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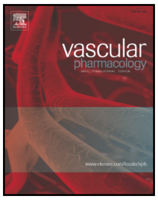

Review

Genetic contribution in sporadic thoracic aortic aneurysm? Emerging evidence of genetic variants related to TLR-4-mediated signaling pathway as risk determinants

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ABSTRACT

Sporadic thoracic aortic aneurysms (TAA) and dissections are one of the major causes of morbidity and mortality worldwide, especially in those older than 65 years. The presentation of TAA is varied and often silent. Thus, sporadic TAA detection is often fortuitous, with identification occurring during a routine physical examination or during an unrelated medical evaluation. Once suspected, confirmation by imaging clinical approaches is needed to allow the choose of the unique treatments for TAA, namely the surgery procedures, including elective surgery or endovascular repair before the onset of catastrophic and fatal complications, such as dissection or rupture. At present, there are no biomarkers available to identify TAAs before visible symptoms. However, recent progresses in understanding of molecular and cellular mechanisms involved in the patho-physiology of sporadic TAA are suggesting different molecular pathways and their genetic variants as potential biomarkers, which might be applied into TAA clinical practice in the near future. Here, we report literature evidence on some disease pathways and their genetic variants on TAA susceptibility and compliances, and their translation as promising TAA preventive and prognostic biomarkers and targets for new personalized therapeutic treatments.

Contents

1. Introduction

Aortic aneurysms occur in thoracic and abdominal sections of aorta and are a deadly late-age-at onset disease with a very complex pathophysiology. Both thoracic aortic (TAA) and abdominal aortic aneurysms (AAA), are also characterized to be silent and asymptomatic diseases and insidious in their progression [1,2]. Despite these common aspects, the two aorta pathologies show a significant heterogeneity in their prevalence, distribution along aorta length, age-at-onset, male:female ratio of disease susceptibility and pathophysiology, as reported in detail in Table 1 [3]. Thus, TAA and AAA are two distinct pathological entities. Accordingly, diverse recommendations for diagnostic imaging evaluations, and medical and surgical treatments for TAA and AAA have been suggested by 2014 European Society Cardiology (ESC) Guidelines (see

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Table 2) [4]. On the other hand, one of the major points of these guidelines is the inclusion of the entire aorta (thoracic and abdominal) study, that led the ESC, and for the first time, to insert indications for AAA diagnosis and management [4].

These clinical measures and concepts all correlate with the rising evidence that the large heterogeneity of TAA and AAA is due to the different embryological origin of cell lineages in two portions of aorta [1,5]. Specifically, thoracic aorta originates from neuronal crests, while abdominal aorta derives from splanchnic mesoderm, as illustrated in Fig. 1 [5]. Disease susceptibility also varies in two aorta sections, with abdominal aorta being more prone to atherosclerosis and aneurysm formation than thoracic aorta. Underlying genetic factors contributing to two diseases also differ based on the site of the clinical manifestation, as well as their weight (see Fig. 1) [1,5–7]. Accordingly, it has been evidenced that genetic determinants may influence AAA development. Heritability estimations as high as 70% have been found, and recently, several genes and loci have been associated with AAA (CNTN3, CDKN2BAS, CDKN2A, HSPG2, CSPG2, and sortilin-1 [*SORT1*] locus) (see Table 3) [8–11]. Furthermore, in 2013 van de Luijtgaarden and coworkers, and for the first time, analyzed the role of nine genes (the transforming growth factor-beta pathway genes — EFEMP2, FBN1, SMAD3, TGBF2, TGFBR1, TGFBR2-, and the smooth muscle cells genes-ACTA2, MYH11 and MYLK) associated with familial TAA in 155 AAA patients. They found only three genetic variants as pathogenetic or likely pathogenic among a total of 47 variants in these genes. Precisely, they observed one pathogenic and segregating variant (the p.Arg491X in *COL3A1* gene), one likely pathogenic and segregating variant (the p.Arg254Cys in *MYH11* gene), and fifteen variant unknown significance (VUS) in two familial AAA patients, and one pathogenic variant (p.Ile525Phefs*18) in *TGFBR2* gene and seven VUS in a patient with sporadic AAA (see Table 2) [11]. These data confirm the reduced contribution of genetic factors in AAA onset and the AAA different pathogenesis than TAA. Indeed, AAA is a very complex disorder, whose onset is linked not only to genetic predisposition, but prevalently to lifestyle-associated risk factors, including hyperlipidemia, hypertension, sex, age. In addition, smoking is generally regarded as the most important risk factor [10].

In contrast, genetic factors lie at the basis of TAA formation [12,13]. Accordingly, about 20% of TAA cases show classic Mendelian inheritance with high or complete penetrance and a positive familial history. Familial TAAD can be subdivided into syndromic presentations that show prominent features of a systemic connective tissue disorder (such as Marfan, Loeys–Dietz and Ehlers–Danlos syndrome) and non-syndromic presentations (such as bicommissural aortic valve with TAA, and isolated familial TAA). Seven susceptibility loci have been associated with TAA syndromic and non-syndromic forms (see Table 3) [12–16]. Related-genes have revealed that perturbed extracellular matrix signaling cascade interactions, deficient intracellular components of the smooth muscle contractile apparatus and deregulation of transforming growth factor-β cytokine (TGF-β) pathway are the key TAA mechanisms (see Table 3) [2,17–20]. The involvement of TGF-β pathway has particularly opened unexpected new investigation ways for familial TAA forms. Pathogenesis of familial TAA forms is, indeed, today better understood. As result, the management strategies for the medical and surgical treatment of familial TAAs are becoming increasingly gene-tailored [4,15]. In addition, these pathogenetic insights have delivered new treatment options (i.e. angiotensin receptor blockers, which are antagonists of TGF-β signaling pathway, to reduce aorta dilatation), that are currently investigating in large clinical trials [21–23]. On the other hand, this is suggested by 2014 ESC guidelines [4].

Table 1
Heterogenous features in two aorta aneurysms (AAA and TAA).

Different features	Abdominal aortic aneurysm (AAA)	Thoracic aortic aneurysm (TAA)
Inheritance	Controversial	20% of cases shows classic Mendelian inheritance with high or complete penetrance
Incidence	3.9% in men (65–75 years) 0.7% in women	10.4/100.000
Gender Prevalence	1.3%8.9% in men 1.0%–2.2% in women	3%–4% in individuals older than 65 years
Male/Female ratio	6:1	1.6:1
Age of onset	75 years	65 years
Anatomical onset aortic tracts	Infrarenal	50% Ascending, 10% arch and 40% descending
Pathophysiology	Inflammation, smooth muscle cell apoptosis, reactive oxygen species, extracellular matrix degradation, and activation of matrix metalloproteinases	Perturbed extracellular matrix signaling cascade interactions, deficient intracellular components of the smooth muscle contractile apparatus and deregulation of transforming growth factor-β cytokine (TGF-β) pathway are the key TAAD mechanisms

Of course this discussion on the genetics of aorta aneurysms would result incomplete without considering sporadic forms of TAA. On the other hand, sporadic TAAs represent the major number of TAA cases. Their incidence is also increasing in our population, especially in aged subjects [17,18,24]. As consequence, sporadic TAA is becoming a common and serious health risk. Despite this, it still is not clear the weight of genetic component in its susceptibility. A very limited number of genetic studies have been until now executed. As consequence, it is difficult to make generalizations about the disease pathways or genetic risk factors contributing to sporadic TAA forms. Molecular and genetics mechanisms of the non-familial TAA forms, still remain largely unknown. Sporadic TAA is, indeed, considered a pathology by unclear mechanisms [17,18,24]. Being silent, its detection is often fortuitous, with identification occurring during a routine physical examination or during an unrelated medical evaluation. Once suspected, confirmation by imaging clinical approaches is needed to allow the choose of the unique treatments for TAA, namely the surgery procedures, including elective surgery or endovascular repair before the onset of catastrophic and fatal complications (i.e. dissection or rupture), as recommended by 2014 ESC guidelines (see Table 2) [4,17,18,24]. In accordance with this, here it describes, as instructive example, a case, a 71 year old man, arrived in the out-patient department for a routine health screening for employment (see Fig. 2 A and B, and related legend). After a meticulous physical examination, TTE was performed and permitted to diagnose an ascending aortic aneurysm of very consistent size (see Fig. 2 A and B).

At present, there are no biomarkers available to identify sporadic TAAs before visible symptoms. However, it is in increasing the opinion to consider the sporadic TAA forms as immune/inflammatory diseases with a strong genetic component [25]. As result, a better characterization at the molecular level of sporadic TAA is necessary. Firstly, it might likely lead (i) to early predict and diagnose these diseases in a more accurate manner. Second, in a near future it might (ii) permit to translate the genomic information to the clinic, and (iii) improve our understanding of the disease processes, help us to develop better preventive and diagnostic tools, and (iv) lead to the design of new ways to manage sporadic TAA in the era of personalized medicine. This review summarizes for the first time the very limited literature data about genetic studies on sporadic TAA in order to identify disease pathways and their genetic variants able to modulate the susceptibility of sporadic TAA. In addition, evidences about potential associations of inflammatory genetic factors with sporadic TAA are also reported in order to support the current theory of the key role of chronic inflammation in sporadic TAA. This might permit to identify an inflammatory pathway having the role of hub, whose its active stimulation might determine as consequence the onset of sporadic TAA. A very contribution in the research on sporadic TAA might derive from this discovery and open new ways, which might be translated in clinical applications as preventive, diagnostic and prognostic biomarkers and targets for personalized therapies.

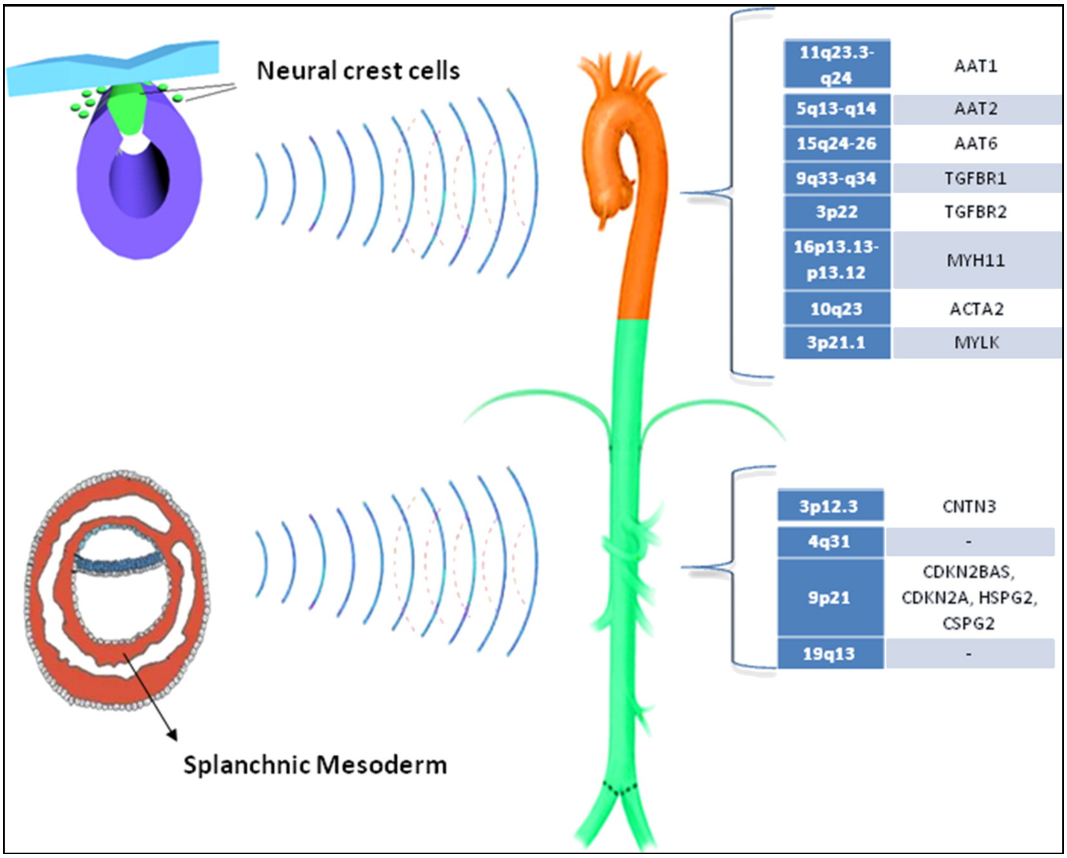
Table 2
Recommendations in diagnostic evaluations and medical and surgical treatments for TAAD and AAA suggested by 2014 ESC Guidelines [4].

Aneurysms	Diagnostic approaches	Medical treatment	Endovascular and surgical treatments
TAAD	The guidelines recommend: transthoracic echocardiogram (TTE) in the assessment of the aortic root and proximal ascending aorta due to the improvements in image quality; the transesophageal echocardiogram (TEE) in evaluation of the rest of the thoracic aorta; caution in using 3-dimensional echocardiography because its incremental value in clinical practice has yet to be evaluated. When an aortic aneurysm is diagnosed, it is important to perform computer tomography (CT) or magnetic resonance imaging to assess the involvement of the rest of the aorta In case of aortic dissection, the guidelines maintain the standard Stanford and De Bakey classifications although, in general, they employ the Stanford classification, based on whether or not the ascending aorta is involved. What is truly novel is the definition of a new time-based classification that distinguishes among acute (<14 days), subacute (up to 3 months), and chronic (more than 3 months) disease. One of the important contributions of the guidelines is the assessment of the a priori probability of finding dissection in a given patient. The 3 sources of information (predisposing conditions, type of pain, and physical examination) are determining factors in the initial evaluation of the patient. The role of D-dimers during the early hours of aortic dissection is also pointed out, although the main limitation is that they are not elevated in intramural hematoma or penetrating aortic ulcer. Computed tomography is undoubtedly the most widely available and accurate technique for the diagnosis of dissection and is especially useful for the study of the extension of the dissection and branch compromise. Nevertheless, TTE is included as the first-line diagnostic technique since a positive result helps to hasten the application of the therapeutic strategy. In unstable patients, the choice between TEE and CT depends on the availability of experts in the center. The latter would be indicated in stable patients	With respect to medical treatment in chronically ill patients, the guidelines stress general measures, including smoking cessation, blood pressure control (<140/90 mm Hg), and avoidance of competitive sports in patients with aortic dilation. There are some novelties in terms of drug therapy: treatment with losartan could reduce both the progression of aortic dilation and aneurysm formation in Marfan syndrome.	Endovascular treatment might be used for TAAD. However there are specific recommendations for thoracic aorta that refer to proximal and distal landing zones, which should be at least 2 cm long and less than 40 mm in diameter. In those patients with chronic aortic aneurysm, the guidelines recommend oversizing of the stent diameter by 10% to 15% with respect to the landing zones. In aortic dissection, oversizing to any extent is advised against. During the procedure, the blood pressure should be lowered and preventive cerebrospinal fluid drainage should be performed in patients at high risk of paraplegia Concerning surgical treatment of aortic dissection, the guidelines insist that it is indicated in all patients with type A acute aortic syndrome and stress that the aim should not only be to save the patient's life, but also to prevent late reinterventions. On the other hand, in the presence of organ malperfusion, a hybrid procedure (surgery and endovascular treatment or fenestration) may be the best option. The guidelines also recommend endovascular treatment in complicated type B aortic dissection (class I recommendation) and their advice is that this option be considered in uncomplicated dissections (class IIa recommendation). The indications for surgery in patients with ascending aortic aneurysm have not changed, except for the definition of this condition as a risk factor in patients with Marfan syndrome or bicuspid aortic valve having an increase in aortic diameter > 3 mm/year (in previous guidelines, a growth of >2 mm/year was considered). Although in patients with Marfan syndrome the indication for surgery is established when the diameter is ≥50 mm, or ≥45 mm in the presence of risk factors, in those with bicuspid aortic valve, surgery is indicated when the diameter of the ascending aorta is ≥55 mm, or ≥50 mm in the presence of risk factors. The guidelines point out the importance of indexing the diameters in patients with a small body surface area, especially in those with Turner syndrome, and recommend surgery with indexed diameters >27.5 mm/m2.
AAA	The guidelines recommend ultrasound as technique of choice for the initial study of the abdominal aorta. Although linear probes are more accurate, those used in echocardiography enable a correct assessment in most cases. The anteroposterior diameter of the abdominal aorta should be measured from outer edge to outer edge in a circular transverse image. Due to the variability in the measurement, it is recommended that variations of less than 5 mm be interpreted with caution One interesting aspect is the screening of AAA by means of abdominal ultrasound. The prevalence among men over 65 years of age has been calculated to be 5.5%. On the basis of recent studies, examination of the abdominal aorta with conventional echocardiography is recommended, as AAA are diagnosed in 3.5% to 4% of men aged over 65 years in less than 1 min.	With respect to medical treatment in chronically ill patients, the guidelines stress general measures, including smoking cessation, blood pressure control (< 140/90 mm Hg), and avoidance of competitive sports in patients with aortic dilation Concerning drug therapy, guidelines underline that the use of statins could reduce the progression of aneurysms	Endovascular treatment might be also used for AAA. The indications for elective surgery are a diameter > 55 mm, a growth rate of >10 mm/year, or the development of symptoms. In smaller aneurysms, the conservative approach is a better option than surgery (open or endovascular). The indication for surgery in women is an especially complex decision. The rates of rupture for a given aortic diameter are 3 to 4 times higher in women than in men, and the aortic diameter at the time of rupture is, on average, 5 mm smaller.16 For this reason, the indication for surgery appears to be justified when the diameter is >50 mm.

2. Focus on the role in sporadic TAA of genetic variants in candidate genes encoding components of pathways associated with extra-cellular matrix remodeling

2.1. MMP-pathways

As well recognized, aorta media is composed by vascular smooth muscle cells (VSMCs) and extracellular matrix (ECM) proteins, primarily elastin and collagen. Maintaining a balanced composition of VSMCs and ECM proteins appears to be critical for preserving the important functional properties of the thoracic aorta, especially its mechanical compliance with pulsatile blood flow. Disturbances in the molecular and cellular balance, resulting in excessive ECM degradation, might lead to progressive aortic wall deterioration, expansion, and rupture [17,18,24]. Recent studies indicate that VSMCs in condition of endothelium dysfunction participate in remodeling of aortic wall by localized



11q23.3-q24	AAT1
5q13-q14	AAT2
15q24-26	AAT6
9q33-q34	TGFBR1
3p22	TGFBR2
16p13.13-p13.12	MYH11
10q23	ACTA2
3p21.1	MYLK

3p12.3	CNTN3
4q31	-
9p21	CDKN2BAS, CDKN2A, HSPG2, CSPG2
19q13	-

Fig. 1. TAAD and AAA are two distinct pathological entities. The large heterogeneity in two aorta diseases is principally due to their different embryological origin. Thoracic aorta derives from neuronal crests and abdominal aorta from splanchnic mesoderm. Disease susceptibility also varies in two aorta sections, with abdominal aorta being more prone to atherosclerosis and aneurysm formation than the thoracic aorta. Underlying genetic factors contributing to two diseases also differ based on the site of the clinical manifestation, as well as their weight.

production of matrix metalloproteinase (MMPs) [17,18,24]. MMPs are a family of more than 20 zinc-dependent proteolytic enzymes [26,27]. They play vital roles in diseases related to ECM metabolism and aortic wall remodeling, which might be relevant to the development of aneurysms or dissection. In addition, this has led to hypothesize that genetic variants affecting expression or activity of MMPs might contribute to thoracic aortic diseases. Accordingly, research groups demonstrated significant associations between polymorphisms in *MMP* genes and sporadic TAA. In particular, in 2006 Chen and colleagues, by genotyping for −8202A/G, IVS4 + 3G/T, and 2003A/G [Q668R] polymorphisms in *MMP-9* gene 28 patients with degenerative TAA, 60 with dissection and 111 control patients, observed increased MMP-9 expression in patients with thoracic aortic diseases. They also found that frequency of the −8202G allele was significantly higher in patients with TAA and dissection than in control subjects (0.36, $P < 0.001$). Patients with TAA and dissection were nearly 5 times more likely than control subjects to have the G allele (adjusted odds ratio, 4.87; 95% confidence interval, 2.04–11.64). There were no significant associations between the IVS4 + 3G/T or 2003A/G polymorphisms and TAA and dissection [28].

Table 3
Susceptibility loci associated with syndromic and non syndromic TAA and AAA.

	Chromosomal location	Genes
Non-syndromic TAA	11q23.3-q24	AAT1
	5q13-q14	AAT2
	15q24-26	AAT6
	9q33-q34	TGFBR1
	3p22	TGFBR2
	16p13.13-p13.12	MYH11
	10q23	ACTA2
	3p21.1	MYLK
Syndromic TAA	2q31	COL3A1
	15q21.1	FBN1
	3p22	TGFBR2
	9q33-q34	TGFBR1
	15q	SMAD3
Abdominal Aortic Aneurysm	3p12.3	CNTN3
	4q31	–
	9p21	CDKN2BAS, CDKN2A, HSPG2, CSPG2
	3p22	TGFBR2
	16p13.13-p13.12	MYH11
	2q31	COL3A1
	1p13.3	sortilin-1 (SORT1)

Subsequently, Lesauskaite and colleagues detected eventual association of 5A/6A polymorphism in the promoter region of *MMP-3* gene with TAA, in 76 patients (age ranged from 31 to 81 years; median age, 64 years) with dilatative TAA and a random sample of the population ($n = 604$). The prevalence of MMP-3 genotypes was similar in the group of patients with TAA and random sample of population. The frequency of 5A allele did not differ significantly between both groups and was 0.506 and 0.514, respectively. Male carriers of 5A/5A genotype were significantly younger compared with those with the 6A/6A genotype [29].

In 2012, Kato and colleagues performed an association study for 95 polymorphisms in 89 candidate genes and TAA in 103 Japanese patients with this condition. Evaluation of genotype distributions by the Chi-square test and subsequent multivariable logistic regression analysis with adjustment for covariates revealed that the −340A → G polymorphism (rs514921) of *MMP-1* gene was significantly ($P = 0.0288$) associated with the outcome of TAA, with the minor G allele being related to a favorable outcome [30].

In 2014, Wang and colleagues assessed the association of 4 single-nucleotide polymorphisms (SNPs) in *MMP-9* and *TIMP-3* genes with

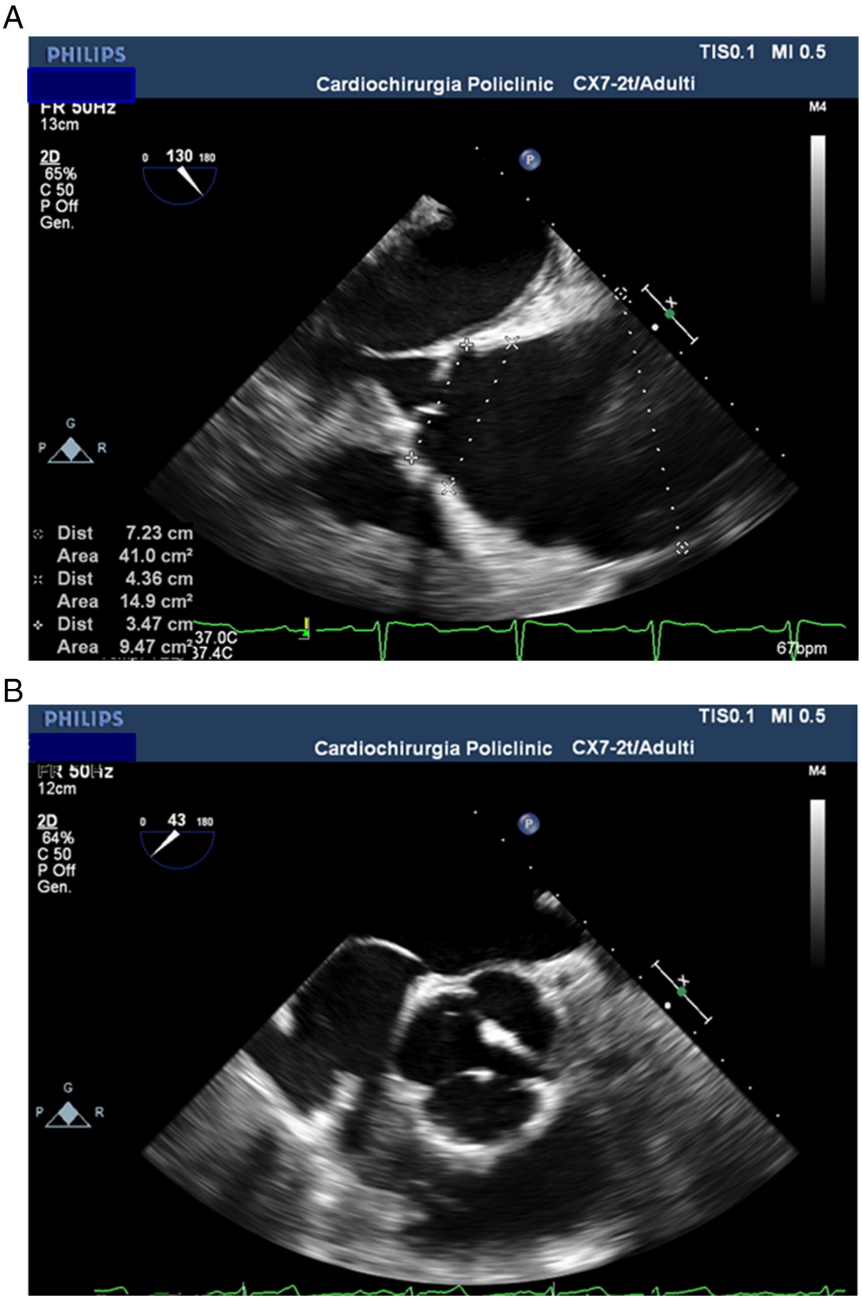


Fig. 2. A and B. A 71-year-old Sicilian man arrived in the out-patient department for a routine health screening for employment. After a meticulous physical examination, TTE was executed and showed ascending aorta aneurysm of a very consistent size, see A. In B, TTE showed tricuspid aortic valve, with a minimal aortic regurgitation and calcification on left aortic cuspid. He had no complaints before presentation. His medical history was significant for hypertension. He had no known allergies. His medications included beta blockers and ACE inhibitors.

TAD risk in Chinese Han population. A total of 206 Chinese patients with thoracic dissection and 180 controls were included in this study. Four SNPs (rs3918249, rs2274756, rs9609643 and rs8136803) were genotyped using high-throughput MALDI-TOF mass spectrometry. The G allele frequency for the *MMP-9* SNP rs2274756 was significantly higher in female patients than in female controls ($P = 0.0099$). Moreover, after adjusting for traditional cardiovascular risk factors (sex, age, hypertension, dyslipidemia, diabetes and smoking habit), the rs2274756 polymorphism (odds ratio: 0.30; 95% confidence interval: 0.11 to 0.79, $P = 0.015$) resulted in an independent susceptibility factor for dissection in females. No associations were found between the other SNPs and this disease [31].

Similar interesting data have been recently obtained in studies performed of my research group on sporadic TAAD. Precisely, we analyzed 161 cases affected by sporadic TAA, 18 cases with thoracic dissection and 128 controls for three SNPs in *MMP-2* and *MMP-9* genes. In particular, we obtained that rs3918242 *MMP-9* and rs2285053 *MMP-2* SNPs are an independent factor for sporadic TAA and dissection [32,33]. Consistent with these data, increased plasma levels of MMP-9 and MMP-2 were detected in the cases carriers of these SNPs, which positively correlate with elastic fragmentation and the elevated amounts of MMP-9 observed in their tissue aorta samples ($r = 0.497$, $P = 0.0001$; $r = 0.267$, $P = 0.03$, $r = 0.342$, $P = 0.006$, respectively, by non-parametrical Spearman correlation test; data not shown)[33].

2.2. TGF-β pathway

TGF-β is stored within the matrix and bound by ligands. Upon release, the peptide dimerizes, binds to cell surface receptors (TGFβ-R I or II), and initiates an intracellular signaling pathway that terminates in the nucleus with direct transcriptional regulation. Classically this signaling pathway utilizes the Smad protein complex and has been associated with increased production of collagen, elastin, and the tissue inhibitors of MMPs (TIMPs) for a net effect of ECM synthesis or stabilization. Conversely, via an alternate pathway, (ERK1/2) TGF-β appears to play a major role in proteolysis and ECM destruction. In its role as homeostatic regulator of the thoracic aortic ECM, recent data suggest that TGF-β contributes to TAA formation through modulation of MMP release and amplified proteolytic activity [34]. These observations also have led several groups to indentify associations between polymorphisms in TGF-β pathway and aneurysm. An increased number of studies demonstrated associations of SNPs in TGF-β pathway and abdominal aorta aneurysms [35,36]. In 2013, Wang and coworkers performed, for the first time, mutational analysis on FBN1, TGFBR1 and TGFBR2 genes (the three most common genes causing familial TAA) in 29 TAA Chinese patients (7 affected families and 22 sporadic patients), and found 21 mutations. In particular, they provided evidence that lack of exon 47 skipping of FBN-1 leads preferentially to cardiovascular defects and human ancestries influence genotype–phenotype correlation in TAA [16].

Subsequently, my research group analyzed the role of ten polymorphisms in genes encoding TGF-β isoforms and receptors in sporadic TAA. Our study included cases affected by sporadic TAA and two control groups. The most relevant finding obtained allows us to propose that rs900 *TGF-β2* SNP is associated with sporadic TAA in women, opening new perspectives for the analysis of sporadic TAA susceptibility factors and prevention [37].

3. Focus on the role in sporadic TAAD of genetic variants in pathways related to endothelium dysfunction

3.1. RAS pathway: focus on D/I ACE genetic variant

Components of the renin-angiotensin system (RAS) are heavily expressed within vascular tissues, and up-regulation of local angiotensin II synthesis is associated with adverse autocrine effects on arterial structure and function, i.e. tissue RAS activity is a major determinant of vascular tone. Angiotensin II promotes hypertension and alters shear stress. Experimental data suggest that activation of the RAS may lead to an increased inflammatory response in the vessel wall and to an activation of MMPs [38].

The synthesis of angiotensin II is performed by a key zinc metallopeptidase, the angiotensin-converting enzyme (ACE), that catalyzes the conversion of angiotensin I to angiotensin II. ACE is highly expressed in the aneurysmal vascular wall, in both human disease and animal models. ACE inhibitors protected against aortic expansion and rupture in animal models of aortic aneurysm. In addition, ACE inhibitors were associated with a decreased risk of aneurysm rupture in a clinical study [38]. Thus, ACE might be critical in aortic aneurysm development because of the relation between the RAS and blood pressure (see above), which is a known risk factor for aortic aneurysm. The *ACE* gene is located in chromosome 17q23.3. In intron 16 of this gene, a polymorphism comprising an insertion (I) or a deletion (D) of a 287-bp Alu repeat sequence has been identified that results in three genotypes: homozygous DD, II and heterozygous ID [39].

This I/D polymorphism within the *ACE* gene has been associated with many diseases, such as myocardial infarction [40]. The *ACE* I/D polymorphism could account for approximately half of the observed variance in ACE levels [39]. Individuals who are homozygous for the D allele have the highest levels of ACE, those who are homozygous for the I allele have the lowest and heterozygous individuals have an intermediate level [39]. Many studies have evaluated the association between *ACE* I/D polymorphism and aortic aneurysm risk, but the results are conflicting. Thus, two recent meta-analysis have been performed by Song and colleagues and Huang and colleagues [41,42]. Song and colleagues included ten studies (our study included; [32]) with 3557 cases and 5231 controls. The association between *ACE* I/D genotype and aorta aneurysm risk was significant (OR = 1.30; 95% CI, 1.07-1.57; $P < 0.01$; $I^2 = 68\%$). When stratified by ethnicity, a significantly elevated risk was observed in Caucasians (OR = 1.31; 95% CI, 1.07-1.61; $P < 0.01$; $I^2 = 71\%$). In the abdominal AA subgroup, a significantly increased risk was observed (OR = 1.29; 95% CI, 1.03–1.62; $P = 0.02$; $I^2 = 73\%$). However, ACE I/D polymorphism was not associated with thoracic aneurysm risk (OR = 1.33; 95% CI, 0.85-2.07; $P = 0.21$; $I^2 = 52\%$). Subgroup analysis on blood pressure status showed that an increased risk was found in hypertensive patients (OR = 1.52; 95% CI, 1.02-2.26; $P = 0.04$; $I^2 = 0\%$) but not in normotensive subjects (OR = 1.46; 95% CI, 0.72-2.96; $P = 0.30$; $I^2 = 25\%$) [41].

In the meta-analysis of Huang and colleagues [42], fourteen case–control studies, including a total of 3938 cases and 5748 controls were included. Among these studies, a study performed of my research group was included [43]. This meta-analysis showed a significant association between ACE I/D polymorphism and aortic aneurysm risk (OR = 1.53, 95% CI 1.26-1.87, $P < 0.01$). In the subgroup analysis by ethnicity, a statistically significant association was found in Caucasians (OR = 1.46, 95% CI 1.20-1.77, $P < 0.01$), but not in Asians. In the subgroup analysis by type of aortic aneurysm, this polymorphism was significantly associated with AAA risk (OR = 1.38, 95% CI 1.10-1.74, $P < 0.01$), TAA risk (OR = 1.59, 95% CI 1.11-2.29, $P = 0.01$) and aortic dissection risk (OR = 2.43, 95% CI 1.07-5.52, $P = 0.03$). Stratification by hypertension status showed that hypertensive patients with this polymorphism were associated with increased aortic aneurysm risk (OR = 1.47, 95% CI 1.03-2.09, $P = 0.03$), whereas normotensive individuals with this polymorphism did not have an increased aortic aneurysm risk [42].

3.2. NO pathway

Nitric oxide (NO) has multiple effects on vessel wall biology that could be important in aneurysm pathogenesis, including vasodilatation and inhibiting smooth muscle migration/proliferation [17,18,24]. The *Nitric Oxide Synthase 3* (*NOS3*) + 894G > T (Glu298Asp) polymorphism has been associated with reduced NO production [44]. In addition, variable number of tandem repeat (VNTR) polymorphism in intron 4 (eNOS 4a/b polymorphism), have been shown to affect NO metabolism and increase the risk for cardiovascular events [44]. Additionally, this *eNOS* gene polymorphism has been also shown to be associated with hypertension [44].

In 2014, Ekmekçi and colleagues assessed the association of *eNOS* gene polymorphisms with aortic dissection. In this study, patients who underwent surgery with the diagnosis of aorta dissection and survived after the operation between May 2007 and June 2011 were recruited retrospectively. Among the polymorphisms, the distribution of eNOS4 a/b gene polymorphism differed significantly from the control group, with higher frequencies of eNOS 4a/a and 4a/b genotypes in the case group ($x(2) = 7.16$, $P = 0.03$) [45].

Significant associations were observed in recent studies performed of my research group between the rs2070744 (−786C/T) *eNOs* polymorphism and sporadic TAA and dissection, by analyzing 18 cases with dissection, 161 cases with sporadic TAA and 128 controls. In particular, higher frequency of −786 T allele was assessed in two case groups than controls ($P = 0.03$ and $P = 0.00007$, respectively by $\chi 2$ test). On the other hand, this SNP located in the promoter region determines a reduced gene transcription [32,33].

4. Focus on the role in sporadic TAAD of genetic variants in pathways related to inflammation

Recent evidence proposes sporadic TAAD as an immune disease with a strong genetic component [25]. An active participation of both innate/inflammatory and clonotypic responses has been evidenced. Infiltration of inflammatory/immune cells has been actually identified through immune-histochemical assays both in the media and adventitia from aorta samples of patients with sporadic TAA [46, 47]. Accordingly, we observed significant increased amounts of CD3 + CD4 + CD8 + CD68 + CD20 + cells in tissue aorta samples from patients with Stanford type A aortic dissection [32]. Increased plasma levels of inflammatory markers, such as C-reactive protein (CRP) and inflammatory cytokines have been observed in aortic dissection and sporadic TAA patients [48]. In accordance with these data, we assessed higher plasma levels of Interleukin-(IL-) 6, Tumor necrosis factor-α (TNF-α), Interferon (INF)-γ, CRP, MMP-2 and-9 plasma levels in Stanford type A aortic dissection and sporadic TAA patients than controls (12.66 ± 2.1 vs. 3.1 ± 0.99, $P < 0.001$; 16.78 ± 1.2 vs. 7.1 ± 2.2, $P < 0.0001$; 12.13 ± 1.7 vs. 2.1 ± 0.5 $P < 0.0001$; 14.66 ± 3.2 vs. 4.6 ± 1.67, $P < 0.0001$; 56.8 ± 3.8 vs. 12.54 ± 1.6, $P < 0.0001$; and 59.7 ± 3.7 vs. 11.7 ± 2.6, $P < 0.0001$, respectively) [32].

Emerging involvement of chronic inflammation is leading to identify inflammatory pathways and their genetic variants, which might operate as key link between the onset of sporadic TAA and immune system. However, no literature data are actually reported about associations between SNPs in *immune/inflammatory genes* and sporadic TAA. Despite this, researchers are focussing their attention on an innate immune pathway, the Toll-like receptor-4 (TLR-4), which has been associated with the pathophysiology of a large number of CVDs, including atherosclerosis, cardiac dysfunction, congestive heart failure and other vascular diseases, as amply stressed by Frantz and colleagues [49]. Rising evidence on TLR-4 signaling pathways and TAAD is described below.

4.1. TLR-4 pathway

The TLR-4 pathway is able to recognize both pathogens and endogenous ligands. Its structure consists of three domains: an extracellular leucine-rich repeat (LRR) domain, a transmembrane domain, and an intracellular Toll-interleukin-1 receptor (TIR) domain. The extracellular LRR domain is involved in recognition of the lipopolysaccharide (LPS) of Gram-negative bacteria, the prototypic TLR-4 ligand. Other exogenous TLR-4 ligands are the fusion protein of respiratory respiratory syncytial virus and the envelope protein of mouse mammary tumor virus [50]. In addition, endogenous molecules can directly or indirectly interact with TLR4 pathway, such as heat-shock proteins (HSPs), hyaluronic acid, β-defensin-2, oxidized-LDL (ox-LDL), fibronectin, and amyloid peptide [50]. Its activation implies a downstream signaling mediated by several intracellular adaptor molecules, inducing the activation of transcription factors, such as Nuclear factor (NF)-kB, and consequently the production of different inflammatory mediators [50,51]. Anti-inflammatory mediators, such as interleukin (IL)-10, are also produced by the parallel activation of anti-inflammatory pathways, which limits potential tissue damage from excessive activation of the innate immune system. TLR-4 pathway also triggers instructive immunity. In antigen-presenting cells, TLR-4 pathway activation induces the expression of costimulatory molecules and the Major histo-compatibility complex class II antigens, molecules which contribute to sustain the activation of instructive responses. Its expression has been also observed on epithelial cells at potential sites of pathogen entry, including skin, respiratory, intestinal and genitourinary tract, and on endothelial cells and smooth muscle cells [50].

As above mentioned, it has been suggested that TLR-4 pathway has a key role in the pathophysiology of several CVDs [50,52–55]. Recently, the group of Pasterkamp has provided an overview of the endogenous molecules, released under cellular cardiovascular stress and damage, the DAMPs, which can trigger innate immunity via TLR-4-mediated signaling pathway and induce CVD onset as consequence [52]. In addition, polymorphisms of *TLR-4* gene (MIM: 603030), and particularly the rs4986790 *TLR-4* polymorphism, have been associated with the risk of several CVDs and other age-related diseases, even if contrasting results have been reported in literature [33,50,52–55].

Recently, it is also emerging its crucial role in age-related aorta dysfunction, aneurysm formation and related complications (dissection or rupture). In particular, recent experimental investigations in animal and ex vivo models report the role of TLR-4 pathway in the vascular aorta alterations (vascular remodeling -VR and medial degeneration-MD) and their complications, such as sporadic TAA. Precisely, they evidence as this pathway evocates or modulates increased expression and activation of endothelium dysfunction and extra matrix remodeling aorta pathways [56–64]. Pryshchep and colleagues demonstrated the TLR4-mediated signaling pathway expression in all cells of arterial wall and particularly in ECs and VSMCs. In addition, they also evidenced its functional importance in both mediating physiological aorta homeostasis and maintaining protection, as well as in inducting pathological aorta phenotypes, such as VR and MD [57,63,64]. Furthermore, Song and colleagues reported that signaling via *TLR*-4 pathway and its signal adaptors (i.e. MyD88) is responsible for the age-elevated basal IL-6 response using VSMCs from aged *TLR-4*-/- *and Myd88*-/-mice [59]. Eissler and colleagues observed an increased hypertension related to increased expression of TLR-4-mediated signaling pathway in vascular cells and consequent activation of ACE pathway in untreated hypertensive rats [56]. The group of Golzales-Ramos underlined that circulating Heat Shock protein 70, associated with an increased cellular aorta's damage, regulates the profibrotic response of human aorta VSMCs through increased transforming growth factor type-1 (TGF-1) expression, evocated by TLR-4 signaling pathway [61]. In addition, Li and colleagues reported the role of TLR-4 signaling pathway in regulating the MMP-9 expression in human VSMCs [58]. Bucci and colleagues recently emphasized as the vascular thoracic aorta homeostasis and its alteration in rats is based on the activity of TLR-4 signaling pathway and its cross talk with other stress and stretch pathways, including ACE, eNOs, and MMP pathways [60]. Furthermore, a recent study demonstrated in apolipoprotein E-deficient mice that it is possible to limit the inflammatory process by blocking TLR-4/c-Jun. N terminal kinase signaling pathway with Rosiglitazone in the initiation stages of aortic aneurysm development [62].

In complex, these literature data suggest that the TLR-4 pathway should seem to have a crucial role in sporadic TAA patho-physiology and represent a crucial link between pathways linked to sporadic TAA development and immune system, as suggested in our reports [33,50, 52–55]. However, this encouraging and increasing evidence is fruit prevalently of animal investigations. In addition, no genetic investigations support its evidence. The gene association study performed by my group in 2014, indeed, represents the first report which, through a human ex vivo approach, evidenced as some polymorphisms related to TLR4-mediated signaling pathway significantly modulate sporadic TAA risk [33]. In particular, we found that the rs4986790 (+896A > G) *TLR-4* polymorphism confers a higher susceptibility for sporadic TAA ($OR = 14.4$, $P = 0.0008$). Cases bearing the +896A TLR-4 allele showed higher systemic inflammatory mediator levels than other cases and control carriers. This effect increased in cases, which also were carriers of DACE/-1562TMMP-9/-735TMMP-2 alleles. Thus, we evaluated eventual differences in the levels of systemic inflammatory mediators between cases carriers of +896ATLR-4/DACE/-1562TMMP-9/-735TMMP-2 alleles (a combined genotype) and cases no carriers, and between cases and controls. Higher levels of systemic inflammatory mediators in patients with +896ATLR-4/DACE/-1562TMMP-9/-735TMMP-2 alleles were observed. In addition, they also had higher plasma levels of MMP-9 and -2 which correlated with the amounts of MMP-9 and elastic fragmentation observed in their tissue aorta samples. A higher chronic inflammatory infiltrate was also found in cases bearing these alleles, which positively correlated with histological abnormalities and levels of mediators [33]. In addition, they showed in their tissue aorta samples a typical morphological phenotype, characterized by elevated cystic medial degeneration, plurifocal medial apoptosis, and increased MMP-9 amounts, and defined in a previous study as phenotype III [65]. Furthermore, we detected that these alleles influence vascular biological aging, evaluating the gold standard aging marker, the telomere length, in a small number of cases and controls, selected randomly, but having the same age and gender. It characterized the 85% of the cases examined, which had lower telomere length, higher levels of mediators, increased amount of chronic inflammatory infiltrate [33,66,67].

Thus, our results emphasize as a combined risk genotype (+896ATLR-4/DACE/-1562TMMP-9/-735TMMP-2) associated with TLR4-mediated signaling pathway is able to modulate the grade of aorta age-related phenotypical, histological, and systemic abnormalities and consequently vascular aorta aging, onset, and progression of sporadic TAA. They also led us to suggest that this signaling pathway might also be an optimal target for new therapeutic treatments able to retard or block the typical aorta age-related changes which determine endothelial dysfunction, MD, and VR. This might open new perspectives for the prevention of both aortic VR and MD and sporadic TAA, by using combined risk genotype (+896ATLR-4/DACE/−1562TMMP-9/−735TMMP-2) as optimal genetic biomarker for the earlier detection of this silent pathology in preliminary phases and to treat with different and specific therapies depending on individual's genotypes [33].

However, future and ulterior more large studies are certainly need to validate the weight of our findings and suggestions, even if our data are the result of a relatively small sample and a very homogenous population. In addition, gene expression analyses, immunohistochemical TLR-4 quantification, and soluble TLR-4 level detection represent further objectives of our future studies.

5. Conclusions and perspectives

As summarized in this report, genetic component appears to play a role in onset and progression of sporadic TAA. Significant associations between some genetic variants in *genes* of ACE, NO, MMP, TGF-β pathways (associated with endothelium dysfunction, extracellular matrix remodeling and chronic inflammation) and sporadic TAA risk have been described (see Fig. 3). However, number of gene association studies reported in literature is very limited and consequently inadequate to evaluate the weight of these genetic factors in susceptibility of sporadic TAA. Particularly reduced to one or two studies are the investigations on the role of immune/inflammatory SNPs in sporadic TAA. Nevertheless, it is in increasing the opinion of scientific community to consider this pathology an immune disease, as well as its evidence [25]. Based on this, we evaluated, for the first time, the role of TLR-4-mediated signaling pathway and ten related genetic variants in the risk of sporadic TAA and dissection [32,33]. Interestingly, we found that their combined genotype was significantly represented in cases with sporadic TAA than controls. As result, they led us to suggest the crucial role of this pathway in the onset of this disease [33]. Likely, activation of TLR-4-mediated signaling pathway expressed both on EC and VSMC cells might determine activation or deregulation of ACE, NO, MMP, TGF-β pathways (associated with endothelium dysfunction, extracellular matrix remodeling and chronic inflammation) and as consequence sporadic TAA (Fig. 3). Accordingly, we postulated a sporadic TAA onset model, that we defined in Ruvolo et al. study as *model of the signaling pathway from the double-face*, given its features (see Fig. 4 of Ruvolo et al., 2014 study [33]; http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4120489/). We foretell that it can lead several researchers to perform additional investigations focused to clear the complex puzzle of this pathology. Revealing the role of TLR-4-mediated signaling pathway in sporadic TAA may serve as a starting point for future studies leading to a better understanding of the pathophysiological basis and perhaps effective treatment of this human disease. Future studies and additional efforts are, indeed, imperative as well as a combination of analysis based on genetic, transcriptomic, proteomic and epigenomic evaluations. Epigenomic, transcriptomic and proteomic approaches could particularly provide valuable insights about disease pathobiology, although it is very difficult obtaining human tissue aorta samples and appropriate controls. Thus, the development of animal models might be a solution to study human aorta diseases by providing the means for testing new pharmacological interventions. There is a considerable debate in the field of aneurysm research about the disadvantages and advantages of the various rat and mouse models in which aneurysms can be generated in an experimental setting. These models have provided useful information, but a model that replicates the chronic disease seen in humans remains to be produced. Given the unresolved questions, unclear answers and numerous gaps about the genetic factors, mechanisms and the clinical management and outcome of sporadic TAA, the solution might be in looking with new eyes in order to make new discoveries, although, the way of research to execute is still long and difficult. On the other hand, Marcel Proust affirmed "*The real voyage of discovery consists not in seeking new landscapes*, *but in having* new eyes."

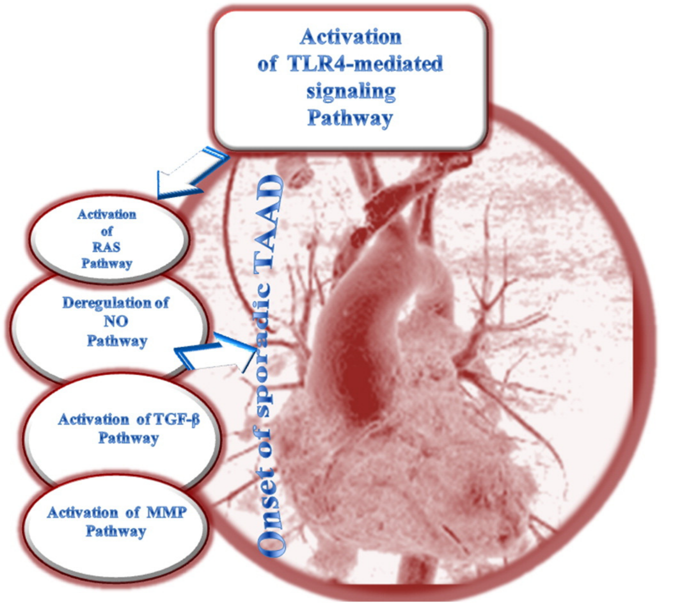


Fig. 3. Activation of TLR-4-mediated signaling pathway expressed both on EC and VSMC cells might determine activation or deregulation of ACE, NO, MMP, TGF-β pathways (associated with endothelium dysfunction, extracellular matrix remodeling and chronic inflammation) and as consequence sporadic TAA onset. (see Figure 4 of Ruvolo et al., 2014 study; http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4120489/).

Conflict of interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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