ASSOCIATION OF ANTIHYPERTENSIVE MEDICATION AND SEVERITY OF DUPUYTREN'S DISEASE

Stella van Bergen
S3496023
University of Groningen

Under supervision of:
Sophie Riesmeijer, MSc
Department of Plastic and Reconstructive surgery
University Medical Center Groningen

Prof. dr. Ruud Bank
MATRIX Research Group, Department of Pathology & Medical Biology
University Medical Center Groningen

Table of Contents

ABSTRACT **3**

INTRODUCTION **4**

Epidemiology *4*

Treatment *5*

Pathogenesis and aetiology *5*

METHODS **7**

Study population *7*

Data selection and editing *7*

Research questions *8*

Outcomes *8*

Analyses *8*

RESULTS **9**

Patient characteristics *9*

Regression analyses *10*

DISCUSSION **11**

Angiotensin receptor blockers and DD *11*

ACE inhibitors and DD *11*

Genetic variation in the TGFβ1 gene *12*

Other antihypertensive medications and DD *12*

Other medications and DD *12*

Strengths of our study *13*

Limitations of our study *13*

Conclusion *14*

Future perspectives *14*

BIBLIOGRAPHY **15**

SUPPLEMENTARY **20**

ABSTRACT

Dupuytren's disease (DD) is a common, fibroproliferative disorder of the palmar fascia that causes flexion deformities of the fingers. It is a complex disease caused by both genetic and environmental factors and has an incompletely understood aetiology. Transforming growth factor beta 1 (TGFβ1) plays an important role in the disease mechanism of DD. Recently, angiotensin II was discovered to modulate TGFβ1 production. We thus hypothesised angiotensin II blockers could be associated with the severity of DD. The aim of this study was to investigate the effect of angiotensin receptor blockers (ARBs) and angiotensin converting enzyme inhibitors (ACEi) on the severity of DD. This retrospective study used a database of 1761 DD patients, including data on patient's clinical characteristics, medication use, flexion deformity clinimetrics, and genotype data. Ordinal logistic regressions were performed to study the association between ARBs or ACEi and DD severity (defined in Tubiana stages). The regression analysis showed a significant negative association between ARB use and DD severity, ACEi's did not show a significant association. In conclusion, we observed a significant association between ARBs and DD severity. This study may provide new insights into medication and DD severity. The results of this study may be used to develop new and improved therapies for treating DD.

INTRODUCTION

Dupuytren's Disease (DD) is a fibroproliferative disease of the palmar and digital fascia of the hand. It initially presents as a firm nodule in the palm of the hand. As the disease progresses the nodule grows in size creating fibrous rope-like collagenous cords that extend into the fingers. Over time these cords can thicken and have the capacity to contract, thereby causing permanent flexion contracture of the joints of the fingers.(DiBenedetti et al. 2011) DD is usually not painful, however, it may progressively hinder the movements of fingers.(Michou et al. 2012) The metacarpal phalangeal joint (MCP) and the proximal interphalangeal joint (PIP) are most commonly affected, the distal interphalangeal joint (DIP) less often.(Nunn and Schreuder 2014) The fourth and fifth digit are most often affected and might depict an early progression of the flexion contracture in the fingers.(Black and Blazar 2011) Regression of flexion contractures is uncommon.(DiBenedetti et al. 2011) DD has a wide array of symptoms and varies in severity, ranging from unnoticed to rapid deterioration.(Moermans 1996) DD may seriously affect activities essential to daily life, and therefore disturbs the quality of life. Commonly expressed problems are gripping, shaking hands and personal care.(Wilburn et al. 2013)

Epidemiology

Lanting et. al (Lanting et al. 2014) calculated a mean estimated prevalence of DD of 12% at 55 years of age, increasing to 29% at age 75 in the general population in Western countries. Men are more commonly affected than women.(Michou et al. 2012) Various studies have suggested both genetic and environmental risk factors.(Michou et al. 2012) Environmental risk factors include alcohol abuse, smoking, aging, injuries to the hands and intense manual labour.(Morelli, Fraschini, and Banfi 2017) Additionally, a high prevalence of DD has been observed in diseases such as hypertension, diabetes mellitus and epilepsy.(Salari et al. 2020)

Hypertension has been associated with DD: various studies have reported hypertension to be one of the diseases most commonly associated with DD.(Mansur, Oliveira, and Gonçalves 2017; Michou et al. 2012) These studies, however, do not comment on the severity of DD or on the use of antihypertensive medication. One study found that patients that used antihypertensive medication developed DD at a later age compared to patients without hypertension.(Shchudlo et al. 2020) Furthermore, other studies have also associated antihypertensive medication with DD: beta-blockers, for example, have been reported to reduce fibroblast proliferation in humans by blocking the process of endogenous beta adrenergic agonists which inhibit fibrosis.(Oliphant and Gouws 2019)

A systematic review and meta-analysis conducted by Broekstra et al. (Broekstra et al. 2018) showed a strong association between the occurrence of diabetes mellitus and DD. DD disease course is however generally milder in the diabetic population and less frequently requires surgery.(Zyluk and Puchalski 2015) These observations may suggest an association between antidiabetic medication and DD severity. Moreover, a shared genetic aetiology has been found between DD and body mass index, type II diabetes mellitus, high-density lipoproteins, and triglycerides. (Major 2019)

An association between a higher risk of DD and epilepsy has been reported (Broekstra et al. 2018), but the evidence of this relationship has been limited and inconsistent. Furthermore, the use of antiepileptic medication has been correlated with the development of

DD.(Critchley et al. 1976) One study reported that phenobarbital was associated with a profibrotic effect. When phenobarbital was substituted by carbamazepine this effect decreased.(Tripoli et al. 2009)

Treatment

Treatment for DD is aimed at reducing flexion contractures, as no cure exists. Treatment can consist of discontinuing a contracted cord (needle fasciotomy), or surgically removing it (fasciectomy).(Layton and Nanchahal 2019) The current guidelines for the management of DD recommend intervention when the flexion contracture of the finger limits the function of the hand, and the MCP or PIP joint are flexed to 30º or more.(Townley et al. 2006) The primary aim of these treatments is to restore hand function. However, complications can occur and there is often a high chance of recurrence.(Desai and Hentz 2011) The more aggressively the disease progresses, the higher the risk of recurrence after treatment and the possibility that the disease will become debilitating.(Moermans 1996) A treatment to prevent or cease disease progression or prevent recurrence does not yet exist. This is mainly because the underlying processes of the development of DD are incompletely understood.

Pathogenesis and aetiology

The disease mechanism of DD remains unclear, however, it likely results from the interaction of genetic, immunological and environmental factors that promote the progression of the disease.(Layton and Nanchahal 2019) In DD, subcutaneous fat is replaced by pathologic cords that emerge at the point of maximal stress between the dermis and the fascia. These cords consist of a dense collagenous matrix that contains fibroblasts. Here, fibroblasts transform into myofibroblasts (characterised by the presence of alpha-smooth muscle actin) due to the mechanical stress and the exposure to cytokines such as transforming growth factor beta 1 (TGFβ1)(Vaughan et al. 2000) Myofibroblasts can build intercellular joints and connect to the collagen fibres in the fascia. If these myofibroblasts experience stress, they contract and result in Dupuytren's contracture. In normal wound healing in non-predisposed individuals, once the stress is resolved the myofibroblasts will go through apoptosis due to the lack of external stimulation. However, for reasons that remain unclear, in individuals with DD, these pathological myofibroblasts do not go through apoptosis but continue to grow and contract, even after the stress source has resolved.(Feldman, Rozen, and Rubin 2017)

Eighty per cent of the disease occurrence of DD (e.g. heritability) can be explained by genetic risk. (Larsen et al. 2015) Although 28 genetic risk loci have been identified by genome wide association studies (Ng et al. 2017; Dolmans et al. 2011), they only explain a small proportion of the heritability.(Ng et al. 2017) Several of these risk loci contain genes that encode proteins in the Wnt-signalling pathway. Abnormal Wnt-signalling has been linked to several diseases, including DD.(Dolmans et al. 2011) The canonical pathway of Wnt-signalling activates β-catenin, resulting in alterations in gene expression, influencing cell proliferation and the regulation of fibroblast differentiation. *TGFβ* is one of the genes related to the Wnt/β-catenin pathway.(Dolmans et al. 2011)

TGFβ1 plays a key role in myofibroblast differentiation during wound healing and in fibrotic diseases like DD.(Tomasek, Vaughan, and Haaksma 1999) Cytokines in the TGFβ family (including SMAD proteins) regulate cell proliferation, migration, differentiation and play an

important role in development and tissue repair.(Ingman and Robertson 2009) In fibrosis, TGFβ1 induces proliferation of genetically abnormal myofibroblasts and increases the contractile force of DD cells.(Bisson et al. 2003)(Iwasaki et al. 1984) TGFβ1 has been shown to be upregulated in DD tissue.(Ratajczak-Wielgomas et al. 2012) Therefore, it is believed to be a promising therapeutic target for DD.(Badalamente et al. 1996; Ratajczak-Wielgomas et al. 2012)

Additional to the immunological effects of TGFβ1, genetic variations in association to the degree of TGFβ1 expression have been studied. Several studies have attempted to determine whether naturally occurring single-nucleotide polymorphisms (SNPs) in the TGFβ1 gene are associated with the production and expression of TGFβ1.(Zheng et al. 2013) A C-to-T SNP rs1800469 was found to be differentially related to the transcription factor that binds to the TGFβ1 promotor, the transcriptional activation of TGFβ1 and TGFβ1 plasma concentration levels.(Grainger et al. 1999) Other SNPs, including variants rs1800471, causing an arginine to proline (protein) change, and rs1800472, causing a threonine to isoleucine change, were also observed to be functional and affect the production and activation of TGFβ1. A change in signal sequence at rs1800470 , causing a leucine to proline variation, was reported to be associated with variations in TGFβ1 production levels.(Grainger et al. 1999; Awad et al. 1998; Suthanthiran et al. 2000) The G-to-A SNP rs11466314 and C-to-T SNP rs14466315 showed differences in the reporter gene expression of TGFβ1.(Shah, Hurley, and Posch 2006)

Recently, it became apparent that production of TGFβ1 might be mediated by the renin-angiotensin system (RAS), as Angiotensin II (Ang II) induces TGFβ1 production and systemic secretion by the mesangial cells of the kidneys.(Kagami et al. 1994; El-Agroudy et al. 2003) Angiotensin-converting enzyme inhibitor (ACEi) and angiotensin receptor blockers (ARB) on the other hand, have been reported to antagonise TGFβ1 expression.(El-Agroudy et al. 2003) The blocking of Ang II with inhibitors in animal models, decreased the overexpression of TGFβ1 in the kidneys and likely also systemically. This suggests that the RAS pathway plays a role in the regulation of TGFβ1, while TGFβ1 in turn is a known key mediator of fibrosis in both rat models and human kidney disease.(Noble and Border 1997) We hypothesised that inhibition of Ang II by either ACEi or ARB is associated with a lesser severity of DD through reduced TGFβ1 production and systemic secretion. Moreover, as several SNPs influence production of TGFβ1, and TGFβ1 in turn influences DD phenotype, we hypothesize that these functional gene variations may influence the association of ACE-I and ARB the severity of DD.

METHODS

Study population

For this retrospective study, we used the Genetic Origin of Dupuytren's Disease and Associated Fibromatoses (GODDAF) database. This database, created in 2007 to investigate the genetic background of DD, contains medical data of over 2000 Dutch patients with a fibroproliferative disorder, such as Dupuytren, Ledderhose, and Peyronie disease. In the study described in this thesis, only patients affected by DD (n=1761) were selected from the GODDAF database. Participants were diagnosed by plastic surgeons with clinical experience in treating DD, based on the presence of characteristic nodules or chords in the palm of the hand or fingers. Participants were asked to complete a questionnaire about their clinical and patient characteristics, including age, gender and medication use. The plastic surgeons completed a separate questionnaire on the clinical characteristics of these patients, including extension deficits of the MCP, PIP and DIP of all affected fingers. Additionally, DNA data were obtained via blood withdrawal in order to perform genotyping, as previously described.(Dolmans et al. 2011)

Data selection and editing

Severity of DD was defined based on the severity of contracture of the affected fingers, expressed in Tubiana stages.(Hindocha et al. 2008) To calculate the Tubiana stages the extension deficits of the MCP, PIP and DIP joints per finger were added up, resulting in a total passive extension deficit (TPED). A Tubiana stage of 0 means TPED of 0º, stage 1 means a TPED between 1 – 45º, stage 2 means a TPED between 46 – 90º, stage 3 means a TPED between 91 – 135º, and stage 4 means a TPED > 135º.(Tubiana 1999) Thus, higher Tubiana stages indicate more severe DD. For statistical analyses, the highest Tubiana stage of either hand was used.

Patient questionnaires, completed at entry, included questions on the use of medications known to be associated with DD (e.g., anticonvulsant drugs) and an open field where patients indicated other medications used. Trade/brand names of medications were converted to generic names and spelling errors were corrected. Medications were grouped based on their class (mechanism of action) including angiotensin-converting-enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB), beta-blockers, diuretics, calcium antagonists and statins. Epilepsy and diabetes medication were also available.

To assess the effect of genetic variation in the TGFβ1 gene on the association of ACEi or ARB with DD severity, we first identified single nucleotide polymorphisms (SNPs) associated with TGFβ1 expression. Genomic reference build 37 (GRCh37) was used in this study. We searched the website OMIM (https://omim.org/) for SNPs occurring in the TGFβ1-, TGFβ1-receptor- and SMAD genes. The SNPs were selected on criteria of missense mutations and protein altering mutations that modify TGFβ1 plasma levels. Fifteen SNPs associated with TGFβ expression were found in the literature.(Grainger et al. 1999; Shah, Hurley, and Posch 2006; Suthanthiran et al. 2000; Awad et al. 1998; Kinoshita et al. 2000; Saito et al. 2001; McGowan et al. 2003; Kotlarz et al. 2018; van de Laar et al. 2011) Of these 15 SNPs, only 6 (rs18004469, rs1800470, rs1800471, rs1800472, rs11466314 and rs11466315) were present in our genotype data, as the other 9 SNPs were not included on the genotyping array and were likely not available in the reference genome during genotype imputation. Allele dosages (ranging from 0 to 2) for the 6 SNPs for each participant were added to the dataset.

Research questions

Primary research question

Is the use of ARBs or ACEi associated with a lesser severity of Dupuytren's Disease?

Secondary research questions

Do genetic variants in the TGFβ1 gene have an effect on the association between ARBs or ACEi use and a lesser DD severity?

Are the other antihypertensive medications beta blockers, diuretics or calcium antagonists associated with DD severity?

Are statins, epilepsy medication or diabetes medication associated with DD severity?

Outcomes

Primary outcome

The association between ACEi and ARBs and the severity of DD.

Secondary outcomes

The secondary outcomes are whether genetic variations in the TGFβ1 gene affect the association of ACEi or ARBs with the severity of DD. Furthermore, we associated other antihypertensive medications with DD severity as a sensitivity analysis of the association between ACEi or ARB with DD severity. Lastly, we aimed to confirm the previously reported association of epilepsy and diabetes medications, and additionally use of statins, with the severity of DD.

Analyses

Descriptive statistics for continuous data were presented using means and standard deviations. Ordinal and nominal data were presented as frequencies and percentages. To test for the association between medications and DD severity, ordinal logistic regression analyses were performed in R version 4.0.4. (Team 2020) with the polr function of the package MASS.(Venables and Ripley 2002) Participants' allele dosage for each SNP was included in additional ordinal logistic regression analyses as a causal effect modifier (one analysis per SNP because of multicollinearity

RESULTS

Patient characteristics

For 1671 of the 1761 participants data on medication use were available. Of these 1671 participants 1247 (74.6 %) were male. The mean age of the participants was 63 (range 21 – 89) years old. The mean age of onset was between 50 – 59 years old. Participants had a mean of 1.1 fingers affected by DD. The age of onset was for all participants available. For 1051 of the 1671 participants data was available on whether both hands were affected. Medication use (grouped) is shown in Figure 1; for the specific medication names within groups, see Supplementary Figure 1a-f. For 260 of the 1671 participants genotype data were not available. Tubiana stage could be determined for 890 cases (53.3%) (see Table 1). In our study, fingers with Tubiana stage 0 were coded as 1, due to the low number of patients' fingers with a Tubiana stage of 0 (Table 1). For the distribution of ARB or ACEi use and allele dosages per Tubiana stage, see Supplementary Figure 2-7.

Table 1. Tubiana staging. Maximum Tubiana stages 0 and 1 were merged, due to the low number of participants with maximum Tubiana stage 0.

		N (%)	N missing (%)
maximum Tubiana stage		890 (53.3)	781 (46.7)
	0	4 (0.4)	
	1	401 (45.1)	
	2	329 (37.0)	
	3	106 (11.9)	
	4	50 (5.6)	

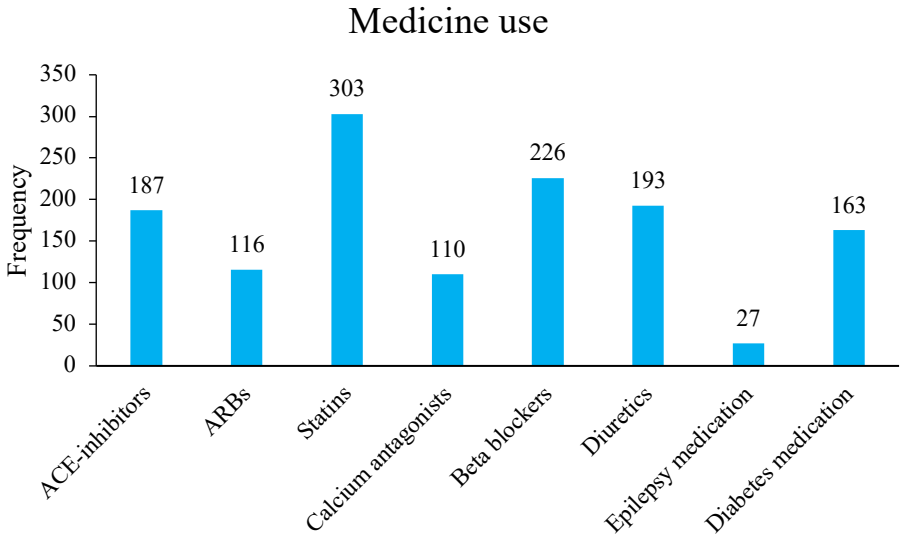

Figure 1. Frequency of medicine used by patients per medication group. Participants may use more than one medication.

Regression analyses

Use of ARBs was significantly associated with a lesser severity of DD (p=0.022). In Figure 2 a larger proportion of ARB use in lower Tubiana stages can be observed. DD patients that do not use ARBs have an odds ratio of 1.88 on having more severe DD compared to patients that do use ARBs. Although the proportion of ACEi use was larger in lower Tubiana stages (Figure 3), the use of ACEi did not show a significant association with DD severity (p=0.288).

In order to determine the influence of genetic variants that may increase TGFβ1 plasma levels on the association of ACEi or ARB use and DD severity, we performed additional logistic regressions, each one with an interaction term of one genetic variant (either rs18004469, rs1800470, rs1800471 or rs1800472) with ACEi and with ARB. None of the interactions between a SNP and use of ARB or ACEi was significant (p= 0.278, p= 0.567, p= 0.344, p= 0.443 and p= 0.0938, p= 0.378, p= 0.174, p= 0.0980 respectively), meaning that simultaneously having TGFβ1 plasma level increasing SNPs and using ARB or ACEi is not associated with a lesser severity of DD. Two SNPs (rs11466314 and rs11466315) were not analysed: as they contained too little non-zero values, the polr package was not able to construct a model with these SNPs.

As a sensitivity analysis, other hypertensive medications were associated with DD severity. Only beta-blockers had a significant positive association with DD severity (p=0.0451). Calcium antagonists nor diuretics had a significant association with DD severity (p=0.705, p=0.478 respectively).

Last, the ordinal logistic regressions of statins, epilepsy- and diabetes medication with severity of DD did not show any significant associations (p=0.468, p=0.460, p=0.995 respectively).

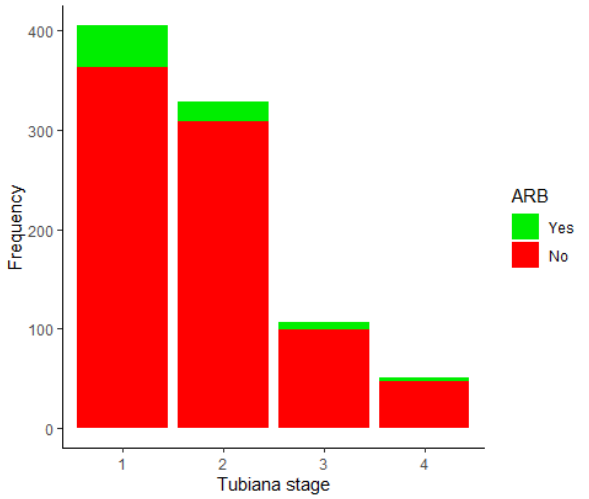

Figure 2. Use of ARBs per Tubiana stage.

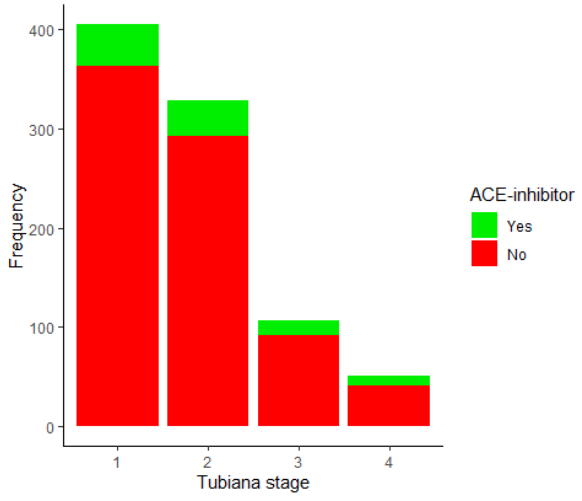

Figure 3. Use of ACEi's per Tubiana stage.

DISCUSSION

The main aim of this study was to investigate the association between the severity of Dupuytren's disease and the antihypertensive medications ARB and ACEi.

Angiotensin receptor blockers and DD

Our results show that the use of ARBs has a significant association with a lesser DD severity. This can be explained through the following mechanism. ARBs block RAS at the level of angiotensin II type-1 receptors (AT1R) (Abadir et al. 2018), irrespective of whether angiotensin II is produced via angiotensin-converting enzymes (ACE) or by non-ACE pathways, such as chymase.(Dzau, Sasamura, and Hein 1993). When Ang II binds to AT1R, systemic TGFβ1 expression is increased, impelling fibroblasts to differentiate into myofibroblasts. These myofibroblasts can then contract and in the case of DD form flexion contracture.(Tomasek, Vaughan, and Haaksma 1999) ARBs reduce the amount of systemic TGFβ1 by blocking AT1R and thus binding of Ang II to AT1R. Treatment with ARBs will therefore reduce the Ang II signalling through AT1R, leading to reduced TGFβ1 expression and thus possibly less severe DD. Moreover, under non-fibrotic conditions fibroblasts in the palmar fascia express TGFβ1 in low concentrations.(Badalamente et al. 1996) TGFβ1 is present in high concentrations in myofibroblasts and fibroblasts in all stages of DD.(Badalamente et al. 1996) Therefore, one could hypothesize that the use of ARBs would be beneficial during all stages of DD.

Another mechanism for the association of ARBs with less severe DD can be via the angiotensin II type-2 receptor (AT2R). With AT1R blocked, Ang II can only bind to AT2R. Less is understood regarding the AT2 receptor. AT2R is known to play a protective role against Ang II-induced fibrosis, as it (1) counteracts the effects of AT1R binding and (2) AT2R activation by Ang II binding inhibits the infiltration of macrophages (a known source of TGFβ1), reducing TGFβ1 stimulation of fibrotic pathways.(Wang et al. 2017)

ACE inhibitors and DD

ARBs and ACEi have different pharmacological mechanisms of action and differ in where they inhibit the RAS pathway. ARBs block AT1R, ACEi inhibit the conversion of Ang I to Ang II, decreasing the amount of available angiotensin II to bind to either AT1R or AT2R. Due to their selectivity, ARBs create a more complete blockade of the RAS pathway than ACEi.(Abadir et al. 2018) In our study, ACEi did not show a significant association with DD severity. This is not in line with an animal study by Abdelrahman et al. (Abdelrahman and Fayed 2019). They demonstrated that treatment with the ACEi perindopril suppressed TGFβ1 expression via Ang II blocking in rats, preventing fibrosis in the liver. A possible explanation for the inconsistency between studies is that the expression of AT2R is more prominent in human DD tissue compared to the expression of AT1R.(Stephen et al. 2018) This may suggest that by inhibiting the conversion to angiotensin II by ACEi, the cascade of AT2R binding is more severely affected than that of AT1R, and thus the anti-fibrotic properties of AT2R are inhibited more than the fibrotic effects of AT1R. The use of ACEi to lessen the severity of DD in humans therefore may have a limited effect.

Genetic variation in the TGFβ1 gene

Next, we studied if genetic variations in the TGFβ gene influenced the association of ARB and ACEi with DD severity. None of the interaction terms of the SNPs in the TGFβ1 gene with ARB or ACEi use showed a significant association with DD severity. In other words, the significant negative association of ARB with DD severity was negated by the TGFβ1 SNPs. This might be explained by the increased TGFβ1 expression that is associated with the SNPs, therefore nullifying the decreasing effect of ARB on DD. In this study, we only were able to study a selection of SNPs influencing TGFβ1 expression, as only data on six SNPs were available. Future studies could also focus on SNPs associated with TGFβ1 expression.

Other antihypertensive medications and DD

To increase our understanding of the association of ARBs and ACEi with DD severity and to take away the uncertainty that the association is in fact a resultant of the disorder hypertension itself; we performed a sensitivity analysis associating other antihypertensive medication with DD severity. Only beta-blockers showed a significant positive association with DD; diuretics and calcium antagonists were not significantly associated. Although to our knowledge few studies have explored the role of beta blockers in fibrosis, those available show contradictory observations. One study by Kobayashi et al. (Kobayashi et al. 2004) observed that beta blockers prevented cardiac fibrosis and improved survival in a rat model. Another study by Nakaya et al. (Nakaya et al. 2012) found the opposite effect, as with our results. They demonstrated the induction of cardiac fibrosis by the beta blocker metoprolol in a mouse model, through a G protein-independent and β-arrestin2-dependent pathway. Metoprolol blocked β_1-adrenergic receptors expressed on cardiomyocytes in vivo, inducing the cardiomyocytes to secrete fibrotic factors. This activated cardiac fibroblasts, resulting in fibrosis. Although, the mechanisms of cardiac fibrosis provide us with an explanation of the workings of beta-blockers in fibrosis, to our knowledge no consistent association between fibrosis and beta blockers has been described in humans. To our knowledge we are the first to report an association between beta-blockers and DD. Additional research is needed to underline the association and understand the mechanisms of beta-blockers and DD.

A study by Rayan et al. (Rayan, Parizi, and Tomasek 1996) reported that calcium antagonists partially inhibit lysophosphatic acid induced contraction of DD fibroblasts in vitro. This suggests that calcium antagonists may contribute to less severe DD. We however observed no association between calcium antagonists and DD severity. This difference might be explained by the fact that our study did not investigate the direct effect of calcium antagonists on DD fibroblasts, but aimed to study its association with disease severity on patient level. We found no known association between the use of diuretics and the severity of DD in literature. The findings of the positive association of beta blockers and the non-significant association of diuretics and calcium antagonists underline that the association found between ARB and DD severity is likely due to the effect of ARB and not a resultant of the disorder hypertensions itself.

Other medications and DD

As DD is genetically correlated with metabolic traits such as high-density lipoprotein and triglycerides, we hypothesised the use of statins could be associated with DD. The use of statins did not show a significant association with DD severity. A possible explanation could

be that statins are usually prescribed in cases of increased low-density lipoprotein, not for high-density lipoprotein or triglycerides. However, an in vitro study showed simvastatin inhibits TGFβ expression in lung fibrosis, suggesting statins might have antifibrotic properties.(Watts and Spiteri 2004) More research needs to be done to elucidate the association between statins and DD.

Our study did not show a significant association between anti-epileptic medication and DD severity. Although previous studies have implicated a link between anti-epileptic medication and the development of DD (Broekstra et al. 2018), there is little conclusive evidence.(Thurston 2003) Broekstra et al. (Broekstra et al. 2018) suggested that this inconsistency is due to the fact that DD is thought to be associated with specific anti-epileptic medications, predominantly barbiturates, however, these medications are not often prescribed anymore. One study demonstrated that the use of phenobarbital resulted in increased occurrence of DD in humans.(Critchley et al. 1976) Additional research, perhaps also investigating different types of anti-epileptics in more detail, needs to be done to draw concrete conclusions.

Last, the use of anti-diabetic medication did not show a significant association with DD severity. Diabetes is one of the suggested risk factors of DD.(Lanting et al. 2013) Although the association between diabetes and DD is well reported (Broekstra et al. 2018), the association between anti-diabetic medication and DD severity is not. Thus, we conclude the association observed between diabetes and DD results from either a shared genetic aetiology (Major et al. 2019) or a causal relationship (Broekstra et al. 2018), but not from the use of anti-diabetic medication.

Strengths of our study

This study is the first to describe the association between ARB or ACEi use and DD severity. Previous studies have investigated angiotensin II receptors and their association with DD (Stephen et al. 2018), however, to our knowledge there has been no research done to explore the effect of ARB or ACEi use on the severity of DD. In addition to the environmental factors, genetics also play an important part in understanding DD. Although genetics are a known to be largely responsible for the development of DD, not much is known of the pharmacogenetics surrounding DD. This study investigated ARBs and DD. Additionally, the combination of genetics and ARBs was studied to endorse the observed effect of ARB use on DD severity. This association between ARB use and DD severity was found to be significant and was supported by the large sample size of this study. The significant negative association between ARBs and DD severity observed in this study provides new targets for follow-up research into better understanding the association between ARBs and DD.

Limitations of our study

A limitation of our study was the use retrospective data, making our data less reliable. In patient research there is always the downside of incomplete patient reporting, in our case medication use via the questionnaire. Also, DD severity was determined by several surgeons, introducing inter-observer variation. This was however unavoidable in a large-scale study as ours and did render a large dataset.

Conclusion

In conclusion, the results presented in this study have shown that the use of ARBs have a significant negative association with DD severity. This conclusion is supported by the fact that this effect of ARBs on DD severity was nullified after adding TGFβ1 plasma-increasing SNPs to the regression analyses. Moreover, the significant positive association of the beta-blockers on DD severity, and the non-significant associations of diuretics and calcium-antagonists underline that the effect seen by the ARBs is not a resultant of hypertension, but in fact of ARBs.

Future perspectives

For future research we would recommend to first confirm the conclusions of our study with another dataset, to see if the association is reproducible. Next, it would be preferable to execute a longitudinal observational study in humans to examine the effect of medication over time. With more information on the effects of the medications used by the participants and a larger dataset, a more thorough analysis can be done. Also, more covariates can then be studied.

In addition, it would be interesting to execute binding studies using radio-labelled angiotensin to identify angiotensin II receptors in DD tissues and cells. This might help gain more insight in the actions of angiotensin II, AT1R and AT2R in DD tissues and thus help scrutinize the mechanisms of effect of ARBs.

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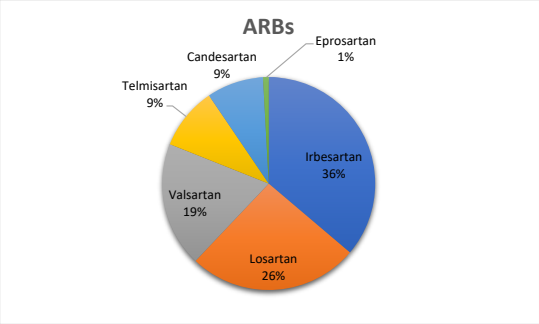
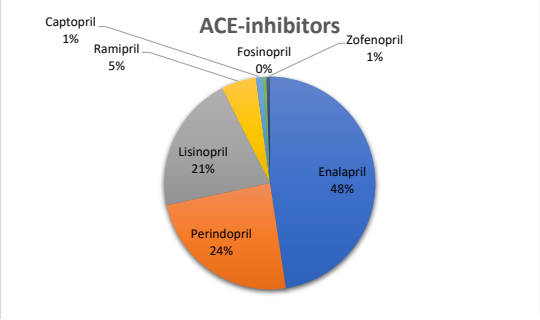
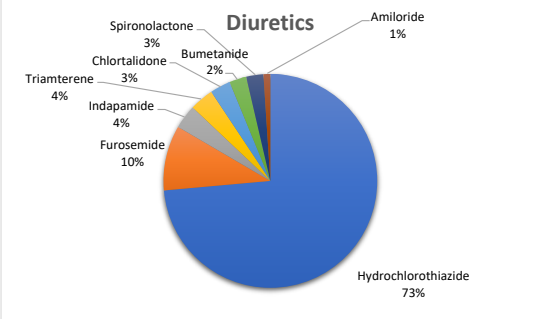
SUPPLEMENTARY



Supplementary Figure 1a. Pie charts representing the ARBs taken by the patients in percentages.



Supplementary Figure 1b. Pie charts representing the ACEi's taken by the patients in percentages.



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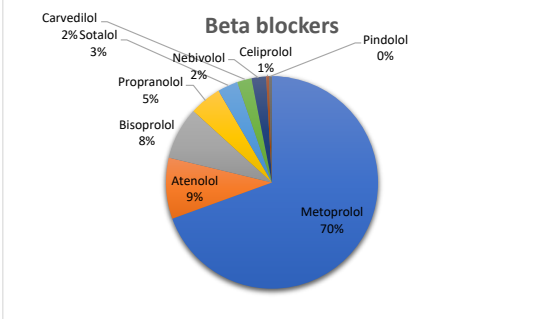
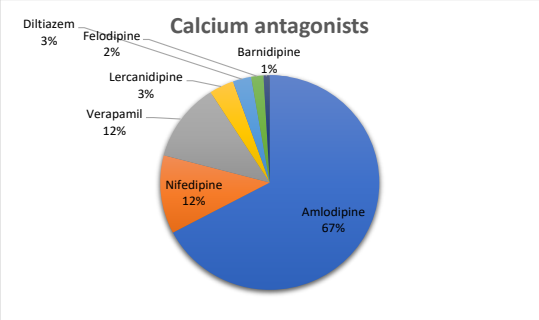


Supplementary Figure 1d. Pie charts representing the beta-blockers taken by the patients in percentages.



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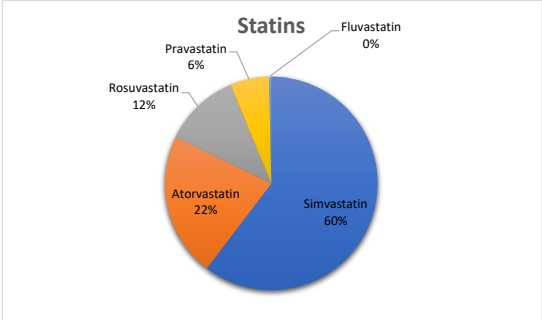

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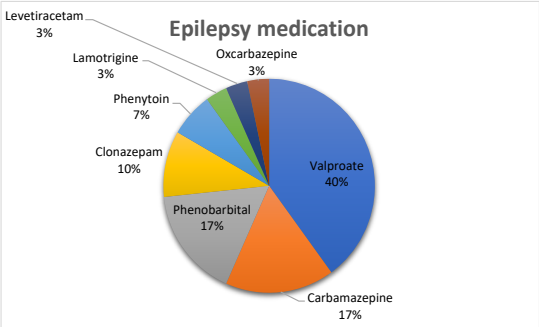
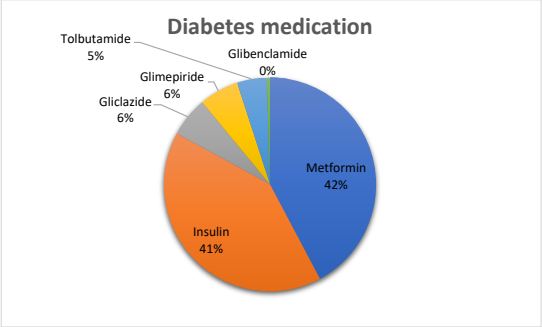
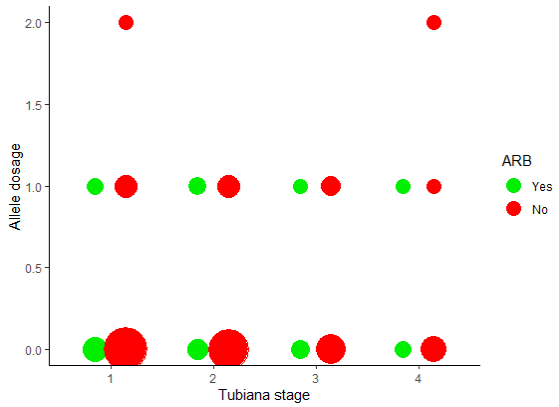


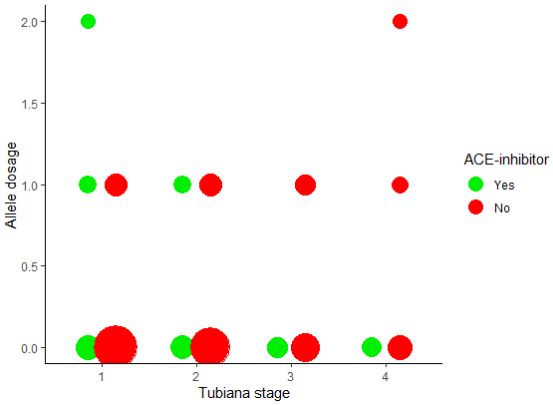
Supplementary Figure 1g. Pie charts representing the epileptics taken by the patients in percentages.



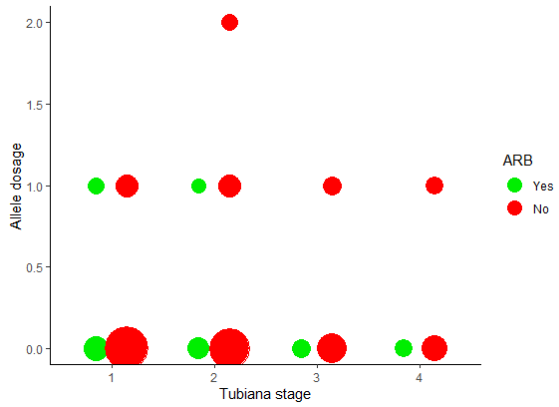
Supplementary Figure 1h. Pie charts representing the diabetics taken by the patients in percentages.



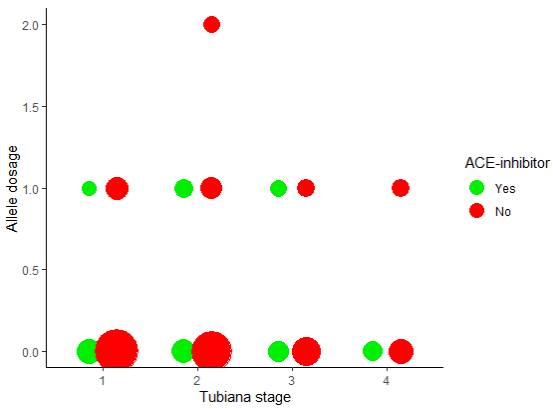
Supplementary Figure 2a. Distribution of ARB use and allele dosage of genetic variant rs1800472 over Tubiana stages. Allele dosages of the imputated SNP are rounded up to the most probable dosage.



Supplementary Figure 2b. Distribution of ACEi use and allele dosage of genetic variant rs1800472 over Tubiana stages. Allele dosages of the imputated SNP are rounded up to the most probable dosage.



Supplementary Figure 3a. Distribution of ARB use and allele dosage of genetic variant rs1800471 over Tubiana stages. Allele dosages of the imputated SNP are rounded up to the most probable dosage.



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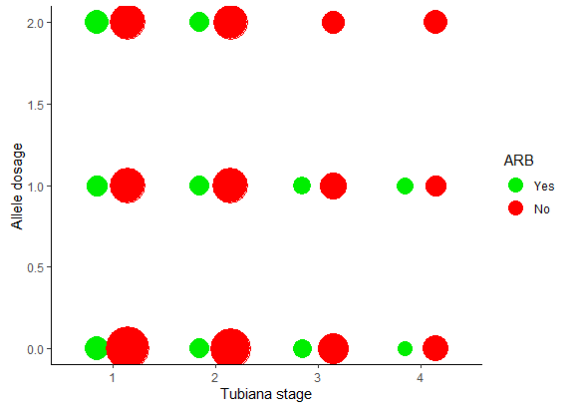

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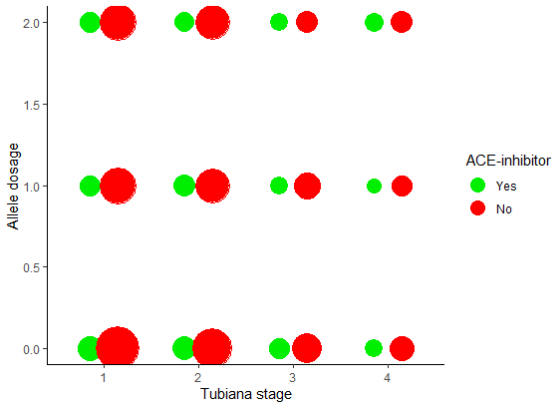

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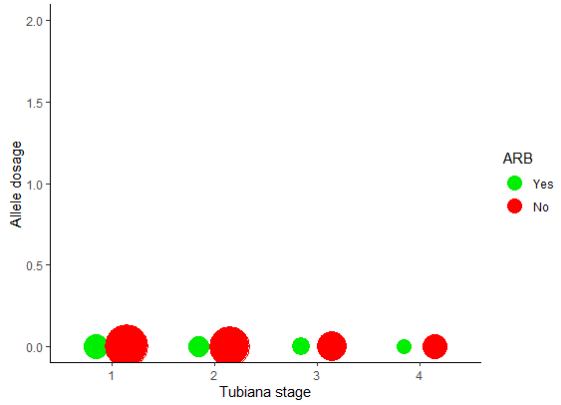

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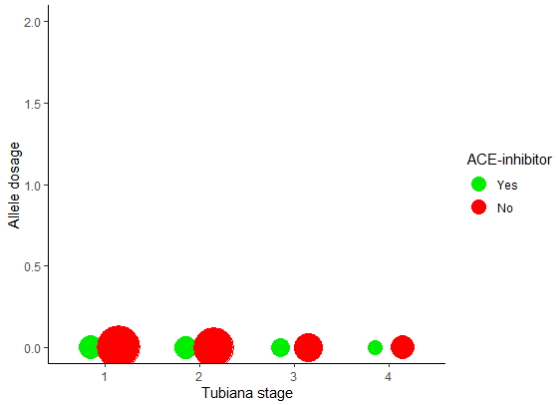

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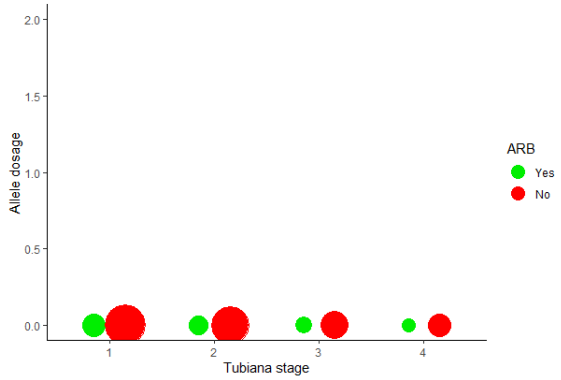
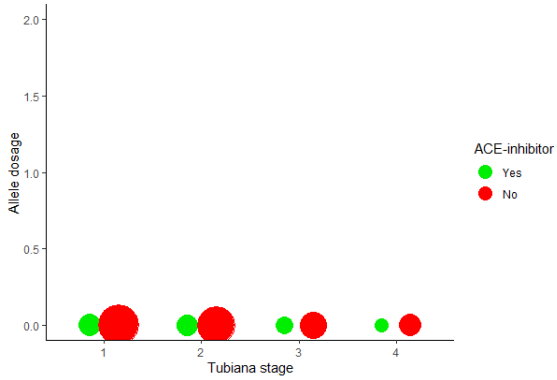
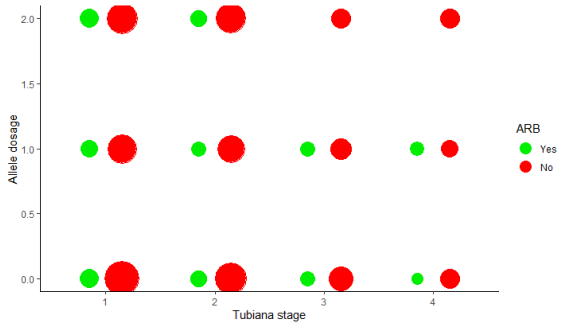


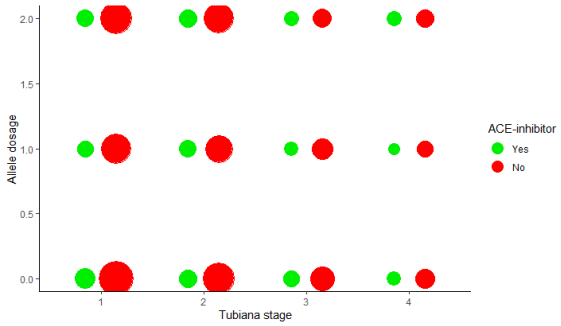
Supplementary Figure 6a. Distribution of ARB use and allele dosage of genetic variant rs11466314 over Tubiana stages. Allele dosages of the imputated SNP are rounded up to the most probable dosage. This SNP was not included in the regression analysis.



Supplementary Figure 6b. Distribution of ACEi use and allele dosage of genetic variant rs11466314 over Tubiana stages. Allele dosages of the imputated SNP are rounded up to the most probable dosage. This SNP was not included in the regression analysis.



Supplementary Figure 7a. Distribution of ARB use and allele dosage of genetic variant rs1800469 over Tubiana stages. Allele dosages of the imputated SNP are rounded up to the most probable dosage.



Supplementary Figure 7b. Distribution of ACEi use and allele dosage of genetic variant rs1800469 over Tubiana stages. Allele dosages of the imputated SNP are rounded up to the most probable dosage.