Descriptive Epidemiology of Haemophilia in Martinique

ON Pierre-Louis[1, 2, 3, 4], J Véronique-Baudin[3], J Rabout[1], MN Yerro[1], S Molcard[1], P Fuseau[3], M Vestris[3], G Aurokiom[3], J Smith-Ravin[2], C Joachim-Contaret[3], MS Pierre-Louis[1, 4]

ABSTRACT

Objective: *Haemophilia is a congenital bleeding disorder characterized by partial or complete deficiency of a clotting factor VIII (FVIII) for Haemophilia A (HA), or factor IX (FIX) for Haemophilia B (HB). In this study, we describe the epidemiology of haemophilia in patients from Martinique, a French Caribbean Island with a population of predominantly Afro-Caribbean origin. This epidemiological study has never been described before, and the associated specific mutations for the corresponding genes were unknown.*

Methods: *We conducted a descriptive study based on the experience of the Martinique Cancer Registry and collected laboratory data with patients' consent.*

Results: *Results showed that about 130 haemophilia patients had been diagnosed in Martinique. Haemophilia A and B were represented, with a higher number of patients with HA. In 2017, three HA patients had inhibitors: this represents a costly complication of treatment for this disease. Also, specific mutations have been found: until now, they were not referenced in any international data base.*

Conclusion: *The Martinique regional centre for haemophilia treatment played a crucial role in global care for patients with clotting factor deficiencies. From the experience of Martinique, it would be useful to develop a collaborative study on the diagnosis and the treatment of haemophilia in the Caribbean area and French Guyana.*

Keywords: Clotting factor deficiency, haemophilia, Martinique

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INTRODUCTION

Haemophilia is a rare X-linked congenital bleeding disorder, affecting approximately 12.2 per 100 000 males for Haemophilia A (HA) and 2.37 per 100 000 males for Haemophilia B (HB) in continental France (1, 2). Male patients manifest, partially or completely, deficiencies of clotting factor VIII (FVIII) for Haemophilia A (HA) and factor IX (FIX) for Haemophilia B (HB), a key component of the clotting regulation cascade. Females carried the deficit but do not usually manifest tissue. Clotting factors could also decrease activity and carried heterogeneous mutations for the corresponding genes. The amount of coagulant activity generate the clinical variability of the phenotype: severe (FVIII: C $\leq$ 1%), moderate (FVIII: C = 2–5%), and mild (FVIII: C $\geq$ 5%). Bleeding into joints and soft tissues are the hallmark of severe and moderate haemophilia and the first clinical manifestations appear during the first years of live.

The severity of manifestations correlates with the clotting factor deficiency level. Treatment is intravenous

From: [1]The Martinique Hospital Centre for Resources and Competence of Haemorrhagic Constitutional Diseases, Fort de France, Martinique, [2]EA929 AIHP-Géode BIOSPHERES Research Group of French West Indies University, Schœlcher, Martinique, [3]The Martinique University Hospital Research Department, Fort de France, Martinique and [4]The Martinique Regional Committee of Hemophilia and Von Willebrand Patients, Schœlcher, Martinique.

Correspondence: Dr ON Pierre-Louis, Regional Treatment Centre of Haemophilia Martinique University Hospital, Pierre Zobda Quitman CS90632, 97261 Martinique. Email: crth.martinique @gmail.com

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injection of the deficient clotting factors, produced by recombinant or plasma-derived strategies (3, 4). Desmopressin (DDAVP) treatment is currently used in mild haemophilia. Clotting factors are synthesized as precursor proteins; the mature chain is obtained by maturation process and proteolytic cleavages. Development of anti-bodies (called inhibitors) directed against clotting factor VIII and IX is the major complication in haemophilia treatment, in relation to their ability to inhibit procoagulant activity and thus represent, a costly complication of therapy (5). A previous report has indicated that patients of African heritage could have higher risk factors for development of inhibitors as compared to Caucasians (6). Gene mutations responsible for haemophilia are mostly represented by point alterations for mild and moderate haemophilia. In severe haemophilia, there is a high prevalence for large gene deletion and inversion (7). Other FVIII antibodies do not interfere with the functional properties of clotting factors: they are called non-neutralizing FVIII antibodies (8 10).

Differently from other countries with ethnic specificities (10–12), in Martinique, a French Caribbean Island with an Afro-Caribbean ascendance, the haemophilia population has never been described. There had been only reports of the risk of hepatitis and HTLV1 (13, 14) in HA patients in Martinique.

The goal of this study was to describe the haemophilia population in Martinique: their clinical and biological specificities, and molecular deficits. These allowed identifying, for the first time, the characteristics of a European associated black population, of Afro-Caribbean origin. We also discuss the role of the Martinique Haemophilia Treatment Regional Centre (CRTH) for haemophilia care in this region.

SUBJECTS AND METHODS

Congenital haemophilia clinics were established since 1989 and acquired haemophilia cases were not considered. Biological data were obtained from the haematology laboratory, of the Martinique University Hospital, in charge of molecular diagnostics. Data included date of registration at the haemophilia clinic, age at diagnosis and registration, type and severity of haemophilia, family history, genetics and molecular clotting factor deficiency. For each sample, clotting factor quantifications were performed in replicates. Pedigree data were used to determine the number of affected relatives in the family. Lists of patients from all haemophilia clinics were cross-checked in order to eliminate overlaps, as many patients had used more than one clinic. The study was performed in accordance with the Declaration of Helsinki and French legislation relating to biomedical research involving human subjects.

Genomic data measurements

Biological samples were conserved in a collection located in the Centre Hospitalier Universitaire (CHU) de Martinique and sent for sequencing in the haemophilia reference Centre (Lyon, France; Pr Négrier C). All patients gave consent for molecular studies. Genomic DNA was extracted by standard procedures from 5–10 mL peripheral blood samples. Results were analysed using Basic Local Alignment Search Tool (BLAST); https://blast.ncbi.nlm.nih.gov/Blast.cgi) programme in comparison with the wild-type F8 gene sequence. All identified mutations were compared to those described in the HAMSTeRS database (http://www.HAMSTeRS.ac.uk/). PolyPhen software (Polymorphism Phenotyping) was used to perform the sequence alignment of the homologous FVIII from four mammalian species and to predict the possible impact of an amino acid substitution on the structure and function of FVIII. Results were also compared to those found on the Variation Society (HGVS) website (http://www.hgvs.org).

Statistical analyses

Quantitative variables were described as mean ± SD or median (Q1, Q3) for quantitative data when appropriate. Qualitative data were described as numbers and percentages. Clotting factor antigen levels were compared by age groups and severity degree of haemophilia, using Mann-Withney U test or Kruskal-Wallis, if applicable. All analyses were performed using Graphpad software.

RESULTS

Descriptive epidemiology of haemophilia in Martinique

The characteristics of the 130 haemophilia patients are shown in Table 1: all have Afro-Caribbean origins. In a population of 176 938 Martinique males, the 108 HA and 22 HB were identified (INSEE 2013). This corresponded to 62.73 per 100 000 males for HA and 12.43 per 100 000 males for HB. Haemophilia A and HB have a five-fold higher frequency in Martinique than in France. Mild HA represents 81.98% of HA, 9.91% for moderate HA and 8.11% for severe HA in Martinique. Haemophilia B minor forms are 72.73% and severe HB represent 27.27% of the global HB population. Severe HA is 5.09 per 100 000 males and severe HB represent 3.39 per 100

000 males. In Martinique, three HA patients had inhibitors: one of them died as a result of this. In contrast, no HB patient developed inhibitor in Martinique.

Table 1: Baseline characteristics for Haemophilia patients, in Martinique

	Haemophilia A n = 108		Haemophilia B n = 22	
	n	%	n	%
Severity				
Severe	6	8.11	6	27.27
Moderate	11	9.91	0	0.00
Mild	91	81.98	16	72.73
History of inhibitor				
Severe HA (Plasmatic)	2	1.80	0	0
Severe HA (Recombinant)	1	0.90	0	0

Causal gene mutations responsible for haemophilia in Martinique

As listed in Table 2, gene mutations responsible for haemophilia consist in point mutations for all degrees of severity for HA and severe or mild HB (No patient with moderate HB was diagnosed in 2014). A specific promoter mutation was detected in minor HA (c.-219C>T). Specific polymorphisms were observed in mild HA (p.Ala343Ala; IVS4 G+17A) and moderate HA (p.Met2257Val). Two patients had inhibitors with specific mutations (p.Arg2228Stop; c.6760delC). Another patient died with inhibitor before the CRTH creation in consequence of an inhibitor associated with inversion 3/22 genotype. In respect of the risk factor for HA patients with Inv. intron 22 to develop inhibitors, therapeutic treatment are closely monitored. Six mutations were not found in the database: p.Asn583Lys; p.Leu2072Phe; p.Met2274Val; p.Cys473Phe; p.His998-Gln; p.Gly2063Arg; another one was in mild HB (p.Phe-345Val).

DISCUSSION

In Martinique, the first case of haemophilia was identified in the 1980s, in a context of non-existent specific hospital care. This is linked to the lack of effective treatments and tests for viral safety of blood products (*eg* HIV and viral hepatitis). Circumstances related to the diagnosis were essentially haemorrhagic manifestations and preoperative assessments. Until this date, epidemiologic data have not been described. After the creation of the regional committee for haemophilia in 1981, health professionals and patients have been educated on haemophilia care. Since creating the Regional Treatment Centre for haemophilia (RTCH) in 1989, the regional hospital responded to the need for multidisciplinary care, clinical monitoring and paramedical support for the benefit of these patients. From 1982 to 2014, we identified 62.73 per 100 000 males with HA in Martinique.

As compared with mainland France (12.2 per 100 000 HA males), this disease has a five-fold higher frequency. Also this Island frequency is five-fold higher for HB, with 14.43 per 100 000 Martinique males, as compared with 2.37 per 100 000 HB males in Martinique and France. Mild haemophilia is highly represented in this statistic because of large family size in an island epidemiological reference. Also, the Island features and mode of inheritance could justify this high number of patients. We cannot highlight significant differences according to severity of the deficit and the age of patients. These suggest that patients have low morbidity according to the severity of their disease. In other Caribbean Islands, haemophilia is poorly represented: this could be related to HA patient's deaths before the 1980s. Inhibitor status is currently a cause of death associated with genetic risk factors (16, 17). In Guadeloupe and French Guyana, a small number of patients was diagnosed (data not shown).

In Guadeloupe, the prevalence of HA is estimated to be 15.96 per 100 000 males. In French Guyana, diagnoses of haemophilia represent less than 4.25 per 100 000 males.

Table 2: Genetic mutations and polymorphism associated with HA/HB, in Martinique

Severity	Haemophilia A	Haemophilia B
Mild	c.-219C>T IVS4 G+17A p.Ala315Thr p.Ala343Ala p.Asn583Lys p.Met633Ile p.Glu702Lys p.Arg717Trp p.Arg1985Gln p.Leu2053Phe p.Arg2178Cys p.Met2274Val	p.Phe55Ile p.Phe345Val
Moderate	p.Cys473Phe p.His998Gln p.Gly2063Arg p.Arg2169His p.Met2257Val	
Severe	Inv. intron 22 p.Val115Phe p.Arg2228Stop p.Met2257Val Del.C6703 del C6703	p.Trp118Stop p.Glu433Lys

Data expressed with HGVS nomenclature

A wide spectrum of causative mutations was observed in Martinique. Haemophilia transmission has been demonstrated by the detection of specific mutations and/or polymorphism: p.Asn583Lys; p.Leu2072Phe; p.Met2274Val; p. Cys473Phe; p. His998Gln; p.Gly2063-Arg for HA and p.Phe345Val for HB. Many atypical atypical genetic polymorphisms were also identified in HA. In association with previous reports (6) showing that black patients with severe HA could have a three-fold higher risk for developing inhibitors, our results suggest that it would be interesting to study the FVIII and FIX haplotype frequency, in respect of the Afro-Caribbean origin of this population. Importance of the genetic factors have been reported in several publications (17–21). In regards to the Martinique genetic profile and risk for developing inhibitors, our results would help physicians to evaluate the most appropriate treatments and improve family management.

CONCLUSION

Before 1982, haemophilia patients from Martinique had a short life expectancy. Prior to 2000, the diagnosis was essentially made during haemorrhagic stroke and haemophilia care was challenging. Patients had severe haemarthrosis which affected their prognosis. The early management of haemophilia patients, from the year 2000, facilitated the early diagnosis and appropriate care for these patients. Today, these patients receive regular follow-up including orthopaedic, psychosocial care, psychotherapy, nursing and autoperfusion. Specialized care protocols in therapeutic education helps reinforce these goals. From the experience of Martinique, it would be interesting to have a collaborative study on the diagnosis and the treatment of haemophilia in the Caribbean area and French Guyana, because very few patients seem to be diagnosed in the Caribbean areas.

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AUTHORS' NOTE

ON Pierre-Louis conceived paper, oversaw data collection, conducted data analysis, wrote manuscript and approved final version. S Pierre-Louis and J Véronique-Baudin participated in study design and approved final version.

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