FINAL DEGREE PROJECT

Clinical genetics of Osler-Weber-Rendu Disease in Gran Canaria

End-of-degree Project: Degree in Medicine

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Ordinary Call

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Infinite thanks
to my parents for giving me wings to fly,
to my tutors for helping me to take the flight.

1. Abstract

Objectives: Hereditary Hemorrhagic Telangiectasia (HHT) is an Autosomal Dominant genetic disease that presents with dysplasia of vascular cells, which causes hemorrhages in different regions, the most prevalent being epistaxis. Several genes whose mutations are responsible for this disorder have been described. This project has allowed the development of direct diagnostic procedures of the most frequent gene variants in Gran Canaria. Likewise, a possible genotype-phenotype correlation has been evaluated both at the inter-family and intra-family levels.

Methodology: It is a descriptive observational study, with an experimental part, in which 74 patients residing in Gran Canaria diagnosed with HHT have been included, grouped by families. Data on the age of onset of symptoms, primary symptoms, concomitant diseases, MAV screening and follow-up by Specialized Care have been collected. In addition, several genetic techniques (PCR, ASA, RFLP) have been put to point to be able to make a correct diagnosis according to the variant of HHT carried by the patient.

Results: The data of the 74 patients were analysed, discovering an association between the symptomatology, its severity and even the concomitant diseases according to the presented variant. The different techniques that were carried out were shown to be highly efficient due to their cost-benefit, the low probability of false negative and positive ones found, as well as their substantive work in the secondary prevention of the health of these patients.

Conclusion: There is a genotype-phenotype association, presenting an inter-family and intra-family variability according to the variant of HHT that is presented. A correct diagnosis is useful and necessary at preconception and postnatal level for the prevention of comorbidity associated with the presence of systemic shunts due to the etiopathogenesis of the disease. Promoting the investigation of this disease and teaching health professionals and patients are essential.

Key words: Hereditary Hemorrhagic Telangiectasia, HHT1, HHT2, Osler-Weber-Rendu disease (OWRD), genetic diagnosis, *ENG*, *ALK1*, inter-family and intra-family variability, epistaxis

2. Resumen

Objetivos: La Telangiectasia Hemorrágica Hereditaria (HHT) es una enfermedad genética Autosómica Dominante que cursa con displasia de las células vasculares, lo que ocasiona hemorragias en distintas regiones, siendo la más prevalente la epistaxis. Se han descrito varios genes cuyas mutaciones son las responsables de este trastorno. Este trabajo ha permitido desarrollar procedimientos diagnósticos directos de las variantes génicas más frecuentes en Gran Canaria. Asimismo se ha evaluado una posible correlación genotipo-fenotipo tanto a nivel interfamiliar como intrafamiliar.

Metodología: Se trata de un estudio observacional descriptivo, con una parte experimental, en el que se han incluido a 74 pacientes residentes en Gran Canaria diagnosticados de HHT, agrupados por familias. Se han recogido los datos de edad de inicio de los síntomas, síntoma prínceps, enfermedades concomitantes, realización de *screening* de MAV y seguimiento por Atención Especializada. Además, se han puesto a puntos diversas técnicas genéticas (PCR, ASA, RFLP) para poder realizar un correcto diagnóstico según la variante de HHT que porte el paciente.

Resultados: Se analizaron los datos de los 74 pacientes descubriéndose una asociación entre la sintomatología, su gravedad e incluso las enfermedades concomitantes según la variante presentada. Las distintas técnicas que se realizaron mostraron ser altamente eficientes por su coste-beneficio, la baja probabilidad de falsos negativos y positivos hallados, así como su importante labor en la prevención secundaria de la salud de estos pacientes.

Conclusión: Existe una asociación genotipo-fenotipo, presentándose una variabilidad interfamiliar e intrafamiliar según la variante de HHT que se presenta. Es útil y necesario un correcto diagnóstico a nivel preconcepcional y postnatal para la prevención de comorbilidad asociada a la presencia de *shunts* sistémicos debidos a la etiopatogenia de la enfermedad. Es fundamental promover la investigación de esta enfermedad y la docencia hacia los profesionales sanitarios y pacientes.

Palabras claves: Telangiectasia Hemorrágica Hereditaria, HHT1, HHT2, Síndrome de Rendu-Osler-Weber, diagnóstico genético, *ENG*, *ALK1*, variabilidad interfamiliar e intrafamiliar, epistaxis.

3. Introduction

Hereditary Hemorrhagic Telangiectasia (HHT) or Osler-Weber-Rendu Disease is a vascular dysplasia characterized by the presence of multiple arteriovenous malformations and telangiectasias at the systemic level, with inheritance of autosomal dominant type of penetrance and variable expression. On the basis of the Curaçao criteria, a definitive diagnosis can be made, such as: spontaneous and recurrent epistaxis, family history of HHT, telangiectasias in characteristic locations (lips, oral mucosa, nose and finger-pulps) and the presence of arteriovenous malformations in viscera. The diagnosis will be definite if it meets three criteria, possible if only two are met and unlikely if fewer than two criteria are present. Therefore, it is established that the diagnosis is purely clinical[1].

This entity was first developed in 1864 by Dr Henry Gawen Sutton, who described it as a hemorrhagic disease "distinct from haemophilia" with a pathophysiology different from those known to explain deficiencies in blood coagulation. It was not until the twentieth century when doctors William Osler in 1901 and Frederick Parkes Weber in 1907 published the first series of cases. In 1909 the term "Hereditary Hemorrhagic Telangiectasia" was first used by Dr Frederick Hanes.

At the beginning of its study, it was considered a benign disease whose main symptoms were recurrent epistaxis and gastrointestinal bleeding, without a clear systemic repercussion. In the 40s, the range of symptoms was widened, since arteriovenous malformations were observed in the lung, brain and liver of some patients, which sometimes caused them death. Over the years, the study of the HHT was expanded thanks to the joint work of several professionals, until in the year 2000 the first scientific meeting of HHT was held on the island of Curaçao. This place was chosen because of its high prevalence of the disease, being 1: 1,330 inhabitants, the highest in the world. From this meeting a consensus document was produced and it defines the clinical criteria to diagnose the syndrome, appearing the Curaçao Criteria. The first consensus guidelines for the diagnosis and management of HHT were published in 2011.

With these requirements, it has been calculated that the worldwide prevalence is 1: 5,000-8,000 people, including within the rare or minority diseases. It is an underdiagnosed pathology due to its lack of knowledge, although it has an important morbimortality and a symptomatic preventive treatment. It should be noted that there are areas of the world where the frequency is higher, such as in the Netherlands Antilles, Funen Island in Denmark, the

Ain region in France, Vermont in the United States, Newcastle in England and the Canary Islands[2].

At present, five pathogenic mutations have been identified in different genes, which explain the disease. These genes modulate TGF-β activity, belonging to the signalling superfamily, in vascular cells, which regulate cell proliferation, differentiation, migration and extracellular matrix formation. The most important mutations for their frequency (> 80%) are in *ENG* located on chromosome 9, which is related to HHT1 and *ALK1* on chromosome 12 (also called *ACVRL1*) that is more associated with the HHT2 subtype. A small proportion of patients have a mutation in the *MADH4* gene as part of a syndrome of superposition of HHT juvenile polyposis.

Both *ENG* and *ALK1* mutations produce unstable proteins with a deficient biological activity, which causes a dysfunction of cell division and migration that limits angiogenesis. Due to this fact, an alteration of the cytoskeleton is caused, resulting a decrease in the cellular resistance of the vascular endothelium. The consequence is a premature lysis of the endothelial cells of the capillaries when they are subjected to an increase in pressure or trauma.

These cellular alterations are responsible for the formation of arteriovenous connections without the participation of the capillary bed. The most visible result is the appearance of multiple mucocutaneous telangiectasias that can occur in any region of the skin and mucous membranes, although they are more frequent in the oral cavity, nose, and conjunctiva and finger-pulp. Especially important are those located in the nose as they lead to epistaxis[3], the most frequent symptom in patients with Osler-Weber-Rendu Disease, up to 90%.

Other arteriovenous malformations (AVMs) are usually located in the lung, brain and liver. In the first case, it is estimated that up to 35% of patients can develop a right-left shunt, which leads to hypoxemia and hemothorax, which can cause serious systemic complications such as AMI or brain abscesses. That is why screening for these malformations should always be done, even if the patient is asymptomatic. It will be of great importance in people who want a pregnancy because during pregnancy these malformations increase in size, becoming more unstable and prone to bleeding.

Malformations at the brain level are less frequent, without reaching 15% of patients. They are usually silent, but they can cause headache, seizures, and both ischemic and haemorrhagic strokes. The screening is crucial and will even mark the prognosis of the disease.

The frequency of liver involvement varies considerably from one patient to another, so that early detection is not performed routinely. It is estimated that its frequency is around 32% of patients. The most frequent pathology is the presence of shunts between the hepatic artery and vein, which is usually asymptomatic. However, it can cause serious pathologies such as portal hypertension and damage to the bile duct.

Arteriovenous malformations in the digestive tract are usually present in elderly patients, what causes microcytic anaemia that can be severe. In young patients, it does not have a greater systemic repercussion than the general symptoms related, such as mucocutaneous pallor and asthenia. In elderly patients, it should be considered a risk factor for the decompensation of other pathologies such as heart failure. It is estimated that the prevalence is 45%.

As already mentioned, in the Canary Islands a high prevalence of this syndrome (1: 1,700) is calculated due to a probable Founder Effect. The genotype varies in comparison with other places. According to preliminary data available in the Clinical Genetics Unit (CGU) of the Insular Maternal and Child Hospital Complex (reference of genetic pathology in the province of Las Palmas) there are probably two frequent mutations in the *ALK1* gene: the variant 353_360dupAGCTGGCC (p. Leu121fsX), and the missense mutation c.1232G> A (p. Arg411Gln). The third prevalent variant is located in the *ENG* gene (c.523 + 1G> T) in which, in the seventh intron of the gene, a change of guanine to thymine occurs in the affections due to the disease. This project aims to identify the genotype of all patients, with sufficient clinical criteria, from Gran Canaria Island, which have been collected in the CGU so far, and associate it with a certain phenotype, observing inter-family and intra-family variability. This would greatly enrich the management of these patients, as well as a correct diagnosis of the disease. The ultimate objective is to perform a protocol that can be carried out in Primary Care where keys are given to recognize the disease and make its correct referral to the CGU and the Consultation of Rare Diseases. In this way, a secondary and tertiary prevention is carried out, pretending the symptomatic control of the patients, as well as recommending some lifestyle habits and especially, implanting an adequate preconceptional and postnatal genetic advice as well as the most indicated reproductive

counselling in each case. A fundamental issue in this process will be to provide knowledge about the disease and encourage interaction between the patient and the association of patients with HHT.

4. Objetives

4.1 General objectives:

4.1.1 Know the HHT subtype and the most prevalent genetic variants in patients living in Gran Canaria.

4.1.2 Identify if there is a correlation between the described genotypes with a specific clinic, thus describing a possible phenotypic variability both at the inter-family and intra-family level.

4.2 Specific objectives:

4.2.1 Update the database of affected patients, specifying data such as age at the onset of the symptomatology, first symptoms, carrying out the different screening and monitoring in Specialized Care.

4.2.2 Develop and implement a cost-effective direct genetic diagnostic method for the most prevalent genetic variants in Gran Canaria.

4.2.3 Encourage patients the importance and need to receive adequate genetic counselling in the CGU, especially prior to a pregnancy.

4.2.4 Act as a bridge between patients and the Canarian association of patients affected by the Osler-Weber-Rendu Disease.

5. Material and methods

To implement this project, we proceeded to update the database of patients (existing in the CGU) to which the genetic study for the diagnosis of Osler-Weber-Rendu Disease has been carried out. Patients have been selected and grouped by different families, following the inclusion criteria: to be carriers of one of the three mutations studied, to be affected by at least two generations of the family tree, or to meet the previously described Curaçao criteria.

Once the accurate genetic diagnosis was confirmed, patients were divided into three groups, according to the genotype described. We proceeded to review the Clinical Histories to complete the data necessary for the study, which were: age of symptom onset; first symptoms and concomitant diseases; follow-up or not in Specialized Care; realization of the screening of liver, lung and brain malformations and its outcome and follow-up by their Primary Care physician. In turn, the Gynecology Service reports were reviewed to know if patients had been correctly informed of the risk posed by this disease during pregnancy and the possibility of a preconceptional diagnosis. In order to objectively evaluate epistaxis (the most prevalent symptom in this disease), the Sadick scale was used (Table 1), which quantifies the frequency and quantity of epistaxis in three grades:

GRADE	**FREQUENCY**	**QUANTITY**
I	Less than once a week	Bleeding is controlled with a handkerchief
II	Several per week	Blood soaks handkerchief / Necessary to use several
III	Several per day	Bowl necessary or medical attention to control bleeding

Table 1. Sadick Scale

Finally, a total of 74 patients who had these data collected in their history were selected.

The genetic diagnosis began with the isolation of DNAg from peripheral blood of patients by using a classical method (salting out). The concentration and quality of it was evaluated by means of nanospectrophotometry (Nanodrop), repeating the extraction when the quality parameters did not meet the standards established in the CGU.

Three different diagnostic approaches were prepared for each of the variants to be investigated:

First of all, with respect to the variant c.1232G> A (p.Arg411Gln) in the *ALK1* gene, the self-designed PCR-RFLP methodology was used. After specific amplification of a fragment containing the nucleotide in question, enzymatic digestion with *MspI* was carried

out under stringent conditions. Finally, an agarose electrophoresis let to know the genotype of the patient and his state (heterozygous and homozygous).

Secondly, the variant 353_360dupAGCTGGCC (p.Leu121fsX) in the *ALK1* gene, was analysed using a simple PCR amplification looking for the highest possible sensitivity and specificity. After obtaining the most suitable conditions, the PCR products were separated in high-resolution agarose at a sufficiently high percentage to differentiate the duplication of the wild-type fragment.

Thirdly, the c.523 + 1G> T variant in the *ENG* gene required the design of an ASA approach (Allele Specific Amplification). By designing specific primers for the nucleotides G (healthy allele) and T (pathological allele), the most stringent PCR conditions were searched in order to differentiate both most certainly. It was necessary to use multiple attempts until finding the maximum specificity. The PCR products (*wt* and *mut*) for each patient are separated in agarose gel allowing an easy, fast and cheap way of diagnosis.

6. Results

In total, 74 people (50% women and 50% men) were included in this database, counting on the index cases and affected relatives that were subsequently studied, fulfilling the Curaçao criteria in all of them. The predominant symptom, regardless of variables such as sex, age and genetic mutation, was epistaxis, found in 80.5% of patients. Other bleeding cases reported by patients were: telangiectasias of oral mucosa, finger and facial pulp (7.7%) and rectum (2.5%).

In 9 patients (55.55 women and 44.44% men) the variant c.1232G> A (p.Arg411Gln) in the *ALK1* gene was found in heterozygosis. The methodological approach chosen managed to identify the mutation efficiently and reproducibly (Figure 1).

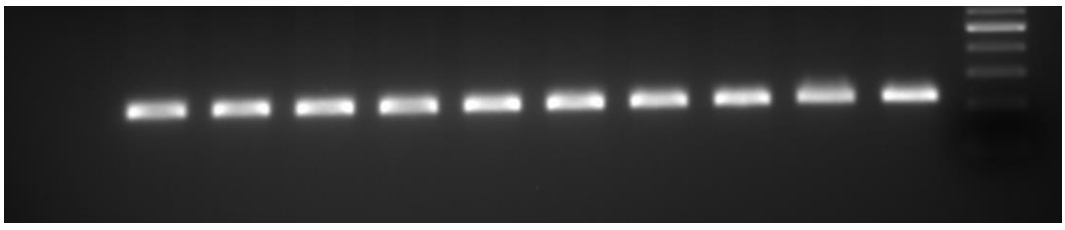

Figure 1A: Amplification by PCR.

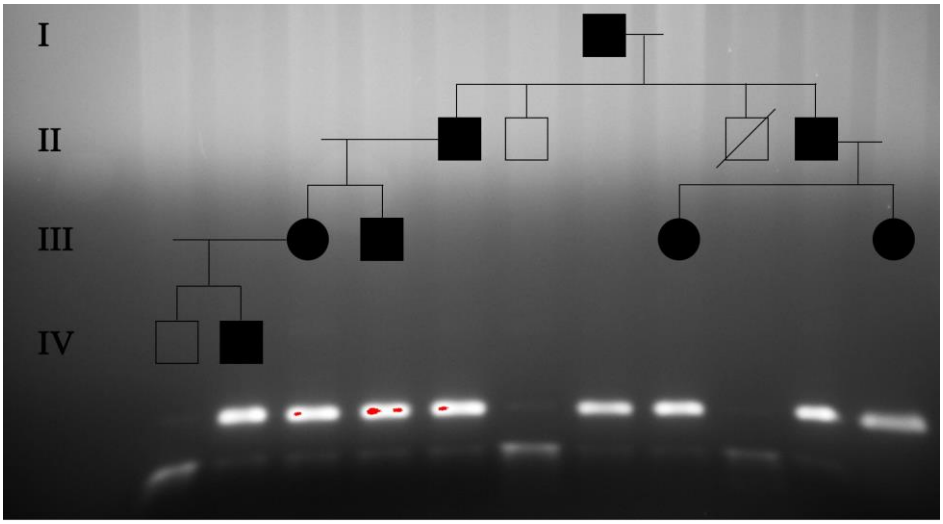

Figure 1B: PCR-RFLP representative in several members of a family.

Eight patients (87.5%) were symptomatic with daily and abundant epistaxis, being catalogued in the grade 3 Sadick scale. They presented several times a day and needed a container or medical attention for the magnitude of bleeding. All patients studied had epistaxis since childhood. 3 members of this family had undergone sclerotherapy in nasal malformations, getting epistaxis to become infrequent (once a month or less) that could be controlled with the use of a handkerchief, defined in the Sadick scale as grade 1. Despite the severity of epistaxis, all had been screened for AVM (its presence being indicators of severity) and only one of the patients had AVM at the liver level and another member of the family at the pulmonary level.

It has to be highlighted the case of a 51-year-old male affected, who had required several blood transfusions due to the severity of his symptoms. Apart from manifesting epistaxis, telangiectasias and oral mucosa bleeding, the patient is afflicted with Charcot-Marie-Tooth disease, a genetically based hereditary neurological disorder that causes both motor and sensory neuropathy. Despite several embolizations of the anterior nasal artery and even the use of sclerotherapy, epistaxis remains very difficult to control.

In 4 patients (50% women and 50% men) the variant c.523 + 1G> T in the *ENG* gene was identified in heterozygosis. The chosen methodological approach was able to identify the mutation, after multiple attempts, in an efficient and reproducible way (Figure 2).

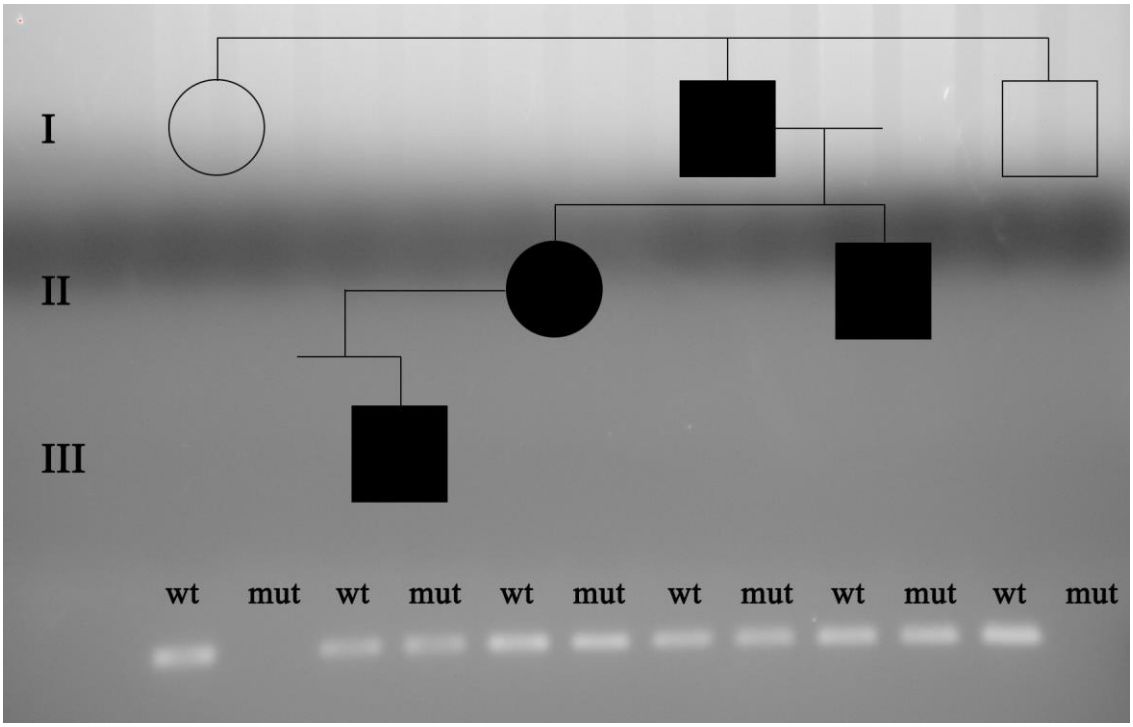

Figure 2: Representative ASA in several members of the same family.

50% of patients studied had grade 1 epistaxis according to the Sadick scale, while the remaining 50% remain asymptomatic at present. Regarding the study of arteriovenous malformations, 2 of the 4 members present malformations at the pulmonary and cerebral levels. One of these patients has not yet manifested any epistaxis symptoms or bleeding in other regions. However, we must emphasize the seriousness of their situation, being a carrier of pulmonary and cerebral AVMs. Although these are of small size, they can condition the patient's health in a serious and deadly way, being necessary a strict control of these by diverse services such as Internal Medicine, Neurology, Pulmonology and Interventional Vascular Radiology.

In 61 patients (distributed in different families) the variant 353_360dupAGCTGGCC (p.Leu121fsX) in the *ALK1* gene was identified in heterozygosis. The chosen methodology, though extremely simple, was able to identify the mutation effectively and reproducibly, after multiple attempts to fine-tune (Figure 3).

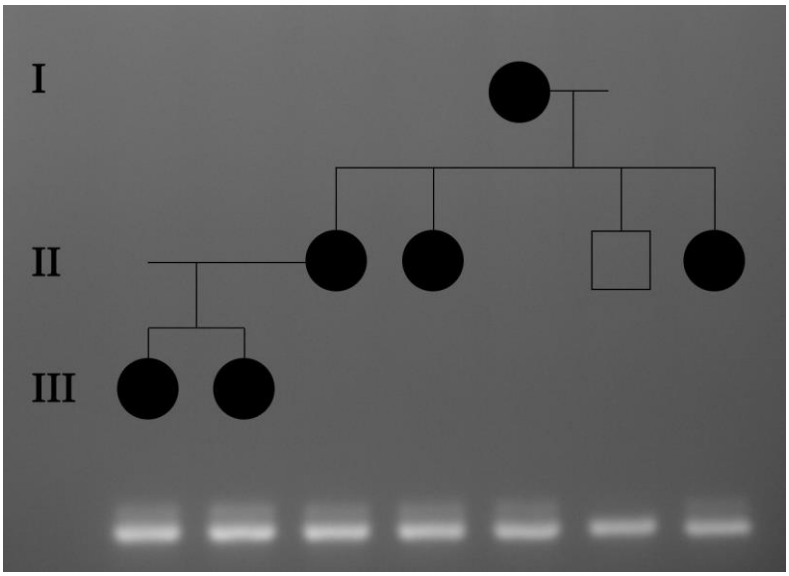

Figure 3. Specific PCR showing the duplication of AGCTGGCC.

The first family studied was composed of 17 members (59% women and 41% men), where 4 members remain totally asymptomatic. The rest of the family manifested grade 1 epistaxis according to the Sadick scale as the main symptom. The age of symptoms onset ranges between 20 and 30 years. No bleeding appears in other locations or concomitant diseases. As for the screening of AVM, only 2 patients, who are still in follow-up due to their small size, presented it at the pulmonary level.

The second family studied is composed of 10 members (50% women and 50% men), all of whom are symptomatic. Five patients present grade 1 epistaxis according to the Sadick scale, 4 of them present grade 2 epistaxis (bleeding occurs several times a week, soaking the tissue used to contain the haemorrhage) and only 1 manifests grade 3 epistaxis. Regarding the study of AVM, two of these patients have been diagnosed with hepatic AVM. In this family, it is worth noting the disparity at the age of onset of symptoms. In 9 of the patients, the debut occurred between 9 and 15 years, although one of them started with epistaxis at 34 years of age when taking an acne treatment whose active principle is isotretinoin.

The third family studied has a total of 13 members studied (46% women and 54% men). All patients manifest symptoms and the main one is grade 1 epistaxis according to the Sadick scale, being its onset between 10 and 25 years. Only one of them has presented rectorrhagia sporadically, two episodes collected in the Clinical History. Regarding the AVM screening, 3 of the patients studied decided not to do it. Of the remaining 10 members of the family, 5 had shown hepatic AVM in CT scan of abdomen without hepatic shunt. However, it

is worth mentioning the variability of concomitant diseases that are expressed in this family. Two members of the family have a personal history of psoriasis, another 2 members suffer from hypothyroidism (one of them also presents Gilbert Syndrome and Diabetes Mellitus type 1) and another patient is diagnosed with fibromyalgia. It should be noted that Mental Health is monitoring 4 patients for endogenous depression with poor response to treatment.

In the fourth family participating in the study, data from 3 affected patients (100% women) were collected. In this case, the first symptom was not epistaxis, but haemorrhages at the digestive level. One of the patients studied debuted the disease with hypovolemic shock due to massive hematemesis. The other two relatives had presented several episodes of rectal bleeding without a determining cause such as external or internal haemorrhoids, anal fissures, polyps, diverticula or an inflammatory bowel disease. In turn, the three subjects presented variable degrees of epistaxis, one of them being asymptomatic and the two family members with grade 2 epistaxis on the Sadick scale. Symptoms had begun in childhood in all subjects with epistaxis, aggravating with upper and lower gastrointestinal bleeding after 50 years. However, all had been negative for the screening of AVM at the pulmonary, hepatic and cerebral levels.

The fifth family participating in the study consisted of 6 members (66.6% women and 33.3% men); all of them are symptomatic. The degree of epistaxis ranged from grade 1 to 2 on the Sadick scale. The symptoms onset ranges from childhood to 35 years. In this case, in addition to the diagnosis of HHT, one of the patients has been diagnosed with Systemic Lupus Erythematosus. Regarding the AVM screening, only one patient came up positive for hepatic and pulmonary AVM, without presenting systemic repercussion.

Six members make up the sixth family included in the study (83.3% women and 16.66% men). The degree of epistaxis is very varied in this family, three members present grade 1, two members grade 2 and only one of them grade 3. The age of symptoms onset is later in this family, beginning in adolescence with 12 years to adulthood with 31 years. What is striking in the study of this family is the concomitance with other diseases: one member suffers from psoriasis, another one from hypothyroidism, two more are diagnosed with celiac disease and another has a personal history of primary hyperparathyroidism, gouty arthritis and depressive syndrome. Analysing the AVM screening, only one of the patients (diagnosed in addition to celiac disease) carries a hepatic and cerebral AVM, without current treatment because of its small size, but in close surveillance by the corresponding departments.

In the seventh family of the study, composed of 3 members (66.6% women and 33.3% men), all of them report grade 2 epistaxis as a cardinal symptom. The age of onset of these is restricted to the decade of 20 years, without any previous symptomatology in childhood. There is no record of concomitant diseases. Only one of the members agreed to the AVM study, being negative for the three locations studied.

In the eighth study family, 3 patients (100% women) were included. The first symptom returns to be grade 2 epistaxis according to the Sadick scale. The onset of symptoms in the four members of the family does not exceed 10 years. As for the concomitant diseases, there is no record of them. However, the numerous complications derived from recurrent haemorrhages characteristic of HHT are evident. One of the patients, aged 70, has suffered two strokes of haemorrhagic aetiology, without leaving neurological sequelae. However, it should be noted that there is no evidence of AVM at the cerebral level, but hepatic, in follow-up by the Digestive Service for hepatic shunt. In addition, in her history the transfusions made are reflected; 7 in the last year because in the control analysis there are figures of Haemoglobin between 6-7 gr/dl with accompanying clinical characteristic of anaemia such as mucocutaneous pallor, asthenia and bradypsychia.

7. Discussion

Currently, the great heterogeneity of the locus and allelicity of this disease is known. To this day, three genes, whose mutated alleles are responsible for producing this entity: *ENG, ALK1* and *MADH-4*, have been identified. Previously, in 2006 Letteboer TG *et al*.[4] already performed a correlation between the mutations in the first two genes in the Dutch population, finding differences in the symptoms presented as well as their severity according to the inherited mutation in heterozygosis. In this project we have proceeded to study the same genes in the most prevalent variants in the population of Gran Canaria.

The HHT type 1 (HHT1) is the result of the mutation of the *ENG* gene, located on chromosome 9, responsible for the manufacture of a protein called endoglin that takes part in the differentiation of arterial and venous embryos and angiogenesis in adults. In the mutation studied in our population an intronic change of a guanine to a thymine appears, generating the variant c.523 + 1G> T. This variant has been studied in multiple investigations due to its high prevalence, especially in countries of northern Europe and North America.

The HHT type 2 (HHT2) is produced by mutations in the *ALK1* gene located on chromosome 12, which encodes a type I specific endothelial receptor for TGF-β. One of the studied variants (c.1232G> A) generates an amino acid change from Arg to Gln at position 411 of the protein. It is one of the most frequent variants worldwide. The other mutation analysed in this same gene (353_360dupAGCTGGCC) supposes a duplication of 8 pairs of bases giving rise to a truncated protein. This variant is not previously described and is, moreover, the most prevalent of those studied in this study.

Mutations have been described in the *MADH 4* gene, located on chromosome 18, which presents a combined syndrome of HHT and hereditary juvenile polyposis, being a minority variant. Given the low prevalence of this genetic mutation in the population studied, no patients who fulfilled the established inclusion criteria were found.

Mutations in both *ALK1* and *ENG* genes act through a haploinsufficiency mechanism, with all patients known to be heterozygous for the mutation. This is enough so that the endothelial cell is not able to perform its functions normally, producing the disease accordingly.

The etiopathogenesis of this syndrome reveals the importance of a correct genetic diagnosis. The previously described procedures have shown to be effective in making an accurate diagnosis. Likewise, they are cost-effective procedures, with a low number of false positive and false negative results. For these reasons, they will become part of the genetic diagnosis of the CGU at the healthcare level of this disease, allowing an efficient and early diagnosis.

The results of this study corroborate that the Osler-Weber-Rendu Disease shows a great variability of symptoms and severity. Furthermore, it can be observed how the symptomatology varies between families, even carrying the same genetic mutation. Within each family, the symptomatology varies among first-degree relatives, thus determining the systemic repercussion of the patient's physical, mental and social. It draws attention to the concomitant diseases that have been found by analysing and studying the clinical histories of patients.

As for the most frequent symptomatology, after epistaxis, digestive haemorrhages are found, which can compromise the patient's physical integrity. Consequently, in 2017 Jackson Sb *et al.*[5] wrote guidelines to follow for the prevention, early detection and treatment of gastrointestinal bleeding in patients with HHT. Despite not being the most frequent clinical

associated with this syndrome, it is true that it can have serious complications that endanger the life of the patient. If the haemoglobin and haematocrit of a patient are low, and the anaemia is of disproportionate magnitude regarding the frequency and quantity of the epistaxis, the study should be completed with upper endoscopy. In addition, it is recommended to start the administration of iron supplements, either orally or intravenously, and endoscopic cauterization can be considered. As for hepatic AVMs, they can be treated with endoscopic cauterization; embolization is contraindicated due to the risk of hepatic necrosis and death.

Although in most cases epistaxis is the most common, striking, and sometimes the only symptom that manifests the disease, HHT is characterized by malformation in the creation of both arterial and venous blood vessels. By no means, the severity of this entity can be catalogued according to the Sadick scale for the assessment of epistaxis. For its correct diagnosis, it is necessary to perform CT at the thoracic level to rule out pulmonary AVMs, at the abdominal level to assess hepatic AVMs and at the cranial level to visualize cerebral AVMs. Thus, it is necessary and essential to explain properly to patients the pathophysiology of this disease and all risks that may involve. Currently, monitoring of patients in order to detect early AVMs is the best way to prevent bleeding at different levels that can lead the patient to suffer secondary comorbidities of a liver shunt, stroke or hemothorax resulting in death.

Of the 74 patients studied, 14 of them (18.9%) refused being screened for the different AVMs. Easey AJ et al.[6] conducted a retrospective cohort study in 2003 comparing the incidence of cerebral haemorrhage in patients with HHT and cerebral AVM with a group of non-affected patients, but with cerebral AVMs. It was shown that cerebral haemorrhages were 20 times more frequent in male HHT subjects under 45 years of age in the general population. It was also extracted that haemorrhages were six times more frequent in women younger than 45 years with HHT compared with women without HHT of the same age range. The big question that the study was trying to solve was: Is it recommended to perform MAV screening in asymptomatic patients, but with a genetic diagnosis? Given the data, it was deduced that due to the devastating effects of the haemorrhages (especially at the cerebral and pulmonary level) it was necessary to carry out an early intervention programme in asymptomatic HHT patients.

It is striking that, of the 74 patients included in the study, none of them was correctly informed about the risks posed by a pregnancy for maternal and fetal health, with the option

of preconceptional advice. There are multiple studies on maternal and fetal risks when the pregnant person is affected by HHT, but none of them concludes with the need for a preconceptional diagnosis that reduces fetal risk in the first instance and then, that affects the quality of life of the child throughout his life.

In general, the results derived from this project indicate the disparity and severity of symptoms in patients despite being carriers of the same mutation or being members of the same family (variable expressivity). Variability in the degree of epistaxis, presence of AVM in different locations, complications of haemorrhage, concomitant diseases and, to a lesser extent, the age of symptoms onset can be observed. All these observational data reflect the need for individual and comprehensive assessment in patients with HHT. That is why genetic diagnosis is fundamental in this disease, since the early detection of AVM can have an impact on the health of patients and their quality of life. This project is contributing to establish structured protocols that involve Primary and Specialized Care for the interconsultation of the CGU when patients are detected with sufficient HHT criteria. The accurate genetic diagnosis of the disease in the CGU will allow the establishment of appropriate genetic counselling, reproductive counselling, family study and referral, from the CGU to the consultation of Rare Diseases for an effective monitoring of the disease.

Currently, in the studied population, the most prevalent mutations are *ALK1* (c.353_360dupAGCTGGCC) in 82.3%; *ALK1* (c.1232G> A) by 12.1% and *ENG* (c.523 + 1G> T) by 5.6%.

Based on data obtained, certain patterns can be observed between the different mutations. The variant p.Leu121fsX presents a wide variety of symptoms, with different degrees of severity, without reaching a clear correlation. However, analysing the observed data, it is the variety that is most associated with other disorders, especially those of autoimmune etiopathogenesis such as DM1, psoriasis, hypothyroidism or LES.

Conducting a review of the literature published so far, there is no evidence that correlates Osler-Weber-Rendu disease with hypothyroidism, psoriasis, Gilbert's syndrome, type 1 diabetes mellitus, celiac disease, primary hyperparathyroidism, arthritis (rheumatoid or gouty) or other autoimmune diseases such as LES or scleroderma. However, the correlation between HHT and depressive syndrome has been exhaustively studied. Chaturvedi *et al.* [7] conducted a cross-sectional study in 2017 in patients affected by the Osler-Weber-Rendu disease where they completed two validated questionnaires: the PTSD checklist for DSM-5 (PCL-5) and Beck Depression Inventory-II (BDI-II). Of the 222 patients included in the study

with an average age of 54 years, with the same percentage of men and women, 185 completed both forms. The diagnosis of depression, generalized anxiety disorder and post-traumatic stress disorder was present in 81% of the patients studied. Zarrabeitia *et al.*[8] in 2017 determined that the 187 adult patients with HHT studied have greater problems than the general population in the five dimensions of the EuroQol 5D-3L (validated descriptive test to determine quality of life), particularly considering pain, discomfort, anxiety and depression. Values similar to those of populations with chronic diseases were obtained.

It is very remarkable that, in the exposed results, there is evidence of a patient, with this HHT2 variant, who began to manifest epistaxis after taking isotretinoin. When reviewing his prospect, it appears as a very rare adverse effect (1 in 10,000 subjects who take it may present it) redness, pain and swelling of the blood vessels, not to mention the possibility of bleeding. A review of the literature published up to the moment where the use of this drug is correlated with continued bleeding at the nasal or oral mucosa level has been made. It has been described that palpebral mucositis, blepharoconjunctivitis, corneal ulcers, nasal mucositis, oral mucositis, epistaxis and pseudotumor cerebri are associated with isotretinoin as adverse effects[9]. However, this would explain the epistaxis during the period of time when the drug acts or the period next to it, not epistaxis continued for years.

As for p.Arg411Gln variant, it is possible to appreciate a specific symptomatology, grade 3 epistaxis according to the Sadick scale. In this case, the severity of this guiding symptom is striking, with sclerotherapy being necessary in 3 of the 8 members of this family (37.5%) for the control of haemorrhage. Despite the severity of epistaxis, it does not correlate with the presence of AVM and the complications derived from them.

Taking into account particular cases, in relation to the patient affected by Charcot-Marie-Tooth disease and by HHT2 variant; no relationship has been found between this disorder and the Osler-Weber-Rendu disease that could explain an aggravation of this patient's symptoms. At the moment, this neurological disorder has been associated with arthropathies in various joints that would worsen the patient clinic. There is also no scientific evidence on the association between HHT and other neurological disorders such as Alzheimer's disease, cerebellar ataxia or Huntington's chorea. In the neurological sphere, a wide variety of complications have been described that can be caused by pulmonary arteriovenous fistulas or associated with vascular malformations of the CNS, thus producing a high morbidity and mortality that worsens the quality of life of the patient.[10]

Finally, in the c.523+G>T variant, epistaxis was classified as grade 1 according to the Sadick scale in 2 family members (50%) while the other 2 members included in the study (50%) do not show symptoms. However, it is worth noting the presence of pulmonary and cerebral AVMs in 50% of the subjects studied in this family. It can be affirmed that, in spite of the little relevance at a systemic level that this degree of epistaxis supposes, it is a serious clinical situation due to the possible complications that these malformations suppose, producing a high comorbidity as already explained above.

At the moment, there are no previous studies with the same working hypothesis to be able to compare the data. Therefore, it can be concluded that it is necessary to carry out more research work on the Osler-Weber-Rendu disease (especially on its correct genetic diagnosis) and scientific dissemination so that health professionals can recognise it, give appropriate information to patients according to their health status and derive them properly. In addition, it is essential to communicate those affected in a precise and detailed manner what this chronic disease entails, and the importance of its prevention, both at the preconceptional level and in childhood and adult life, without being relevant the presence of symptoms. As a result, the intensification of the interaction between specialists with Patient Association will be fundamental.

8. Conclusions

The most prevalent mutations in the population of Gran Canaria have been extracted, these being: *ALK1* c.353_360dupAGCTGGCC (p. Leu121fsX) in 82.3%; *ALK1* c.1232G> A (p. Arg411Gln) by 12.1% and *ENG* c.523 + 1G> T by 5.6%. These data submitted with the previously described diagnostic tests let establish an accurate and cost-effective diagnosis of this syndrome in the CGU.

Data show that there is a certain correlation between genotype and phenotype according to the genetic variant expressed in the patients. However, an important clinical heterogeneity has been found at inter-family level (still carrying the same mutation for the development of HHT) and at intra-family level. Analysing these observations, we can conclude in the importance of an individual assessment of the affected patient, being exhaustive in terms of symptomatology, severity of the same and concomitant diseases.

In conclusion, it is necessary to do more research work on this entity, at ethiopathogenic and diagnostic level; as well as its teaching diffusion among different health professionals. The ultimate aim is to provide the best and most accurate information to

affected patients so that they can understand whatever implies suffering from this disease and its correct follow-up to avoid future adverse events.

9. Bibliographical references

1. Hunter BN *et al.* An evaluation of the severity and progression of epistaxis in hereditary hemorrhagic telangiectasia (HHT) 1 vs. HHT 2. Laryngoscope. 2016;126(4): 786–790.
2. Cabrera DR. González MV. Chocano BT. Fernández CA. Puente RZ. Telangiectasia hemorrágica hereditaria: Enfermedad de Rendu-Osler-Weber. FMC. 2016;23(8): 446-58. Spanish.
3. McDonald J, Wooderchak-Donahue W, VanSant C, Whitehead K, Stevenson D, Bayrak-Toydemir P. Hereditary hemorrhagic telangiectasia: genetics and molecular diagnostics in a new era. Front. Genet. 2015; 6(1): 1-8.
4. Letteboer TG *et al.* Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia. J Med Genet. 2006;43(4):371-377.
5. Jackson SB *et al.* Gastrointestinal Manifestations of Hereditary Hemorrhagic Telangiectasia (HHT): A Systematic Review of the Literature. Dig Dis Sci. 2017; 62(10):2623-2630.
6. Easey AJ *et al.* Should asymptomatic patients with hereditary haemorrhagic telangiectasia (HHT) be screened for cerebral vascular malformations? Data from 22 061 years of HHT patient life. J Neurol Neurosurg Psychiatry. 2003;74 (6):743-748.
7. Charurvedi S *et al.* Depression and post-traumatic stress disorder in individuals with hereditary hemorrhagic telangiectasia: A cross-sectional survey. Thromb Res. 2017; 153: 14-18.
8. Zarrabietia R *et al.* Quality of life in patients with hereditary haemorrhagic telangiectasia (HHT) Health Qual Life Outcomes. 2017;15 (1) 19.
9. Gorpelioglu C, Ozol D, Sarifakioglu E. Influence of isotretinoin on nasal mucociliary clearance and lung function in patients with acne vulgaris. Int J Dermatol. 2009;49 (1): 87-90.
10. Donaldson J, McKeever T, Hall I, Hubbard R, Fogarty A. Complications and mortality in hereditary hemorrhagic telangiectasia. Neurology. 2015; 84(18):1886-1893.