque interdisciplinario durante los tres últimas décadas. En los años ochenta, una encuesta epidemiológica permitió establecer su prevalencia en Francia a un nivel más elevado que el que hasta entonces se había supuesto, y también señaló la existencia de zonas de fuerte concentración. Los estudios genealógico y de demografía histórica ulteriores, focalizados en el principal polo de concentración, no demostraron la existencia de un fundador único a escala nacional, pero permitieron hacer ciertas hipótesis sobre la fecha de aparición de una mutación local. En fin, durante estos últimos años, la identificación de los genes responsables de esta enfermedad ha permitido establecer su gran heterogeneidad genética y constatar la existencia de un gran número de mutaciones diferentes en el territorio francés. Sin embargo, numerosos enfermos residentes en el principal polo de concentración estudiado anteriormente poseen une mutación única asociada a un haplotipo específico. Al término de esta serie de estudios, excepcional por su dimensión interdisciplinaria y por su duración, los diferentes enfoques convergen en afirmar la existencia de un efecto fundador local, y están de acuerdo sobre la datación aproximada de esta mutación.

Translated by Catriona Dutreuilh.