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ASSESSMENT OF THE CONTRIBUTION OF GENETIC FACTORS IN ACUTE DIVERTICULITIS

Dissertation

Dissertation for the conclusion of MSc in Clinical Laboratory Genetics, oriented by MA Francisco Laranjeira, co-oriented by Professor Dra Joana Barbosa and presented to Faculty of Medicine of University of Coimbra.

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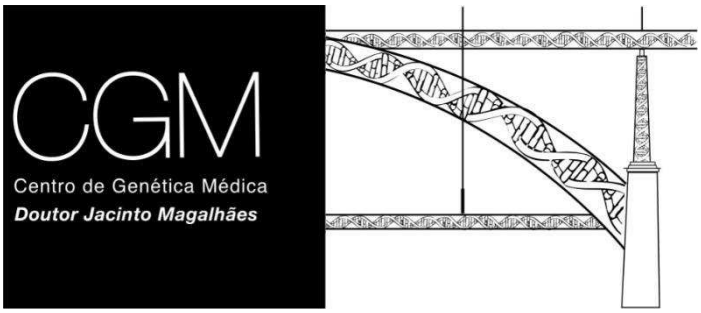

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Abbreviations Index

%	per cent
µL	microliter
A	adenine
ACD	acute complicated diverticulitis
ACDg	Acute Complicated Diverticulitis Group
AD	acute diverticulitis
AUD	acute uncomplicated diverticulitis
AUDg	Acute Uncomplicated Diverticulitis Group
C	cytosine
CRP	C-reactive protein
CTRL	Populational Control Group
DNA	deoxyribonucleic acid
dsDNA	double strand DNA
ENS	enteric nervous system
et al.	*et alia*
EUR	European
F	female
G	guanine
gDNA	genomic DNA
ICC	interstitial Cajal cells
M	male
MHC	Modified Hinchey Classification
mL	millilitre
MP	myenteric plexus
Na+	calcium
NALCN	Na+-leak channel
NS	nervous system
PCR	polymerase chain reaction
pmol	picomole
SCFAs	short-chain fatty acids
sd	standard deviation

SNP	single nucleotide polymorphism
SNV	single nucleotide variant
ssDNA	single strand DNA
T	thymine
USA	United States of America
V	volts
vs	versus
WT	wild type

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Abstract

Acute diverticulitis is a gastrointestinal disorder with various risk factors and organ systems involved on its etiology, followed by a wide spectrum of symptomatology. Recent studies suggest that genetic factors may play a significant role on the development, severity, recurrence, and selection for elective surgery in acute diverticulitis. Here, the results come from genotyping of risk associated variants to acute diverticulitis, though Capillary Sanger Sequencing. Several associations were identified, with the *FAM155A* gene being the one with most potential to serve as a tool for follow-up on patients. The other studied gene variants still have potential, but further analysis with bigger sampling is needed.

Key-words: Acute Diverticulitis, Capillary Sanger Sequencing, Genotyping, Association Study

1. Introduction

Diverticulitis is a gastrointestinal multifactorial disorder with higher incidence in individuals over 70 years old - about 50% - but can also affect younger people, where the phenotype is usually more severe (Coble *et al*. 2017). There is a higher prevalence of this condition in industrialized countries when compared with less developed ones, where is rarely reported. In the occidental regions of the globe, diverticulitis affects mainly the sigmoid colon, but in oriental regions is the ascending colon that is mostly affected (Violi *et al.* 2018). Data shows that there has been an increase of cases per year worldwide, in various age groups because of the overall constant lifestyle changes and industrialization (Tursi 2019).

The etiology of diverticulitis is the macroscopic peridiverticular inflammation of diverticula - herniations of the colonic mucosa and submucosa, resulting from age - followed by acute or chronic symptomatology. Possible complications go from abscesses, intestinal obstruction, fistulas, peritonitis, and haemorrhages, which can be deadly if not treated (Camilleri *et al.* 2020; Tan *et al.* 2016; Violi *et al.* 2018).

Aim

This project has the goal of identifying genetic variants, previously referenced in the literature, on *LAMB4*, *TNFSF15*, *ARHGAP15*, *FAM155A* and *COLQ* genes, with statistical significance for the development of acute forms of diverticulitis. Additionally, the correlation between genotype-phenotype will be reviewed for these variants with the recurrence of episodes and probability for surgical intervention in acute sigmoid diverticulitis.

2. Acute Diverticulitis

Acute diverticulitis (AD) is a complication of the diverticular disease, characterized by severe inflammation in a restricted portion of the colon, and as a result, there's a wide clinical spectrum of symptoms. These reside mainly in acute abdominal pain, tenderness in the inferior left or right quadrant of the intestine, fever and bowel movement alterations. Over time, symptoms such as fistulas and stenosis become chronic manifestations of the condition (Camilleri *et al.* 2020; Humes and Spiller 2016; Reichert and Lammert 2015).

This pathology can subcategorize into two large groups that are established on the Modified Hinchey Classification (MHC) (Table 1): uncomplicated and complicated diverticulitis. The acute uncomplicated diverticulitis (AUD) form is characterized by abdominal pain and persistent fever that can be managed through passive treatments such as antibiotics 0 and Ia on the MHC (Table 1).

On the other hand, acute complicated diverticulitis (ACD) is a more severe form of the condition, where life threatening complications might emerge, which requires a more invasive and aggressive approach of treatment. All the levels of severity of possible complications are established on the MHC (Table 1), from Ib to IV, where IV is the most severe of the scale (Ceresoli *et al*. 2018; Köckerling 2015).

Table 1. Modified Hinchey Classification for Acute Diverticulitis (Wasvary *et al*. 1999).

Stage	Description
0	Mild Clinical Diverticulitis
Ia	Confined pericolic inflammation or phlegmon
Ib	Confined pericolic abscess
II	Pelvic, distant intra-abdominal or retroperitoneal abscess
III	Generalized purulent peritonitis
IV	Generalized fecal peritonitis

2.1. Pathophysiology of Acute Diverticulitis

Through the years, several studies have been conducted with the goal of determining the etiology and pathogenicity behind AD, but most of them remain just theories. Since AD is a multifactorial disorder, there are many systems involved in the triggering process, such as the nervous system, microbiome, immune system, and genetic factors (Camilleri *et al*. 2020). The various factors involving AD can be found illustrated in Figure 1.

There are two theories for the starting mechanism of AD, the traumatic event, and the ischemic event. At this time, the traumatic event theory is the most accepted by the scientific community, being based on a traumatic damage to the diverticula, as an onset mechanism. The damage might be caused by hard faecal matter clusters that cause harm on the diverticular mucosa as it goes through the intestine. The resulting injuries predispose a higher risk for opportunist infections that result in an inflammatory response in order to control the bacterial proliferation (Piscopo and Ellul 2020).

In contrast, the ischemic event theory is based on the intense and abnormally prolonged neuromuscular contractile impulses in response to normal stimuli. These impulses of idiopathic etiology compress the blood vessels of the diverticular wall, resulting in ischemia on the mucosa, where posteriorly emerge micro-perforations (Piscopo and Ellul 2020). Although, Strate and Morris (2019) suggest that the ischemia is a result of the traumatic event and not the main cause for the development of AD.

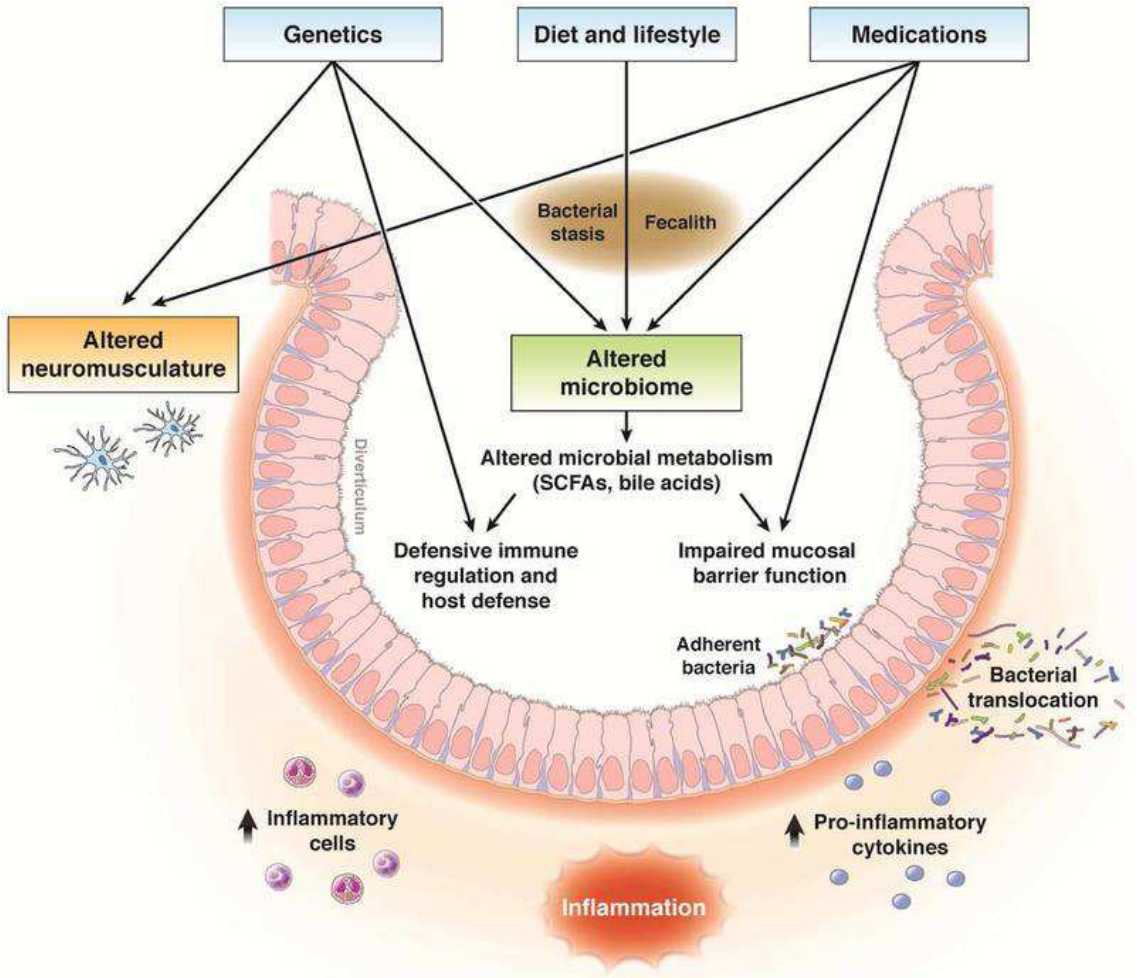

Figure 1. Proposed pathophysiological mechanisms for the development of acute diverticulitis. *Strate and Morris (2019).*

2.1.1. Enteric Nervous System

It is known that the gastrointestinal tract is closely connected with the nervous system (NS), and evidence for this fact is the existence of a NS that acts independently from the rest of the autonomic nervous system, the enteric nervous system (ENS). The ENS is formed by the myenteric and submucosal plexus and the most relevant for AD is the myenteric plexus (MP). The MP resides on the colon and is mainly constituted by afferent neurons that support the intestinal motility process. Another major main component of the MP is the interstitial Cajal cells (ICC) that work as an intestinal pacemaker, activating fazed contractions on the smooth intestinal muscle (Cochet-Bissuel *et al*. 2014; Rühl 2009).

Studies point to a reduction on all subtypes of ICC, along with morphologic changes of glial cells in patients with AD. The glial cells, along with the ICC, belong to a group of non-nervous cells whose ENS interacts, which guarantees the maintenance of the integrity of the intestinal epithelial barrier and colonic motility regulation (Rühl 2009; Tursi 2019). It's important to notice that during the inflammatory process, the ICC are the first affected cells, with direct repercussions on the gastrointestinal functioning (Rühl 2009). Overall, this data indicates a possible correlation between the deficient functioning of the ENS and a higher predisposition for the development of AD.

2.1.2. Microbiome

The combination of worldwide studies show that the prevalence of AD has a direct correlation with the composition of the intestinal microbiome, being affected by diet and lifestyle. It has been described that the occidental diet decreases the intestinal microbiota diversity, which then alters the functions and homeostasis of this microsystem. On the other hand, rich fiber diets have showed to increase the biodiversity on the intestine (Camilleri *et al*. 2020; Strate and Morris 2019).

Fiber is a complex carbohydrate that serves has a power source for microorganisms, and after metabolized, short-chain fatty acids (SCFAs) are obtained (Strate and Morris 2019). The metabolization of fiber into SCFAs is an important process for mucous production, a key role for the protection of the intestinal wall, maintenance of cellular proliferation and immunological homeostasis.

Analysis of the microbe population in AD patients showed that there was a reduction on the microbial population of *Clostridiales* bacteria- SCFAs producers- but there was an increase on protein degrading bacteria's, the *Proteobacteria* (Strate and Morris 2019; Tursi 2019). Based on this information, there is a possible positive correlation between the fluctuation of bacterial populations and the integrity and health of the intestinal mucosa, leaving it more susceptible to damage (Ceresoli *et al*. 2018; Raimondi *et al*. 2021). Although this data goes according to other studies of inflammatory intestinal diseases, these still need statistical significance to determine the actual influence on the onset and prognosis for AD.

2.1.3. Immune System

Another system that is equally important for the development of AD is the immune system. Although the immune response is mainly due to ongoing infection on the colon, some studies surrounding AD suspect that there might exist some genetic factors that alter the proper regulation of the immune system (Connelly *et al*. 2014; Nasef and Mehta 2020).

Normally, the maintenance of homeostasis is responsibility of the innate immune system, which is the first activated pathway and results in inflammation. The innate immune system has the main function of the distinction of the pathogens and activation of phagocytic cells, such as macrophages. On the other hand, the adaptive immune response has the function to give a proper response to the threat, being mediated by T and B cells. As expected, in AD there's an increase of activity of macrophages on the colon that leads to an increase of inflammatory response (Tursi 2019).

Likewise, chronic immunosuppression is a risk factor for the development of AD because there isn't an effective inflammatory response that then leads to severe complications, such as abscesses and perforation (Camilleri *et al*. 2020). Although most of the inflammation is a response to the bacterial proliferation, it's correct to assume that an underlying malfunction of the immune system is also a contributor for the damages that might emerge from severe inflammation (Nasef and Mehta 2020; Tursi 2019). Nonetheless, this fact still needs more statistical support.

2.2. Risk Factors

The multifactorial nature of AD determines that there are several environmental factors that influence the onset and development of the condition, such as age, gender, diet, drug intake, hormonal and genetic factors (Sigurdsson *et al*. 2017).

2.2.1. Diet

It has been proven that diet has a major influence on prevalence/incidence of AD, so much that some studies suggest that changes on the Western diet, might contribute to a lower risk of developing the disorder. Theoretically, this would happen by actively increasing microbial diversity populations that could even help prevent progression of the condition into ACD.

Some of the dietary adjustments consist in the increase of fiber intake, nuts, corn and vitamin D, as well as decrease of red meat intake (Reichert and Lammert 2015). At the same time, obesity due to unhealthy diets, aggravates the risk for complications associated with AD, and a higher risk for the development of the condition on obese women (Camilleri *et al*. 2020; Reichert and Lammert 2015).

2.2.2. Drugs

It has been identified that regular intake of drugs such as nonsteroidal anti-inflammatory drugs, corticosteroids, and opium's are promotors for the development of AUD and worst prognosis of ACD. Studies speculate that these medications might damage or interfere directly with the mucous barrier of the intestine, making it more permeable and susceptible to infections (Ceresoli *et al*. 2018; Collins and Winter 2015). On the other hand, calcium antagonists have showed to provide protection against complications (Camilleri *et al*. 2020; Humes and Spiller 2016).

2.2.3. Physical Activity, Smoking and Alcohol Consumption

Nowadays, physical inactivity is considered a risk factor, but it's not really understood if regular physical activity might prevent AD, although it has been related to the decrease of colonic pressure and intestinal transit (Reichert and Lammert 2015). Meanwhile, there's a strong correlation between smokers, alcohol drinkers and the incidence of AD, with a bigger susceptibility for complications, especially for female smokers (Ceresoli *et al*. 2018; Collins and Winter 2015).

2.2.4. Hormonal Factors

Camilleri *et al*. (2020) refers a study that identified a possible negative correlation between steroid ovarian hormones and the prevalence of AD in women. This study points out that there are less cases of the condition on pre-menopause women in comparison to men with the same age. These hormones act on collagen and elastin, which are molecules that constitute connective tissue, including the one on the intestinal tract.

In post-menopause, the quantity of these molecules declines and the number of cases of AD increases significantly. This study gives a possible explanation for the fact that men have a higher probability of developing the condition before 50 years old, than women (Collins and Winter 2015).

2.2.5. Genetic Factors

It's estimated that the genetic contribution for AD is around 40-50%, however its etiology is mainly unknown (Sigurdsson *et al*. 2017). There are several genetic disorders associated with higher probability of developing AD, and this is due to their etiology that affects the conjunctive tissue. Some of these are Ehlers-Danlos Type IV, Coffin-Lowry, Marfan and Williams-Beuren (Reichert and Lammert 2015).

Likewise, several studies point to some variants on the *LAMB4* and *TNFSF15* genes with high statistical significance for the development of AD. There are also other genes with some correlation with AD, the *ARHGAP15*, *FAM155A* and *COLQ* genes that were also included in this study.

LAMB4 Gene

The *LAMB4* gene (7q31.1) encodes a molecule called laminin subunit β4 (NC_000007.14), which belongs to the laminin family that constitutes the extracellular matrix. This protein family is mainly expressed on the colon and has functions on cellular adhesion and differentiation. More specifically, the laminin subunit β4 plays a role on the development on the ENS. All of these activities allow the maintenance of the intestinal barrier integrity and motility (Coble *et al*. 2017; Strate and Morris 2019; Tursi 2019).

Until the present day, several variants have been described in patients with AD, with the addition of a reported decrease of protein levels of laminin subunit β4 on the MP, on colonic wall biopsies. Although this has also been observed in other gastrointestinal disorders, it's still relevant to study *LAMB4* gene SNVs in order to identify some specificity for AD on the Portuguese population (Coble *et al*. 2017). The described SNVs in literature are shown on Figure 2 and Table 2.

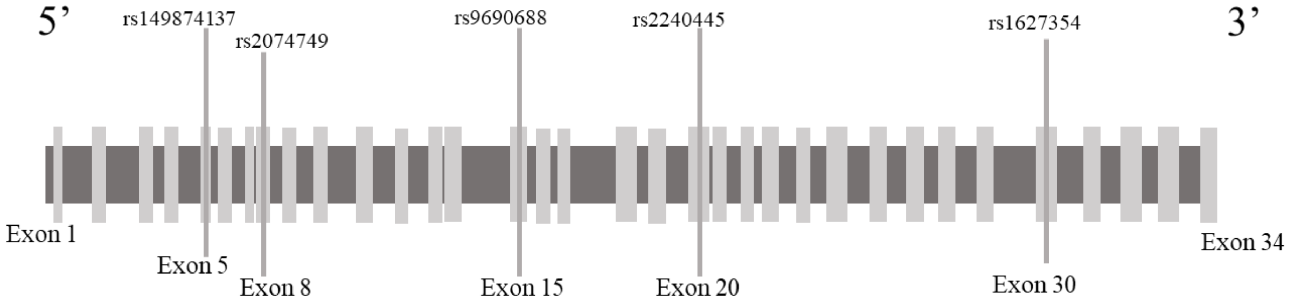

Figure 2. *LAMB4* gene variants. Adapted from Connely *et al.* 2014.

Table 2. Studied variants of the *LAMB4* gene.

Variants	WT Alleles	"Disease related" alleles	Classification	Frequency (EUR)	Clinical Significance
rs149874137	C	G	Exonic/Missense	C:99% G:1%	Benign/Likely Benign
rs2074749	G	A	Exonic/Missense	G:100%	Benign
rs9690688	C	A	Exonic/Missense	C:92% A:8%	Benign
rs2240445	T	C	Exonic/Missense	T:99% C:1%	Benign
rs1627354	G	A	Exonic/Missense	G:92% A:8%	Benign

TNFSF15 Gene

The *TNFSF15* gene (9q32) encodes the TL1A protein (NC_000009.12), also known as the vascular endothelial growth inhibitor (VEGI)-251. This is a transmembrane type II protein, belonging to the superfamily of TNF ligands member 15, a tumour necrosis family. The TL1A protein is expressed on the endothelial cells, macrophages, and gut lamina propria lymphocytes together with the main function of immunoregulation and anti-angiogenesis (Connelly *et al*. 2014; Desplat-Jégo *et al*. 2014; Tursi and Elisei 2019).

It has been described on these SNPs that upon the presence of pro-inflammatory cytokines, there is an upregulation of the *TNFSF15* gene, stimulating the proliferation of T cells. Also, downregulation of this gene compromises the normal functions of this protein, being possibly related with AD (Connelly *et al*. 2014). The *TNFSF15* gene as also been associated to other inflammatory intestinal disorders and autoimmune disorders, suggesting that a similar haplotype of these variants might specify for AD (Desplat-Jégo *et al*. 2014; Kadiyska *et al*. 2018; Reichert and Lammert 2015). The variants of this gene that have been associated to AD in other populations are displayed on Table 3 and Figure 3.

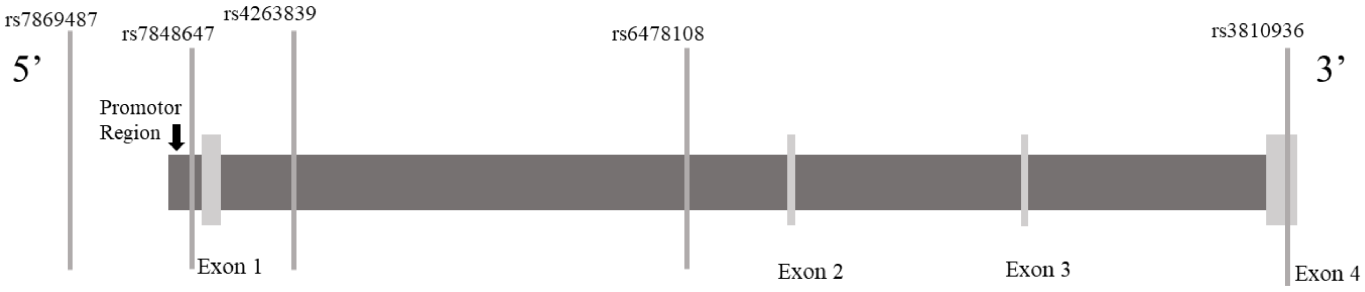

Figure 3. *TNFSF15* gene variants. Adapted from Connely *et al.* 2014.

Table 3. Studied Variants of the *TNFSF15* Gene.

Variants	WT Alleles	"Disease related" alleles	Classification	Frequency (EUR)	Clinical Significance
rs7869487	A	G	Intergenic	A:69% G:31%	Likely Benign
rs7848647	G/A	G	Intergenic	G:67% A:33%	Benign
rs4263839	C	T	Intronic	C:68% T:32%	Possible splice site alteration
rs6478108	T/C	T	Intronic	T:66% C:34%	Possible splice site alteration
rs3810936	G	A	Exonic/Synonymous	G:67% A:33%	Benign

ARHGAP15 Gene

The *ARHGAP15* gene (2q22.2-q22.3) encodes the Rho-GTPase-activating protein 15 (NC_000002.12) that belongs to the Rac family, specifically from the GTPases activating proteins (GAP). These proteins are essential to cell proliferation, apoptosis, motility, and ligation. The GTPase 15 protein has also phagocytic and inflammatory functions, through the regulation of selective neutrophils (Nasef and Mehta 2020; Strate and Morris 2019).

The normal expression of the *ARHGAP15* gene results in the activation of the Rac, which then interferes with the cytoskeleton actin and cell morphogenesis. Upon a case of upregulation of this gene, there is an increase of actin fiber stress and higher cellular contractibility (Sigurdsson *et al*. 2017). Considering this fact, and present studies with high statistical significance for AD, it's relevant to determine if the same correlation is identified on the Portuguese population. The most significant SNP identified on the literature for AD is exhibited at Table 4.

Table 4. Studied Variant of the *ARHGAP15* gene.

Variant	WT Alleles	"Disease related" allele	Classification	Frequency (EUR)	Clinical Significance
rs4662344	C	T	Intronic	C:82% T:18%	Possible splice site alteration

FAM155A Gene

The *FAM155A* gene (13q33.3), also known as *NALF1* or *NLF-1*, encodes the Na+-leak channel (NALCN) auxiliary factor 1 A (NC_000013.11) cientists think that this protein works as a chaperon that facilitates the formation and normal functioning of the NALCN channel. Its transcription occurs mainly on the brain, serving the purpose of helping the neuron's basal excitability and modulation through this channel (Cochet-Bissuel *et al*. 2014).

Studies indicate that the NALCN channel influences the regulation of the intestinal pacemaker activity on the ICCs, which makes it a study interest for AD, because variants in the *FAM155A* gene might impair its normal functioning. However, according to Cochet-Bissuel *et al*. (2014), the NALCN channel isn't required for basal activity of the ICCs. Despite this incoherence, there is a statistical significance for AD with variant represented on Table 5.

Table 5. Studied variant of the *FAM155A* gene.

Variant	WT alleles	"Disease related" alleles	Classification	Frequency (EUR)	Clinical Significa
rs67153654	T	A	Intronic	T:80% A:20%	Distant from splicing sites

COLQ Gene

The *COLQ* gene (3q25.1) (NC_000003.12) codes the collagen like tail subunit of the asymmetric acetylcholinesterase protein (ColQ). This protein establishes a connection with acetylcholinesterase (AChE), which allows the union to the basal lamina of the neuromuscular junctions (Nasef and Mehta 2020; Sigurdsson *et al*. 2017).

Camilleri *et al*. (2020) has demonstrated that there's downregulation of this gene with homozygous mutations - or compost heterozygous mutations - which result in the decrease of levels of AChE. Consequently, there is a prolonged signalling between the nerve and the muscle, resulting on muscle weakening (Sigurdsson *et al*. 2017). Nonetheless, these studies point out that this gene can take part on the development of AD, and the most significant variant for the *COLQ* gene for this disorder is on Table 6.

Table 6. Studied variant of the *COLQ* gene.

Variant	WT Allele	"Disease related" allele	Classification	Frequency (EUR)	Clinical Significance
rs7609897	C	A	Intronic	C:76% A:24%	Possible splice site alteration

3. Diagnosis of Acute Diverticulitis

According to the American Society of Colon and Rectal Surgeons and the European Society of Coloproctology, the diagnostic guidelines of AD, revolve around individualized recognition of the clinical history of the patient, symptomatology, and presence of inflammatory biomarkers (Sartelli *et al*. 2020).

Some of the biochemical inflammatory markers are the levels of C-reactive protein (CRP) that are good predictors of severity of the inflammation (Bolkenstein *et al*. 2017; Sartelli *et al*. 2020). Likewise, faecal calprotectin (FC) is an inflammatory colonic mucosal marker quantified on stool that allegedly, can distinguish AUD from other gastrointestinal inflammatory diseases (Tursi and Elisei 2019; Tursi *et al*. 2009). On the other hand, Jeger *et al*. (2018) present in their study the possibility of procalcitonin being used to distinguish AUD from ACD. Procalcitonin is a biomarker for bacterial infections and is expected to aid with the decision on whenever antibiotic treatment is really required.

Additional testing such as imaging exams are also essential to guarantee the authenticity of the diagnosis. Computed tomography scan is one of the exams that allows to determine with great precision the severity of the disease (Collins and Winter 2015). Nevertheless, a large range of testing and physical examination of the patients, increases the precision of the diagnosis and consequently provide a more efficient course of treatment (Bolkenstein *et al*. 2017; Sartelli *et al*. 2020; Strate and Morris 2019; Tan *et al*. 2016).

4. Therapeutic Guidelines for Acute Diverticulitis

The standard treatment for AD after episodes revolves around the implementation of a strict regime of dietary changes and antibiotics. The dietary changes consist of increase of fiber intake, low fat and white meats, or even liquid diets, although there isn't very strong evidence that this practice brings significant benefits for the patient. At the same time, the administration of antibiotics is also a controversial topic for its efficiency and safety on the various MHCs (Bolkenstein *et al*. 2017; Strate and Morris 2019). Yet, data shows that patients with AUD are the ones that benefit the most with this regime of treatment, despite other studies showing otherwise (Ceresoli *et al*. 2018; Köckerling 2015).

In the case of ACD, it's required more invasive procedures such as percutaneous drainage, laparoscopic lavage, surgery and/or pain control (Ceresoli *et al*. 2018). The percutaneous drainage and laparoscopic lavage are techniques that allow the removal of big dimension abscesses, for a better management of the ongoing infection and postpone possible surgery (Collins and Winter 2015; Köckerling 2015).

Although initial conservative management for ACD has become a standard, with percutaneous drainage and/or antibiotics, the long-term management strategy remains unclear. As the necessity of elective resection is a matter of ongoing debate, so the guidelines advise to consider elective surgery in an individualized approach (Strate and Morris 2019; Tursi and Elisei 2019). The indication is based on the risk factors for each patient, such as age, other comorbidities, and severity (Köckerling 2015; Piscopo and Ellul 2020; Strate and Morris 2019).

Despite all the vast guidelines, there isn't a defined course of action to every stage of AD, reinforcing the importance for the study of the genetic influence on AD. This will allow, in addition to other existing variables, to develop a more accurate guideline of treatment.

5. Recurrence of Acute Diverticulitis

Nowadays, the most reliable predictor model for recurrence of AD consists of the determination of endoscopic and histological inflammation levels, on patients follow up appointments (Tursi and Elisei 2019). However, this isn't a regular practice amongst clinicians and with no guarantees of its precision on the prediction of another episode.

About $^{2}/_{3}$ of treated patients suffer from recurrence of AD, with unknown predictive risk factors. Data suggests that there is a 90% chance of recurrence 5 years after the first episode and increases considerably with each episode (Tursi *et al*. 2014). Meanwhile, surgery is one of the used methods to prevent the recurrence of the condition (Humes and Spiller 2016; Strate and Morris 2019).

According to Buchs *et al*. (2013), CRP levels are highly related with recurrence in AUD, where levels above 240 mg/L, on the first AUD episode, have a close link for recurrence after 6 months. Another biomarker suitable for the prediction of recurrence is the FC, where Tursi *et al*. (2014) identified that there was an increased FC on 35% of patients after an episode of AUD. In general, inflammatory biomarkers seem to be good predictors of recurrence, since high inflammatory levels on the colon are indicative of a higher probability of another episode, in comparison to lower levels of inflammation.

6. Materials and Methods

6.1. Study Population and Control Criteria

For this study, it was stablished 2 main groups, the Populational Control Group (CTRL) and the Study Groups (AUDg and ACDg). The samples from the CTRL have no known history of AD, serving for the present study for determination of the population frequency of these variants. On the other hand, the study group samples are from individuals with a confirmed clinical diagnosis of AD for the corresponding subtypes of the disorder, AUD and ACD. It's in this group that there's the intention to determine some correlation between the studied variants and the parameters of severity and prognosis.

In order to advance with the study, it was established inclusion criteria for the admission, such as being ≥ 18 years old, not having personal or familiar history of other inflammatory bowel disorders and inherited connective tissue disorders. Finally, the patient must be able to sign the informed consent form.

6.2. Study Variables

The relevant variables for this study are age, gender, disease severity, recurrence, number of episodes and elective surgery associated with the pathology. The total sample size is 89 participants, for which there is the CTRL group (n=47; 52,8%), the AUDg (n=19; 21,3%) and the ACDg (n=23; 25,8%). The frequencies of each variable in study can be found at Table 7 and on Figure 4 it's represented the distribution of AD in each age group.

Table 7. Frequency of the variables in study.

	CTRL (n=47)		**AUDg (n=19)**		**ACDg (n=23)**	
			Min/Max	M(sd)	Min/Max	M(sd)
Age	—	—	32-85	54,4 (13,1)	35-77	57,0 (11,4)
Nº Episodes	—	—	1-8	2,2 (1,6)	1-4	1,6 (0,7)
	N	%	N	%	N	%
Gender						
Male	22	46,8	8	42,1	10	43,5
Female	25	53,2	11	57,9	13	56,5
Modified Hinchey Classification						
0	—	—	5	26,3	—	—
Ia	—	—	14	73,7		
Ib	—	—	—	—	11	47,8
II	—	—	—	—	8	34,8
III	—	—	—	—	4	17,4
Recurrence						
Yes	—	—	11	57,9	12	52,2
No	—	—	8	42,1	11	47,8
Surgery						
Yes	—	—	1	5,3	10	43,5
No	—	—	18	94,7	13	56,5

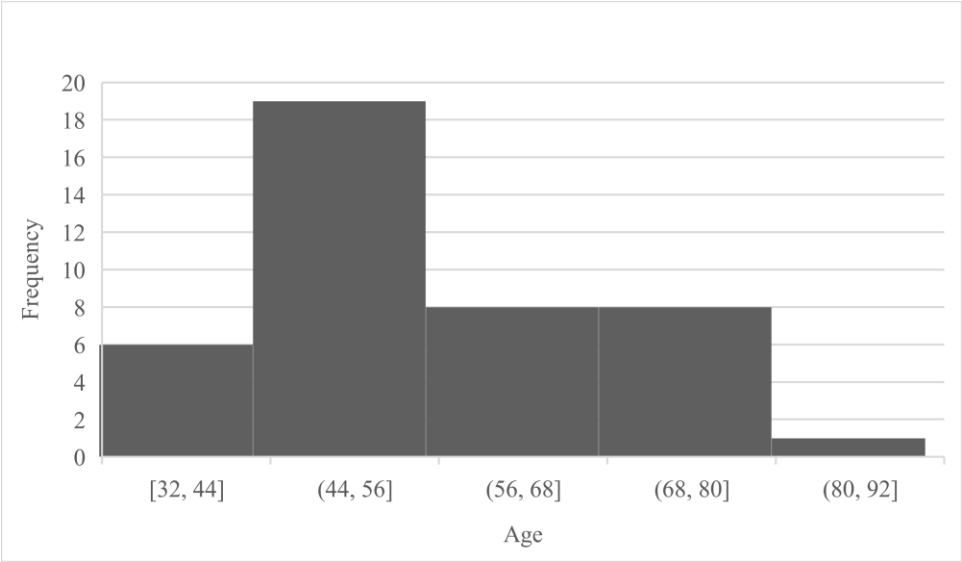

Figure 4. Histogram regarding the frequency of AD in each age group.

6.3. Genotyping

6.3.1. Experimental Design

For this study, it was stipulated the use of specific primers for each variant's alternatives on PCR Multiplex. Since this approach didn't come out as expected, the next step was to use the new designed primers to then sequence.

Before sequencing, the first step is to amplify the fragment of interest through PCR. This Nobel Prize winning technique revolutionized molecular diagnosis, allowing to amplify one single fragment by the addiction of two primers, the forward and reverse primers, by denaturation the dsDNA into two ssDNAs through temperature. The presence of the Taq polymerase and dNTPs allows to synthesize new DNA strands using the original strand as a template, and with each cycle, obtain a high detectable and workable amount of the fragment of interest (Zhu *et al*. 2020). The developed optimized PCR program was: 95ºC 7', (95ºC 45'', 61ºC 45'', 72ºC 1'30'') x30, 72ºC 7', 8ºC ∞.

After the PCR program is finished, the samples are then loaded into an agarose gel (Sea Kem LE at 2%), with an electrophoretic race of 30 minutes at 140 V accompanied with

a molecular marke This methodology allows to separate the DNA fragments by size in a porous gel, throughout an electrical current that goes from a negative charge pole to a positive one. After confirming the presence of the expected band, the PCR product was prepared for sequencing by the removal of the unused primers and nucleotides through the usage of the ExoSap enzyme. This is followed by the Sanger sequencing reaction with BigDye terminators, and finally there's a last purification with silica gel columns and resuspension in formamide.

The available sequencing method at Centro de Genética Médica Dr. Jacinto Magalhães, at the Molecular Genetics Department was the Sanger Sequencing by Capillary Electrophoresis. This method of sequencing has a 96-column format, where each capillary has the capacity of separate fragments based on size through an electric current. Once the sample is on the capillary, another electric field is applied so the DNA fragments can go through it. As they go through, a laser-induced fluorescence detector recognizes the fluorescence signal for each base pair, that is labelled with a distinct dye. The data is then turned into a file that can then be opened at Chromas or Finch TV for further analysis (Dovichi and Zhang 2000). An example of these type of file analysis is on Figure 5.

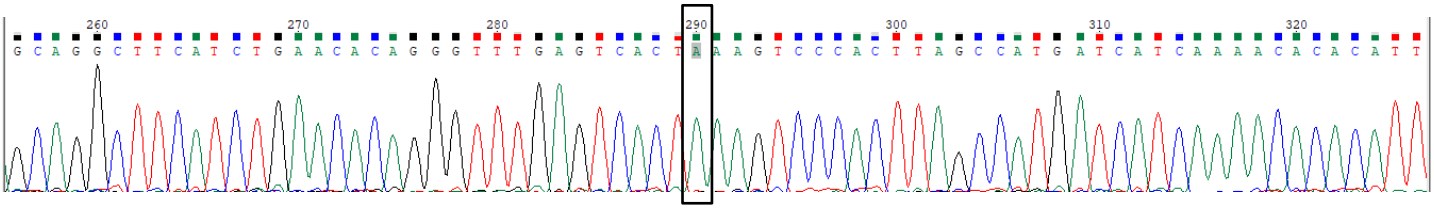

Figure 5. Chromas software with the forward sequence of the *TNFSF15* gene, rs7869487 variant in one of the samples. On this example, the black square evidence the SNP of interest. Each peak represents one nucleotide with the following nomenclature: T- Red; A- Green; C- Blue; G- Black.

6.3.2. Primer Design and Validation

Initially, the primer design was based on the concept of specificity provided by the last base of the primer, to identify by PCR the study variants. However, the resulting primers had very low specificity and were discarded. Some of the non-specific primers were kept and other pair mates were designed upstream or downstream of the variant, in order to generate a PCR product in order to identify them by Sanger Sequencing.

The actual primer design was based on the human reference genome sequence (GRCh38) provided by the data bases of Ensembl and NCBI. Primer Blast from NCBI and the PerlPrimer softwares were employed for design of primers forward and reverse for PCR amplification of genomic regions surrounding each variant of interest, with similar PCR conditions. The primer synthesis was then ordered from Thermo Fisher Scientific.

Upon reception of the primers, these were resuspended in water according to the manufacturer's instructions, and then aliquoted in 10 pmol/μL dilutions. Every primer was tested, and the PCR conditions optimized, until one clear band of the intended size was obtained. After optimized, every fragment corresponding to each variant was then sequenced through Sanger Sequencing by Capillary Electrophoresis (ABI-3130XL; Filter D; Dye ROX500). All the sequencing results were then inserted on NCBI Blast, in order to determine if the primers amplified the region in question, and if so, they were considered validated. The final primers that were used for this project are listed on Table 8.

Table 8. Primers used for the studied variants.

	Variants	Forward Primer	Reverse Primer
LAMB4	rs149874137	5'-ATAGTGGCCATGTGTGTTGC-3'	5'-AGGCAGTGAAATTCCAGATAAAC-3'
	rs2074749	5'-TCCCTTGAACTGATGCACCTC-3'	5'-GCTGCAGAATCCACAAGCAC-3'
	rs9690688	5'-TTGCAGCAACACAGGCAAAA-3'	5'-TGTCAGCTTTTCACCACCAAAG-3'
	rs2240445	5'-TCCAGTGACTCAGTGAATTGTT-3'	5'-GCATATGAATTCCATACCTTTCACA-3'
	rs1627354	5'-AGTATTCAGCACTCAGTGAGCA-3'	5'-GGGCACTGAACTATCAGAAGG-3'
TNFSF15	rs7848647	5'-TGCATGATCCAGCAGTACC-3'	5'-CGGCTTGGAGTTGTAACCTCT-3'
	rs7869487	5'-CCAATGAAGGGCAGTAATCAA-3'*	5'-GAGACCCATCACGAACTTGC-3'
	rs3810936	5'-AAATCAGACAAGCAGGCCGA-3'	5'-AGCTAAACCGTTGTCCCTGT-3'
	rs4263839	5'-AAGAACCGCAGGTCATGGG-3'	5'-ACCCCCATTTCCCTCTCCTTT-3'
	rs6478108	5'-TTCCCAGCAGAAAGCCTTGAT-3'	5'-TGCTGCTCTCCTGGATTCTT-3'*
ARHGAP15	rs4662344	5'-GGAAGCAGAGCCCTGAAGTA-3'	5'-GGAAGCAGAGCCCTGAAGTA-3'
FAM155A	rs67153654	5'-TAGCGTGTTCTCGGAACTTCA-3'	5'-GGTACCTTAGCACGTCAACT-3'
COLQ	rs7609897	5'-CCTGGGAAAAGATGGAGAGCC-3'	5'- GGCTCTGTGACCCCAACAAC -3'

A/T/G/C- Nucleotides *Connelly *et al*. (2014)

6.4. Sample Processing

For each individual participating in this study on the study group, peripheral blood was collected in lithium heparin tubes from Santo António Hospital, Department of Colorectal Surgery and from CICAP (Centro Integrado de Cirurgia de Ambulatório, Porto). All the samples arrived pseudo-anonymized and were categorized and processed on the Laboratory of Biochemical Genetics, at Centro de Genética Médica Dr. Jacinto Magalhães.

The gDNA was extracted by an automated DNA extractor (EZ1 Advanced, QIAGEN), and the obtained product stored at -20ºC, until usage. The DNA samples were then processed as previously described on point 6.3.1. On Figure 6 there's an example of an agarose gel with the PCR products of several study samples, before these were sent for sequencing.

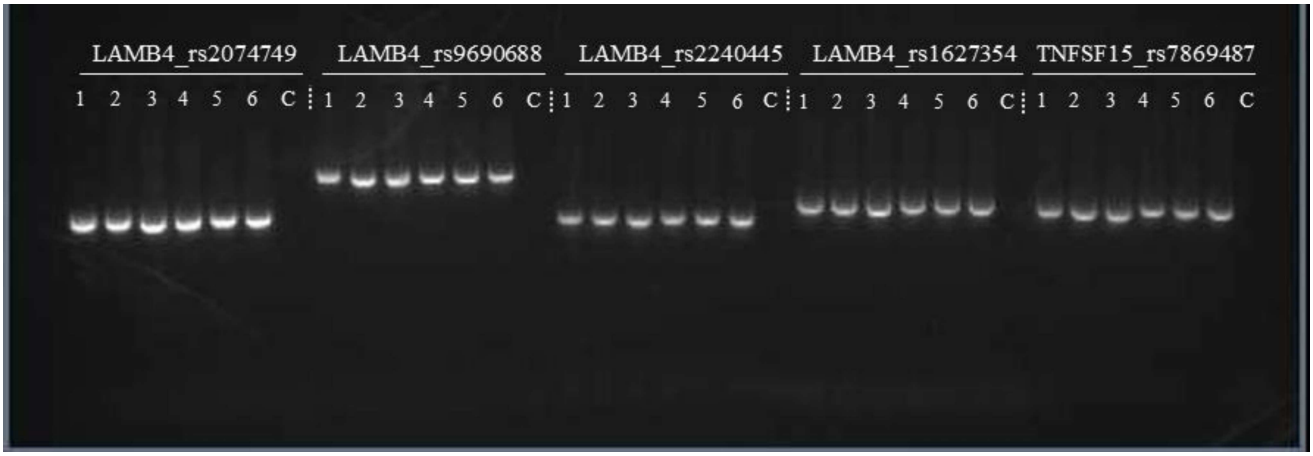

Figure 6. PCR products for sequencing. 1-6: Samples; C- Negative Control.

7. Results and Statistical Analysis

The statistical data analysis was performed through descriptive and inferential statistics with the SPSS-24.0 software (Statistical Package for the Social Sciences). Due to the low sampling and the results from the Kolmogorov Smirnov normality test, it was determined that the sampling doesn't follow a normal distribution, resulting on the usage of non-parametric tests.

To compare the study variables in function of the groups, it was applied the Mann-Whitney test (Tables 22, 23 and 28) and for the correlation between the quantitative variables in study, the correlation coefficient of Spearman was applied (Table 24). Additionally, the Qui-Square test (X^2) was enforced to associate the categorical variables (Tables 9-20; 25-27) and finally, in order to compare the study variables in function of more than two groups, the Kruskall-Wallis test was performed (Table 21). To determine statistical significance from these tests, the *p-value* has to be lower than 0,05 (Marôco, 2014).

7.1. Genetic Profile vs Study Groups

The first analysis represented in this study consists in determining if there is any statistical significance between the genetic profile of each gene variants in study and the groups. First of all, the rs149874137, rs2074749, rs2240445 and rs1627354 SNVs from the *LAMB4* gene, doesn't show any tendency to possibly assist on distinguishing each group. However, on the rs9690688 variant, there's a slight deviation of percentage on the AUDg for the heterozygous profile (C/A), about 26,3% of all AUDg samples (Table 9). Nonetheless, this difference isn't significant.

Table 9. Crosstab of the genetic profile on the *LAMB4* gene variants and the Groups (CTRL, AUDg, ACDg).

			CTRL	**AUDg**	**ACDg**	*Total*	*p*
***LAMB4_* rs149874137**	C/C	N	47	19	22	88	
		%	100,0%	100,0%	95,7%	98,9%	
	G/G	N	0	0	1	1	
		%	0,0%	0,0%	4,3%	1%	0,234
***LAMB4_* rs2074749**	G/G	N	44	19	23	86	
		%	93,6%	100,0%	100,0%	96,6%	
	G/A	N	3	0	0	3	
		%	6,4%	0,0%	0,0%	3,4%	0,250
***LAMB4_* rs9690688**	C/C	N	40	14	22	76	
		%	85,1%	73,7%	95,7%	85,4%	
	C/A	N	7	5	1	13	
		%	14,9%	26,3%	4,3%	14,6%	0,133
***LAMB4_* rs2240445**	T/T	N	47	19	23	89	
		%	100,0%	100,0%	100,0%	100%	—
***LAMB4_* rs1627354**	G/G	N	37	16	21	74	
		%	78,7%	84,2%	91,3%	83,1%	
	G/A	N	10	3	2	15	
		%	21,3%	15,8%	8,7%	16,9%	0,414
	Total	N	47	19	23	89	
		%	**100%**	**100%**	**100%**	**100%**	

(CTRL-Control Group, AUDg-Acute Uncomplicated Diverticulitis Group, ACDg- Acute Complicated Diverticulitis Group)

An overview of Table 10 exhibits a trend, in every SNP, for the predominance of the heterozygous profile on the disease related groups, compared to the control group. Yet, the

same can't be said with the possibility of distinguishing the AUDg from the ACDg. The lack of statistical significance doesn't allow to determine with confidence the truthfulness of this data.

Table 10. Crosstab of the genetic profile on the *TNFSF15* gene variants and the Groups (CTRL, AUDg, ACDg).

			CTRL	**AUDg**	**ACDg**	*Total*	*p*
***TNFSF15_* rs7869487**	A/A	N	31	10	13	54	
		%	66,0%	52,6%	56,5%	60,7%	
	G/G	N	4	1	0	5	
		%	8,5%	5,3%	0,0%	5,6%	
	A/G	N	12	8	10	30	
		%	25,5%	42,1%	43,5%	33,7%	0,347
***TNFSF15_* rs7848647**	A/A	N	5	1	1	7	
		%	10,6%	5,3%	4,3%	7,9%	
	G/G	N	31	7	12	50	
		%	66,0%	36,8%	52,2%	56,2%	
	G/A	N	11	11	10	32	
		%	23,4%	57,9%	43,5%	36,0%	0,089
***TNFSF15_* rs4263839**	C/C	N	31	8	12	51	
		%	66,0%	42,1%	52,2%	57,3%	
	T/T	N	5	1	0	6	
		%	10,6%	5,3%	0,0%	6,7%	
	C/T	N	11	10	11	32	
		%	23,4%	52,6%	47,8%	36,0%	0,072
***TNFSF15_* rs6478108**	C/C	N	6	2	0	8	
		%	12,8%	10,5%	0,0%	9,0%	
	T/T	N	30	8	12	50	
		%	63,8%	42,1%	52,2%	56,2%	
	T/C	N	11	9	11	31	
		%	23,4%	47,4%	47,8%	34,8%	0,098
***TNFSF15_* rs3810936**	A/A	N	2	2	0	4	
		%	4,3%	10,5%	0,0%	4,5%	
	G/G	N	33	8	13	54	
		%	70,2%	42,1%	56,5%	100,0%	
	G/A	N	12	9	10	31	
		%	25,5%	47,4%	43,5%	34,8%	0,139
	Total	N	47	19	23	89	
		%	**100%**	**100%**	**100%**	**100%**	

On Table 11 there's the same preceding analysis but for the *ARHGAP15, FAM155A* and *COLQ* genes. In the genotype of the *ARHGAP15* gene SNP, the data doesn't show any shift on the percentages that could infer some type of influence on this topic. However, on the *FAM155A* gene, 19% of the sampling has a homozygous profile for the "disease related allele" (A). Although, this isn't statistically significant due to transgression of the test parameters.

On the other hand, the *COLQ* gene data for the CTRL group has a surprisingly lower frequency for the "disease related allele" in comparison with the other groups. Since the sampling is very low, these contradictory results have no statistical significance.

Table 11. Crosstab of the genetic profile of the variants on the *ARHGAP15*, *FAM155A*, *COLQ* genes and the Groups (CTRL, AUDg, ACDg).

			CTRL	**AUDg**	**ACDg**	*Total*	*p*
***ARHGAP15_* rs4662344**	C/C	N	31	12	14	57	
		%	66,0%	63,2%	60,9%	64,0%	
	T/T	N	1	0	1	2	
		%	2,1%	0,0%	4,3%	2,2%	
	C/T	N	15	7	8	30	
		%	31,9%	36,8%	34,8%	33,7%	0,901
***FAM155A_* rs67153654**	A/A	N	0	8	9	17	
		%	0,0%	42,1%	39,1%	19,1%	
	T/T	N	35	7	10	52	
		%	74,5%	36,8%	43,5%	58,4%	
	T/A	N	12	4	4	20	(*)
		%	25,5%	21,1%	17,4%	22,5%	0,000
***COLQ_* rs7609897**	A/A	N	7	1	1	9	
		%	14,9%	5,3%	4,3%	10,1%	
	C/C	N	21	12	17	50	
		%	44,7%	63,2%	73,9%	56,2%	
	C/A	N	19	6	5	30	
		%	40,4%	31,6%	21,7%	33,7%	0,168
	Total	N	47	19	23	89	
		%	**100%**	**100%**	**100%**	**100%**	

(*) 3 cells (33,3%) expected a score lower than 5. The minimum expected score is 3,63.

7.2. Genetic Profile vs Modified Hinchey Classification

In this analysis, there's the proposal of the possibility that the genetic profile of the studied variants can determine a predisposition for the severity of AD.

On Table 12, there's the data analysis of the *LAMB4* gene variants, and because of the homogeneity of profiles, the rs149874137, rs2240445 as well as rs2074749, are not statistically significant. Yet, on both rs9690688 and rs1627354 variants, the heterozygous profile has some preponderance in the AUDg.

Table 12. Crosstab of the genetic profile on the *LAMB4* gene variants and Modified Hinchey Classification (0, Ia, Ib, II, III).

				Modified Hinchey Classification						
				0	Ia	Ib	II	III	*Total*	*p*
***LAMB4_* rs149874137**	AUDg	C/C	N	5	14				19	
			%	100%	100%				100%	—
	ACDg	C/C	N			10	8	4	22	
			%			90,9%	100%	100%	96%	
		G/G	N			1	0	0	1	(*)
			%			9,1%	0,0%	0,0%	4%	0,000
***LAMB4_* rs2074749**	AUDg	G/G	N	5	14				19	
			%	100%	100%				100%	—
	ACDg	G/G	N			11	8	4	23	
			%			100%	100%	100%	100%	—
***LAMB4_* rs9690688**	AUDg	C/C	N	4	10				14	
			%	80,0%	71,4%				73,7%	
		C/A	N	1	4				5	
			%	20,0%	28,6%				26,3%	1,000
	ACDg	C/C	N			11	8	3	22	
			%			100%	100%	75,0%	95,7%	
		C/A	N			0	0	1	1	
			%			0,0%	0,0%	25%	4,3%	0,083
***LAMB4_* rs2240445**	AUDg	T/T	N	5	14				19	
			%	100%	100%				100%	—
	ACDg	T/T	N			11	8	4	23	
			%			100%	100%	100%	100%	—

Table 12. Crosstab of the genetic profile on the *LAMB4* gene variants and Modified Hinchey Classification (0, Ia, Ib, II, III) (continuation).

				Modified Hinchey Classification						
				0	Ia	Ib	II	III	*Total*	*p*
***LAMB4*_ rs1627354**	AUDg	G/G	N	4	12				16	
			%	80,0%	85,7%				84,2%	
		G/A	N	1	2				3	
			%	20,0%	14,3%				15,8%	1,000
	ACDg	G/G	N			10	7	4	21	
			%			90,9%	87,5%	100%	91,3%	
		G/A	N			1	1	0	2	
			%			9,1%	12,5%	0,0%	8,7%	0,768
		Total	N			11	8	4	23	
			%	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	

* 4 cells (66,7%) expected a score lower than 5. The minimum expected score is 0,17.

The same analysis on the *TNFSF15* gene variants, shows that all variants have the heterozygous profile in majority on the AUDg. However, on the ACDg, the percentages with the "disease related alleles" are inconsistent and doesn't provide much indication on if they contribute to severity in AD (Table 13).

Table 13. Crosstab of the genetic profile on the *TNFSF15* gene variants and Modified Hinchey Classification (0, Ia, Ib, II, III).

				Modified Hinchey Classification						
				0	Ia	Ib	II	III	*Total*	*p*
***TNFSF15*_ rs7869487**	AUDg	A/A	N	4	6				10	
			%	80,0%	42,9%				52,6%	
		G/G	N	0	1				1	
			%	0,0%	7,1%				5,3%	
		A/G	N	1	7				8	
			%	20,0%	50,0%				42,1%	0,348
	ACDg	A/A	N			6	5	2	13	
			%			54,5%	62,5%	50,0%	56,5%	
		A/G	N			5	3	2	10	
			%			45,5%	37,5%	50,0%	43,5%	0,903
***TNFSF15*_ rs7848647**	AUDg	A/A	N	1	0				1	
			%	20,0%	0,0%				5,3%	
		G/G	N	3	4				7	
			%	60,0%	28,6%				36,8%	
		G/A	N	1	10				11	
			%	20,0%	71,4%				57,9%	0,065
	ACDg	A/A	N			1	0	0	1	
			%			9,1%	0,0%	0,0%	4,3%	
		G/G	N			7	3	2	12	
			%			63,6%	37,5%	50,0%	52,2%	
		G/A	N			3	5	2	10	
			%			27,3%	62,5%	50,0%	43,5%	0,546
***TNFSF15*_ rs4263839**	AUDg	C/C	N	3	5				8	
			%	60,0%	35,7%				42,1%	
		T/T	N	1	0				1	
			%	20,0%	0,0%				5,3%	
		C/T	N	1	9				10	
			%	20,0%	64,3%				52,6%	0,096
	ACDg	C/C	N			7	3	2	12	
			%			63,6%	37,5%	50,0%	52,2%	
		C/T	N			4	5	2	11	
			%			36,4%	62,5%	50,0%	47,8%	0,528

Table 13. Crosstab of the genetic profile on the *TNFSF15* gene variants and Modified Hinchey Classification (0, Ia, Ib, II, III). (continuation)

				Modified Hinchey Classification						
				0	Ia	Ib	II	III	*Total*	*p*
***TNFSF15*_ rs6478108**	AUDg	C/C	N	1	1				2	
			%	20,0%	7,1%				10,5%	
		T/T	N	3	5				8	
			%	60,0%	35,7%				42,1%	
		T/C	N	1	8				9	
			%	20,0%	57,1%				47,4%	0,338
	ACDg	T/T	N			6	4	2	12	
			%			54,5%	50,0%	50,0%	52,2%	
		T/C	N			5	4	2	11	
			%			45,5%	50,0%	50,0%	47,8%	0,977
***TNFSF15*_ rs3810936**	AUDg	A/A	N	1	1				2	
			%	20,0%	7,1%				10,5%	
		G/G	N	3	5				8	
			%	60,0%	35,7%				42,1%	
		G/A	N	1	8				9	
			%	20,0%	57,1%				47,4%	0,338
	ACDg	G/G	N			7	4	2	13	
			%			63,6%	50,0%	50,0%	56,5%	
		G/A	N			4	4	2	10	
			%			36,4%	50,0%	50,0%	43,5%	0,805
		Total	N			11	8	4	23	
			%	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	

As previously seen on Table 11, both *ARHGAP15* and *COLQ* gene variants genotypes have no obvious connection to the onset of AD, which is proven by data on Table 14. Alternatively, on the *FAM155A* gene, the WT homozygous profile (T) and the "disease related allele" (A) have proximate percentages in each group (AUDg and ACDg), which shows that the allele (A) has a higher prevalence in AD than in the general population. However, this data doesn't have statistical significance for AD severity (Table 14).

Table 14. Crosstab of the genetic profile of the variants on the *ARHGAP15*, *FAM155A*, *COLQ* genes and Modified Hinchey Classification (0, Ia, Ib, II, III).

				Modified Hinchey Classification						
				0	Ia	Ib	II	III	*Total*	*p*
***ARHGAP15_* rs4662344**	AUDg	C/C	N	3	9				12	
			%	60,0%	64,3%				63,2%	
		C/T	N	2	5				7	
			%	40,0%	35,7%				36,8%	1,000
	ACDg	C/C	N			7	4	3	14	
			%			63,6%	50,0%	75,0%	60,9%	
		T/T	N			0	1	0	1	
			%			0,0%	12,5%	0,0%	4,3%	
		C/T	N			4	3	1	8	
			%			36,4%	37,5%	25,0%	34,8%	0,679
***FAM155A_* rs67153654**	AUDg	A/A	N	2	6				8	
			%	40,0%	42,9%				42,1%	
		T/T	N	2	5				7	
			%	40,0%	35,7%				36,8%	
		T/A	N	1	3				4	
			%	20,0%	21,4%				21,1%	0,986
	ACDg	A/A	N			4	3	2	9	
			%			36,4%	37,5%	50,0%	39,1%	
		T/T	N			6	3	1	10	
			%			54,5%	37,5%	25,0%	43,5%	
		T/A	N			1	2	1	4	
			%			9,1%	25,0%	25,0%	17,4%	0,796
***COLQ_* rs7609897**	AUDg	A/A	N	1	0				1	
			%	20,0%	0,0%				5,3%	
		C/C	N	3	9				12	
			%	60,0%	64,3%				63,2%	
		C/A	N	1	5				6	
			%	20,0%	35,7%				31,6%	0,212
	ACDg	A/A	N			0	0	1	1	
			%			0,0%	0,0%	25,0%	4,3%	
		C/C	N			9	6	2	17	
			%			81,9%	75,0%	50,0%	73,9%	
		C/A	N			2	2	1	5	
			%			18,2%	25,0%	25,0%	21,7%	0,260
		Total	N			11	8	4	23	
			%	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	

7.3. Genetic Profile vs Recurrence

In this set of statistical analysis, the purpose is to establish if the genotype has an impact on the predisposition for recurrent episodes of AD. The first thing to notice is that there isn't a significant difference in the number of individuals that had recurrence between groups (AUDg-57,9% and ACDg-52,2%). Therefore, the same occurs on those that hadn't recurrence (AUDg- 42,1% and ACDg- 47,8%).

Likewise, on Table 15, the homogeneity of the genotype of the individuals doesn't allow to determine if the SNVs on the *LAMB4* gene have any role on the recurrence of AD.

Table 15. Crosstab of the genetic profile on the *LAMB4* gene variants and Recurrence.

				Recurrence			
				Yes	No	*Total*	*p*
***LAMB4_* rs149874137**	AUDg	C/C	N	11	8	19	
			%	57,9%	42,1%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	—
	ACDg	C/C	N	12	10	22	
			%	54,5%	45,5%	100%	
		G/G	N	0	1	1	
			%	0,0%	100,0%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	0,478
***LAMB4_* rs2074749**	AUDg	G/G	N	11	8	19	
			%	57,9%	42,1%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	—
	ACDg	G/G	N	12	11	23	
			%	52,2%	47,8%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	—

Table 15. Crosstab of the genetic profile on the *LAMB4* gene variants and Recurrence. (continuation)

				Recurrence			
				Yes	No	*Total*	*p*
***LAMB4*_ rs9690688**	AUDg	C/C	N	8	6	14	
			%	57,1%	42,9%	100%	
		C/A	N	3	2	5	
			%	60,0%	40,0%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	1,000
	ACDg	C/C	N	12	10	22	
			%	54,5%	45,5%	100%	
		C/A	N	0	1	1	
			%	0,0%	100,0%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	0,478
***LAMB4*_ rs2240445**	AUDg	T/T	N	11	8	19	
			%	57,9%	42,1%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	—
	ACDg	T/T	N	12	11	23	
			%	52,2%	47,8%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	—
***LAMB4*_ rs1627354**	AUDg	G/G	N	9	7	16	
			%	56,3%	43,8%	100%	
		G/A	N	2	1	3	
			%	66,7%	33,3%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	1,000
	ACDg	G/G	N	12	9	21	
			%	57,1%	42,9%	100%	
		G/A	N	0	2	2	
			%	0,0%	100,0%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	0,217

For the *TNFSF15* gene (Table 16), on rs7848647 and rs6478108 variants, the "disease related allele" which is also the WT, prevails on the AUDg for individuals with recurrence. Additionally, this can also be observed on rs7869487 and rs3810936 variants, on the ACDg for the heterozygous profile. Once more, there isn't statistical significance.

Table 16. Crosstab of the genetic profile on the *TNFSF15* gene variants and Recurrence.

				Recurrence			
				Yes	No	*Total*	*p*
***TNFSF15*_ rs7869487**	AUDg	A/A	N	7	3	10	
			%	70,0%	30,0%	100%	
		G/G	N	0	1	1	
			%	0,0%	100,0%	100%	
		A/G	N	4	4	8	
			%	50,0%	50,0%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	0,336
	ACDg	A/A	N	6	7	13	
			%	46,2%	53,8%	100%	
		A/G	N	6	4	10	
			%	60,0%	40,0%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	0,68
***TNFSF15*_ rs7848647**	AUDg	A/A	N	0	1	1	
			%	0,0%	100,0%	100%	
		G/G	N	5	2	7	
			%	71,4%	28,6%	100%	
		G/A	N	6	5	11	
			%	54,5%	45,5%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	0,377
	ACDg	A/A	N	1	0	1	
			%	100,0%	0,0%	100%	
		G/G	N	6	6	12	
			%	50,0%	50,0%	100%	
		G/A	N	5	5	10	
			%	50,0%	50,0%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	0,619
***TNFSF15*_ rs4263839**	AUDg	C/C	N	6	2	8	
			%	75,0%	25,0%	100%	
		T/T	N	0	1	1	
			%	0,0%	100,0%	100%	
		C/T	N	5	5	10	
			%	50,0%	50,0%	100%	
		Total	N	11	8	19	0,274
			%	**57,9%**	**42,1%**	**100%**	

				Recurrence			
				Yes	No	*Total*	*p*
***TNFSF15*_ rs4263839**	ACDg	C/C	N	6	6	12	
			%	50,0%	50,0%	100%	
		C/T	N	6	5	11	
			%	54,5%	45,5%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	1,000
***TNFSF15*_ rs6478108**	AUDg	C/C	N	0	2	2	
			%	0,0%	100,0%	100%	
		T/T	N	6	2	8	
			%	75,0%	25,0%	100%	
		T/C	N	5	4	9	
			%	55,6%	44,4%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	0,155
	ACDg	T/T	N	6	6	12	
			%	50,0%	50,0%	100%	
		T/C	N	6	5	11	
			%	54,5%	45,5%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	1,000
***TNFSF15*_ rs3810936**	AUDg	A/A	N	1	1	2	
			%	50,0%	50,0%	100%	
		G/G	N	6	2	8	
			%	75,0%	25,0%	100%	
		G/A	N	4	5	9	
			%	44,4%	55,6%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	0,432
	ACDg	G/G	N	6	7	13	
			%	46,2%	53,8%	100%	
		G/A	N	6	4	10	
			%	60,0%	40,0%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	0,680

On the other hand, the heterozygous genotype tends to be the majority on recurrent AD, ACDg on *ARHGAP15* and *COLQ* gene SNPs, yet in the *FAM155A* gene SNP, it is found prevalent on the AUDg (Table 17). In addition, in the *FAM155A* gene, ACDg, the

homozygous genotype (A) is preponderant on recurrency when compared to the WT allele. None of this data has, however, any significance.

Table 17. Crosstab of the genetic profile of the variants on the *ARHGAP15*, *FAM155A*, *COLQ* genes and Recurrence.

				Recurrence			
				Yes	No	*Total*	*p*
***ARHGAP15*_ rs4662344**	AUDg	C/C	N	7	5	12	
			%	58,3%	41,7%	100%	
		C/T	N	4	3	7	
			%	57,1%	42,9%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	1,000
	ACDg	C/C	N	6	8	14	
			%	42,9%	57,1%	100%	
		T/T	N	1	0	1	
			%	100,0%	0,0%	100%	
		C/T	N	5	3	8	
			%	62,5%	37,5%	100%	
		Total		12	11	23	
				52,2%	**47,8%**	**100%**	0,418
***FAM155A*_ rs67153654**	AUDg	A/A	N	3	5	8	
			%	37,5%	62,5%	100%	
		T/T	N	5	2	7	
			%	71,4%	28,6%	100%	
		T/A	N	3	1	4	
			%	75,0%	25,0%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	0,306
	ACDg	A/A	N	6	3	9	
			%	66,7%	33,3%	100%	
		T/T	N	4	6	10	
			%	40,0%	60,0%	100%	
		T/A	N	2	2	4	
			%	50,0%	50,0%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	0,507

Table 17. Crosstab of the genetic profile of the variants on the *ARHGAP15*, *FAM155A*, *COLQ* genes and Recurrence. (continuation)

				Recurrence			
				Yes	No	*Total*	*p*
COLQ_ rs7609897	AUDg	A/A	N	0	1	1	
			%	0,0%	100,0%	100%	
		C/C	N	8	4	12	
			%	66,7%	33,3%	100%	
		C/A	N	3	3	6	
			%	50,0%	50,0%	100%	
		Total	N	11	8	19	
			%	**57,9%**	**42,1%**	**100%**	0,385
	ACDg	A/A	N	1	0	1	
			%	100,0%	0,0%	100%	
		C/C	N	8	9	17	
			%	47,1%	52,9%	100%	
		C/A	N	3	2	5	
			%	60,0%	40,0%	100%	
		Total	N	12	11	23	
			%	**52,2%**	**47,8%**	**100%**	0,544

7.4. Genetic Profile vs Surgery

In the following analysis, it is intended to determine if there is any relation between the genotype of the studied SNPs, with the probability of the patient being selected for elective surgery. Hence that AUD has a lower incidence of surgery, so it's not possible to infer if the data is significant, with any tendencies or not.

On Table 18, as evidenced before, the genotype data of the *LAMB4* gene variants, doesn't provide enough difference on the percentages to determine if these variants play a role on determining the course of surgery. Although the rs9690688 variant has more variability, the results show no significant shift of the values.

Table 18. Crosstab of the genetic profile on the *LAMB4* gene variants and Surgery.

				Surgery			
				Yes	No	*Total*	*p*
***LAMB4*_ rs149874137**	AUDg	C/C	N	1	18	19	
			%	5,3%	94,7%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,750
	ACDg	C/C	N	9	13	22	
			%	40,9%	59,1%	100%	
		G/G	N	1	0	1	
			%	100,0%	0,0%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	0,435
***LAMB4*_ rs2074749**	AUDg	G/G	N	1	18	19	
			%	5,3%	94,7%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,750
	ACDg	G/G	N	10	13	23	
			%	43,5%	56,5%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	0,750

Table 18. Crosstab of the genetic profile on the *LAMB4* gene variants and Surgery (continuation).

				Surgery			
				Yes	No	*Total*	*p*
***LAMB4_* rs9690688**	AUDg	C/C	N	1	13	14	
			%	7,1%	92,9%	100%	
		C/A	N	0	5	5	
			%	0,0%	100,0%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	1,000
	ACDg	C/C	N	10	12	22	
			%	45,5%	54,5%	100%	
		C/A	N	0	1	1	
			%	0,0%	100,0%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	1,000
***LAMB4_* rs2240445**	AUDg	T/T	N	1	18	19	
			%	5,3%	94,7%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,750
	ACDg	T/T	N	10	13	23	
			%	43,5%	56,5%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	0,750
***LAMB4_* rs1627354**	AUDg	G/G	N	1	15	16	
			%	6,3%	93,8%	100%	
		G/A	N	0	3	3	
			%	0,0%	100,0%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	1,000
	ACDg	G/G	N	10	11	21	
			%	47,6%	52,4%	100%	
		G/A	N	0	2	2	
			%	0,0%	100,0%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	0,486

In the case of the rs7869487 and rs3810936 variants of the *TNFSF15* gene (Table 19), ACDg the WT homozygous genotype has a higher frequency on patients that hadn't been submitted to elective surgery. However, the "disease related allele" doesn't have a higher frequency on patients submitted to surgery. The remaining variants have no compelling differences between patients with and without surgery.

Table 19. Crosstab of the genetic profile on the *TNFSF15* gene variants and Surgery.

				Surgery			
				Yes	No	*Total*	*p*
TNFSF15_ rs7869487	AUDg	A/A	N	0	10	10	
			%	0,0%	100,0%	100%	
		G/G	N	0	1	1	
			%	0,0%	100,0%	100%	
		A/G	N	1	7	8	
			%	12,5%	87,5%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,484
	ACDg	A/A	N	5	8	13	
			%	38,5%	61,5%	100%	
		A/G	N	5	5	10	
			%	50,0%	50,0%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	0,685
TNFSF15_ rs7848647	AUDg	A/A	N	0	1	1	
			%	0,0%	100,0%	100%	
		G/G	N	0	7	7	
			%	0,0%	100,0%	100%	
		G/A	N	1	10	11	
			%	9,1%	90,9%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,681
	ACDg	A/A	N	0	1	1	
			%	0,0%	100,0%	100%	
		G/G	N	5	7	12	
			%	41,7%	58,3%	100%	
		G/A	N	5	5	10	
			%	50,0%	50,0%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	0,619

Table 19. Crosstab of the genetic profile on the *TNFSF15* gene variants and Surgery (continuation part1).

				Surgery			
				Yes	No	*Total*	*p*
***TNFSF15_* rs4263839**	AUDg	C/C	N	0	8	8	
			%	0,0%	100,0%	100%	
		T/T	N	0	1	1	
			%	0,0%	100,0%	100%	
		C/T	N	1	9	10	
			%	10,0%	90,0%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,622
	ACDg	C/C	N	5	7	12	
			%	41,7%	58,3%	100%	
		C/T	N	5	6	11	
			%	45,5%	54,5%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	1,000
***TNFSF15_* rs6478108**	AUDg	C/C	N	0	2	2	
			%	0,0%	100,0%	100%	
		T/T	N	0	8	8	
			%	0,0%	100,0%	100%	
		T/C	N	1	8	9	
			%	11,1%	88,9%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,556
	ACDg	T/T	N	5	7	12	
			%	41,7%	58,3%	100%	
		T/C	N	5	6	11	
			%	45,5%	54,5%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	1,000

Table 19. Crosstab of the genetic profile on the *TNFSF15* gene variants and Surgery (continuation part2).

				Surgery			
				Yes	No	*Total*	*p*
TNFSF15_ rs3810936	AUDg	A/A	N	0	2	2	
			%	0,0%	100,0%	100%	
		G/G	N	0	8	8	
			%	0,0%	100,0%	100%	
		G/A	N	1	8	9	
			%	11,1%	88,9%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,556
	ACDg	G/G	N	5	8	13	
			%	38,5%	61,5%	100%	
		G/A	N	5	5	10	
			%	50,0%	50,0%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	0,685

Equally, the WT homozygous genotype of *ARHGAP15* and *COLQ* gene variants, have higher frequencies on patients without surgery on the ACDg, but the “disease related alleles” doesn’t have significant differences on patients with surgery (Table 20). On the *FAM155A* gene SNP, the “disease related allele” (A) has a high frequency on patients with elective surgery, however this isn’t statistically significant due to transgressions on the statistic test parameters, resulting from low sampling.

Table 20. Crosstab of the genetic profile of the variants on the *ARHGAP15*, *FAM155A*, *COLQ* genes and Surgery.

				Surgery			
				Yes	No	*Total*	*p*
***ARHGAP15*_ rs4662344**	AUDg	C/C	N	1	11	12	
			%	8,3%	91,7%	100%	
		C/T	N	0	7	7	
			%	0,0%	100,0%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	1,000
	ACDg	C/C	N	5	9	14	
			%	35,7%	64,3%	100%	
		T/T	N	1	0	1	
			%	100,0%	0,0%	100%	
		C/T	N	4	4	8	
			%	50,0%	50,0%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	0,410
***FAM155A*_ rs67153654**	AUDg	A/A	N	0	8	8	
			%	0,0%	100,0%	100%	
		T/T	N	1	6	7	
			%	14,3%	85,7%	100%	
		T/A	N	0	4	4	
			%	0,0%	100,0%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,405
	ACDg	A/A	N	7	2	9	
			%	77,8%	22,2%	100%	
		T/T	N	1	9	10	
			%	10,0%	90,0%	100%	
		T/A	N	2	2	4	
			%	50,0%	50,0%	100%	
		Total	N	10	13	23	(*)
			%	**43,5%**	**56,5%**	**100%**	0,011

Table 20. Crosstab of the genetic profile of the variants on the *ARHGAP15*, *FAM155A*, *COLQ* genes and Surgery. (continuation)

				Surgery			
				Yes	No	*Total*	*p*
COLQ_ rs7609897	AUDg	A/A	N	0	1	1	
			%	0,0%	100,0%	100%	
		C/C	N	0	12	12	
			%	0,0%	100,0%	100%	
		C/A	N	1	5	6	
			%	16,7%	83,3%	100%	
		Total	N	1	18	19	
			%	**5,3%**	**94,7%**	**100%**	0,319
	ACDg	A/A	N	1	0	1	
			%	100,0%	0,0%	100%	
		C/C	N	7	10	17	
			%	41,2%	58,8%	100%	
		C/A	N	2	3	5	
			%	40,0%	60,0%	100%	
		Total	N	10	13	23	
			%	**43,5%**	**56,5%**	**100%**	0,506

(*) 4 cells (66,7%) expected a score lower than 5. The minimum expected score is 1,74.

7.5. Age vs Modified Hinchey Classification

In this analysis it was expected to determine if there is any relation between the age of the participants and the severity of the disorder (Table 21). With this data, it isn't possible to establish that age plays a role on the severity of AD. Although there's a higher mean of age on Hinchey II, the other ACDg MHCs doesn't follow the same example.

Table 21. Comparison of the Age according to the Modified Hinchey Classification, in each Group (AUDg, ACDg).

	Modified Hinchey		**Age**		
Group	**Classification**	N	Mean	Sd	*p*
AUDg	0	5	55	23,31	
	Ia	14	54	8,45	0,963
ACDg	Ib	11	54	7,14	
	II	8	62	13,78	
	III	4	56	15,45	0,345

7.6. Age vs Recurrence

The following analysis allows to determine if there is any relation between age and recurrence within each group (Table 22). Despite the fact that recurrent episodes on the ACDg having a higher mean of age, the difference to the AUDg isn't significant.

Table 22. Comparison of the Age according to the Recurrence, in each Group (AUDg, ACDg).

			Age		
Group	**Recurrence**	N	Mean	Sd	*p*
AUDg	Yes	11	52	11,10	
	No	8	57	15,82	0,508
ACDg	Yes	12	60	12,29	
	No	11	54	9,98	0,295

7.7. Age vs Number of Episodes

Furthermore, the analysis represented on Table 23 uses the correlation coefficient to determine if age has a correlation to the number of episodes within each group. The results evidence no correlation between the two variables.

Table 23. Correlation coefficient between Age and the Number of Episodes, in each Group (AUDg, ACDg).

Group		Age
AUDg	Number of Episodes	-0,258
ACDg	Number of Episodes	0,282

7.8. Age vs Surgery

This analysis provides a sight on if age has any effect on the probability for an individual with AUD or ACD being submitted to elective surgery. With the presented data on Table 24, there isn't any significant correlation between both groups.

Table 24. Comparison of the Age according to Surgery, in each Group (AUDg, ACDg).

			Age		
Group	**Surgery**	N	Mean	Sd	*p*
AUDg	Yes	1	53	—	
	No	18	55	13,50	0,927
ACDg	Yes	10	57	13,64	
	No	13	57	9,89	0,901

7.9. Gender vs Modified Hinchey Classification

This statistical analysis intends to determine if gender has any correlation to the severity of AD (Table 25). Data shows that there's a higher percentage of women with Hinchey III, but with the small sampling it's not possible to determine that women tend to have a severer form of AD. These results indicate that the variables are independent from each other.

Table 25. Crosstab of the Gender and the Modified Hinchey Classification, in each Group (AUDg, ACDg).

			AUDg				ACDg			
			Male	Female	*Total*	*p*	Male	Female	*Total*	*p*
Modified Hinchey Classification	0	N	2	3	5					
		%	**40,0%**	**60,0%**	100%					
	Ia	N	6	8	14					
		%	**42,9%**	**57,1%**	100%					
	Ib	N					5	6	10	
		%					**45,5%**	**54,5%**	100%	
	II	N					4	4	8	
		%					**50,0%**	**50,0%**	100%	
	III	N					1	3	4	
		%					**25,0%**	**75,0%**	100%	
Total		N	8	11	19		10	13	23	
		%	**42,1%**	**57,9%**	**100%**	1,000	**43,5%**	**56,5%**	**100%**	0,563

7.10. Gender vs Recurrence

Similar to the previous point, the crosstab from Table 26 shows the relation between the gender and recurrence. In the AUDg, most females have recurrence of the disease, and on the ACDg are mainly the male individuals that have recurrence. Again, the small sampling results in non-significant results.

Table 26. Crosstab of the Gender and Recurrence, in each Group (AUDg, ACDg).

			AUDg				ACDg			
			Male	Female	*Total*	*p*	Male	Female	*Total*	*p*
Recurrence	Yes	N	3	8	11		6	6	12	
		%	37,5%	72,7%	57,9%		60,0%	46,2%	52,2%	
	No	N	5	3	8		4	7	11	
		%	62,5%	27,3%	42,1%		40,0%	53,8%	47,8%	
Total		N	8	11	19		10	13	23	
		%	**100%**	**100%**	**100%**	0,181	**100%**	**100%**	**100%**	0,680

7.11. Gender vs Number of Episodes

In this case, there's the comparison between gender and the number of episodes (Table 27). The data shows that the mean of episodes is higher on the AUDg, but not significantly different from the ACDg. However, there isn't any difference between male and female individuals, which indicates that the two variables are most likely independent from each other.

Table 27. Comparison of the Number of Episodes between Gender, in each Group (AUDg, ACDg).

			Nº Episodes		
Group	**Gender**	N	Mean	Sd	*p*
AUDg	Male	8	2,13	2,42	
	Female	11	2,18	0,87	0,149
ACDg	Male	10	1,60	0,52	
	Female	13	1,69	0,86	0,944

7.12. Gender vs Surgery

In conclusion of the statistical analysis, there's the crosstab between gender and surgery in search of some relation (Table 28). The results in the AUDg are bias due to the low number of individuals that go through surgery in this group. On the other hand, the distribution of males and females that were submitted to elective surgery are very similar in the ACDg.

Table 28. Crosstab of the Gender and Surgery, in each Group (AUDg, ACDg).

			AUDg				ACDg			
			Male	Female	*Total*	*p*	Male	Female	*Total*	*p*
Surgery	Yes	N	1	0	1		5	5	10	
		%	12,5%	0,0%	5,3%		50,0%	38,5%	43,5%	
	No	N	7	11	18		5	8	13	
		%	87,5%	100,0%	94,7%		50,0%	61,5%	56,5%	
Total		N	8	11	19		10	13	23	
		%	**100%**	**100%**	**100%**	0,421	**100%**	**100%**	**100%**	0,685

8. Discussion

This study aimed to identify if the genotype of previously referenced SNPs on the literature, had any influence on the onset of AD, prevalence on different MHCs, risk of recurrence and link to selection for elective surgery. Other independent variables such as age and gender were also evaluated for future references. Since AD is a multifactorial disorder, the obtained results became non surprising, because it's the overall combination of all these factors that determine the occurrence and outcome of the disorder.

Firstly, the *LAMB4* gene SNVs have very low populational frequencies for the "disease related alleles" (Table 2), which then translates to a lack of genetic variability in our results, that outcomes as non-significant. However, the rs9690688 variant is the one that has more variability of all SNPs, but the differences of percentages in each variable are not clear for AD. Therefore, it's not possible to confirm or oppose other studies findings.

8.1. Onset of Acute Diverticulitis

When comparing this study results to other scientific studies, some similarities can be found. The most evident concordance is on the *FAM155A* gene, rs67153654 variant, where the homozygous (A) profile has a suggestive association with the development of AD (Table 11 and 14), just as referenced in an Iceland population by Sigurdsson *et al.* (2017). At the same time, but not previously described on the literature, all *TNFSF15* gene variants evidenced that the heterozygous genotype prevailed on the disease control groups (Table 10), which may indicate that the presence of the "disease related allele" has an influence on the onset of AD.

According to what was described by Cochet-Bissuel *et al*. (2014), the allele (A) of the *FAM155A* gene is reported as affecting the regulation of the pacemaker activity of the ICCs on the MP. Since in this study the homozygous (A) genotype is associated to the onset of AD, and assuming that this results on some loss of function of the ICCs, there's the possibility that the epithelial barrier is compromised and for that reason, more likely to result in AD.

In the case of the *TNFSF15* gene, Desplat-Jégo *et al*. (2014) describes that the haplotype that includes the SNPs rs7869487-G, rs7848647-G, rs6478108-T and rs3810936-A increases promotor activity, resulting in stimulation of T cells. This could be one of the contributing factors of the occurring inflammation in AD. Despite this relation being found in heterozygosity of all variants, this suggest that the increase of inflammation can be triggered even in heterozygosity.

8.2. Modified Hinchey Classification

This part of the study is fairly new to the scientific community, where there isn't data for the influence of this studies' selected variables on the severity of AD. When intersecting the genetic data to the MHC on each group, the results are inconclusive (Table 12-14). Since there isn't a homogeneous pattern throughout the various MHCs, the data doesn't allow to determine any association between the genotype and severity, not even to distinguish AUD from ACD. These results prove that the severity of the condition is effectively multifactorial and other factors play major roles on determining the outcome of the disease.

8.3. Recurrence

The etiology of recurrence is not yet understood, yet it is known that various environmental risk factors play major roles on the probability for recurrence, nonetheless, the genetic contribution is another topic that hasn't any literature data.

Our data shows that *TNFSF15, ARHGAP15, FAM155A* and *COLQ* genes, are the ones that might have some influence on recurrence. It appears that the presence of "disease related alleles" on recurrent episodes patients, ACDg, suggests that genetics might play a minor role on recurrence. The etiology of both *TNFSF15* and *FAM155A* genes that was previously described, can also be applied to the possible influence on recurrence.

The mutations on *ARHGAP15* gene, just as Sigurdsson *et al*. (2017) described, increases contractibility and actin fiber stress, which might explain why this variant can have some influence on the recurrence of AD. On the other hand, in the case of the *COLQ* gene, the encoded protein decreases with homozygous mutations, which impairs intestinal motility through the neuromuscular junctions (Camilleri *et al.* 2020). However, in this study, the heterozygous profile also has possibly to some level of association to recurrence.

8.4. Elective Surgery

As previously stated, the criteria for selection to elective surgery is evaluated case by case by the medical practitioner. There are various factors that influence this decision, such as other comorbidities, recurrence, number of episodes, and also severity. Overall, patients from the AUDg have a lower incidence on elective surgery due to minor severity, so the results in this group are very bias, which doesn't allow to determine any possible association.

The study of Connelly *et al.* (2014) found in a USA population, a positive correlation between the homozygous (G) profile and surgical diverticulitis for the rs7848647 SNP from *TNFSF15* gene. Yet, this association wasn't found in this study, which may be due to the low sampling and/or the fact that Connelly's study was performed only with surgical patients, which might be bias. Alternatively, in this study, we found that the *FAM155A* gene SNP was the only one that had a somewhat significance with the "disease related allele" (A) on homozygosity on patients selected for elective surgery.

8.5. Other considerations

The other variables inserted in this study, age and gender were classified as independent variables from the ones that they were compared to. Although the increase of age is usually associated to a rise of comorbidities of various origins, it hasn't a significant association to the development of AD. The same occurs to gender, where males usually develop the disorder sooner than females, but in this study, that wasn't identified with statistical significance.

Additionally, an overall observation of the genetic data from *LAMB4* and *TNFSF15* genes (Figure 2 and 3), indicates that the *TNFSF15* gene SNPs function as a haplotype. This deduction comes from the fact that individuals tend to have a homozygous or heterozygous profile for every variant. This is backed up with the study of Connelly *et al.* (2014), which references that there is a linkage between the *TNFSF15* gene variants. Nevertheless, this association is only an overall observation, and it would be interesting to explore if in the Portuguese population, this haplotype is still valid and if it plays role as a hole, in AD. On the other hand, the issue underlying the *LAMB4* gene SNVs are their populational frequencies of the "disease related alleles", which made it difficult to identify them on the small sampling of this study.

8.6. Future perspectives

A lot can still be done regarding determining the role of genetics in AD, to then be used as a tool to establish new and more personalized guidelines for the welfare of these patients. Since, one of the most reliable parameters of follow-up of patients are the biochemical parameters, this data could be used to cross with the genetic profile of these variants. This analysis could provide an insight of how genetics influence the inflammatory process of AD, and therefore severity and recurrence.

Additionally, more functional studies for each one of these genes are needed to acknowledge the dynamics of gene expression on the context of AD, more specifically on the systems involved on the pathophysiological process. To these studies, it can be incorporated the determination of the impact of each variant to the mRNA and resulting protein, and how this changes its functionality. Since most variants are missense, the protein structure might be changed, but also the intronic and intergenic mutations can affect gene expression and splicing. Finally, the possibility of these variants having potential contribution for AD in haploinsufficiency, is a hypothesis that could be explored by mechanistic studies.

9. Conclusions

In the present study, the SNP rs67153654 from the *FAM155A* gene is the one that has proven to associate more with the development, recurrence, and selection for elective surgery out of all variants. This finding is followed by the association of *TNFSF15* variants to development of AD and recurrence, as well as *ARHGAP15* and *COLQ* gene SNPs for recurrence. All of these variants provided an insight of what determines the onset and prognosis of AD, where each one of them causes alterations on the involved systems. In the meantime, more studies with bigger sampling are necessary to understand AD and how this data can be used for patient care and management.

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