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Preventing the aortic complications of Marfan syndrome: a case-example of translational genomic medicine

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The translational path from pharmacological insight to effective therapy can be a long one. We aim to describe the management of Marfan syndrome as a case-example of how pharmacological and genomic insights can contribute to improved therapy. We undertook a literature search for studies of Marfan syndrome, to identify milestones in description, understanding and therapy of the syndrome. From the studies retrieved we then weaved an evidence-based description of progress. Marfan syndrome shows considerable heterogeneity in clinical presentation. It relies on defined clinical criteria with confirmation based on *FBN1* mutation testing. Surgical advances have prolonged life in Marfan syndrome. First-line prophylaxis of complications with β-adrenoceptor blockers became established on the basis that reduction of aortic pressure and heart rate would help. Over-activity of proteinases, first suggested in 1980, has since been confirmed by evidence of over-expression of matrix metalloproteinases (MMP), notably *MMP-2* and *MMP-9*. The search for MMP inhibitors led to the evaluation of doxycycline, and both animal studies and small trials, provided early evidence that this widely used antimicrobial agent was useful. Identification of the importance of TGF-β led to evaluation of angiotensin II type I receptor (AT_1R) blockers with highly promising results. Combination prophylactic therapy would appear rational. Pharmacological and genomic research has provided good evidence that therapy with losartan and doxycycline would prevent the aortic complications of Marfan syndrome. If on-going well designed trials confirm their efficacy, the outlook for Marfan syndrome patients would be improved considerably.

Introduction

On palpating Jefferson Hope, the avenging murderer's chest, Doctor Watson, who was accompanying Sherlock Holmes, recounted that he 'became at once conscious of an extraordinary throbbing and commotion which was going on inside. The walls of his chest seemed to thrill and quiver as a frail building would do inside when some powerful engine was at work. In the silence of the room I could hear a dull humming and buzzing noise which proceeded from the same source'. Whereupon Watson cried out, 'Why, you have an aortic aneurysm'. This passage from the 1887 novel, 'A study in scarlet' by Sir Arthur Conan Doyle, is thought to be the first description of Marfan syndrome, years ahead of that given in 1896 by Antoine Marfan. Hope, the murderer, commented, 'I went to a Doctor last week about it, and he told me that it is bound to burst before many days passed. It has been getting worse for years'. In the Edinburgh Medical School-trained Conan Doyle's time, most patients did not live as long as Hope, who was then in his late thirties. Modern day diagnosis and management of Marfan syndrome has undergone considerable progress over recent years and as we describe below, even Sherlock Holmes would not have said, 'Elementary my dear Watson'.

Marfan syndrome, a serious disorder affecting connective tissues, is inherited as an autosomal dominant disease. Although Weve [1] named the disease after Antoine Marfan, Gabrielle P, the 5-year-old patient he described with elongated spider-like limbs (arachnodactyly), did not have all the characteristic features of the syndrome [2]. The clinical manifestations of the disease, which affects about 1

in 5000 to 1 in 10 000 individuals, were summarized by Victor McKusick, way back in 1984 as affecting three organ systems: (i) Ectopia lentis, a hallmark of ocular involvement, (ii) skeletal features including overgrowth with long thin limbs and spider fingers and (iii) weakness of the aortic media [3]. With closer study of patients with Marfan syndrome, other clinical features such as dura ecstasia [4, 5], myopathy [6] and obstructive sleep apnoea [7], which may accelerate disease progression, are also being identified. Today, diagnosis of Marfan syndrome is usually done using the revised Ghent diagnostic criteria with the classical syndrome characterized by clinical manifestations of the cardiovascular, skeletal and ocular systems, with the greatest weight placed on aortic root aneurysm and ectopic lentis [8, 9]. These ophthalmological and cardiovascular abnormalities are the most common cause of morbidity and mortality. The clinical phenotype of the syndrome is highly variable and there is some evidence suggesting that the syndrome shows ethnic variability in clinical presentation [10, 11] with skeletal involvement being less common among Koreans and Japanese patients [10, 11]. Generally, across all populations, even the cardiovascular phenotype may show considerable variability [12] and the clinical manifestations increase with age [13]. However, aortic rupture or dissection, from an aortic root aneurysm, suspected by Dr Watson, is generally regarded as the main cause of morbidity and mortality in patients with the syndrome. Without treatment death often results in early adult life [14] but with advances in surgery [15, 16], life expectancy has improved considerably [17]. However, the most widely-used pharmacotherapy with β-adrenoceptor blockers, is still unsatisfactory [18]. Angiotensin converting enzyme (ACE) inhibitors and calcium channel blockers are also used, but like β-adrenoceptor blockers, they too do not arrest abnormal aortic growth, or obviate the need for eventual surgical intervention [19]. After a long period characterized by lack of progress in the pharmacological management of the disease, we explain how recent molecular genetic and pharmacological insights are ushering in a new era of potentially more effective prophylactic treatments against the physical ravages of the disease. In particular, we highlight how recent data suggest that by using drugs, long-established for other diseases, and hence with well-defined safety profiles, the translational road from scientific insights to effective therapy can be considerably shortened. Losartan, a widely used antihypertensive angiotensin II type 1 receptor blocker, and doxycycline, a tetracycline antibiotic, first introduced in 1988 and 1958, respectively, are likely to become first-line drugs in the management of Marfan syndrome [20, 21].

Methods

We undertook a literature search for studies of Marfan syndrome from its first description to its present day management to identify, qualitatively, milestones in description, understanding and therapy of the syndrome. Both primary studies and reviews were scrutinized to ensure comprehensive coverage of relevant issues. Improved coverage was undertaken though iterative searches of reference lists of articles identified earlier. From the studies retrieved we then weaved an evidence-based description of the progress made.

Results and discussion

Genetics of Marfan syndrome

In most cases, Marfan syndrome is caused by heterozygous mutations in *FBN1*, the gene that encodes fibrillin-1, one of the major components of extracellular microfibrils [22, 23]. In the recently revised nosology, identification of mutations in *FBN1* is given increasing importance [11]. Fibrillin-1 is not needed for elastogenesis but is critical for maintenance of elastic fibre [24, 25]. Diagnostic confirmation of the disease is through identification of pathogenic mutations of the gene. More than 1000 mutations have been identified and most affected families seem to have mutations specific to them (private mutations) [26]. Originally it was thought that the clinical syndrome was solely caused by the formation of weaker connective tissue as a result of the formation of defective fibrillin-1. While this would account for many of the features of the syndrome such as the typical aortic root dilatation and lens dislocation, it did not explain satisfactorily other signs such as thickening of the heart valves and bone overgrowth. Moreover subjects with the same mutation may often present with variable phenotypes [27, 28]. Animal studies subsequently suggested that a decrease in the amount of normal fibrillin-1 may be as important as, and in some cases more than, the formation of a mutant protein [29]. This inference is supported by human genetic studies [30, 31] with disease severity correlating with expression level of normal fibrillin-1 [31]. Recent studies suggest that intra-familial variability in Marfan syndrome might also in part be due to the presence of compound-heterozygous mutations [32]. Other genetic modifiers are expected to be discovered.

Surgical advances

Without a clear understanding of the disturbed molecular pathways, and hence identification of specific targets for pharmacotherapy, the dramatic improvements, over the last four decades, in the longevity of patients with Marfan syndrome, has hitherto been largely due to surgical advances [15–17, 33, 34]. A report estimated that by 1993, the mean (± SD) age of survival had increased to 41 ± 18 years from 32 ± 16 years in 1972 and that the median survival age had increased to 72 years from 48 years over the same period. Only relatively recently

has the evidence for effective pharmacotherapy against the cardiovascular complications of Marfan syndrome emerged.

Early and standard pharmacotherapy

β-adrenoceptor blockers were the first prophylactic therapy investigated in Marfan syndrome. The pharmacological rationale includes reduction in pressure and heart rate, both of which are expected to reduce stress on the aortic wall. The clinical rationale was the early evidence suggesting that blood pressure lowering with β-adrenoceptor blockers improved survival in patients in the general population with acute dissection of aortic aneurysms. This seemed to be supported by a few small studies in Marfan syndrome. However, a recent systematic review of the evidence provided little support for their effectiveness [35]. Although the authors attempted to estimate the magnitude of effect, the evidence uncovered was highly heterogeneous, subject to considerable bias, and unsuitable for meta-analysis. For example, the specific β-adrenoceptor blocker drugs used were variable across the studies. Only one of the six studies included in the meta-analysis was a randomized controlled trial (RCT) involving 70 patients. Thirty-two were randomized to propranolol, and the others to no-treatment, in the control arm. In the RCT, blinding was not possible and the patients were followed-up for an average of 9.3 years in the control group and 10.7 years in the propranolol group. While the rate of aortic root enlargement was slowed, the combined clinical endpoint (death, acute aortic dissection, acute regurgitation, cardiovascular surgery, congestive heart failure, and aortic root diameter larger than 6 cm) was not significantly different in the two groups. This lack of effect is probably a reflection of the low power of the study to identify clinically meaningful differences in event rates [18]. With a relatively rare disease, recruitment rate to trials to generate enough subjects for a well-powered study is a particularly challenging problem. Despite the limited amount of solid supportive evidence, β-adrenoceptor blockade is still widely used, probably on the basis of best evidence-synthesis and emphasis on the better conducted trial [18]. β-adrenoceptor blockers are in fact still considered first-line prophylaxis and the gold-standard comparator in randomized controlled trials of new treatments by experts [34, 36], most notably by those involved in generating, probably the best and most influential trial, supportive of the use of β-adrenoceptor blockers [18].

ACE vs. AT_1R inhibitions

Angiotensin converting enzyme inhibitors (ACEi) are also used either alone or in combination with β-adrenoceptor blockers. The pharmacological rationale is the involvement of the renin-angiotensin system (Figure 1) in the development of aortic stiffening, dilatation and rupture in Marfan syndrome. While some limited clinical evidence in 10 subjects, suggests that ACEi may reduce both aortic stiffness and aortic root diameter [37], more recent pharmacological studies indicate that selective inhibition of the angiotensin II type 1 (AT_1R) receptors may be more appropriate [20, 34].

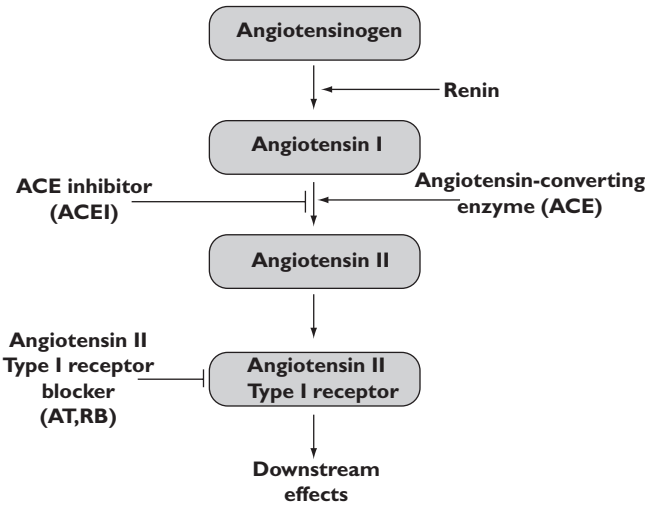

Figure 1

The renin-angiotensin system and sites of action of ACE inhibitors and angiotensin II type I receptor blockers. Angiotensinogen synthesized in the liver, and released into the bloodstream, is cleaved by renin to form angiotensin I. This is converted to angiotensin II in the lungs by angiotensin converting enzyme (ACE). Angiotensin II activates AT_1 receptors in arterioles to cause vasoconstriction. ACE inhibitors inactivate ACE while angiotensin AT_1 receptor blockers block receptor activation and hence downstream signalling

The molecular pharmacological rationale for the use of AT_1R blockers, often abbreviated as ARBs, in Marfan syndrome, developed from the observation that in addition to its structural function, fibrillin-1 interacts with, and regulates, the transforming growth factor beta (TGF-β) family of growth factors. Proof of the importance of TGFβ came with the demonstration that a neutralizing antibody for this growth factor rescued lung septation in animal models of the disease [38]. The fibrillins contain a motif homologous to latent transforming growth factor-β binding protein (LTBP). These LTBPs sequester TGF-β into an inactive form. Defective fibrillin-1 leads to poorer binding of these LTBPs and as such to an increased TGF-β activity. Dysregulation of the TGF-β signalling leads to inhibition of the maturation of satellite cells into myoblasts, and further disintegration of fibre matrix follows (Figure 2). This may explain the myopathy and the inability of many patients with Marfan syndrome to increase muscle mass, despite physical exercise. Indeed animal studies show that AT_1R blockade attenuates TGF-β-induced failure of muscle generation in different myopathic states [39]. Most importantly, blocking of the increased TGF-β by neutralizing antibodies also rescued the aortic root dilatation and restored vessel wall architecture. With these new insights, translational work progressed rapidly.

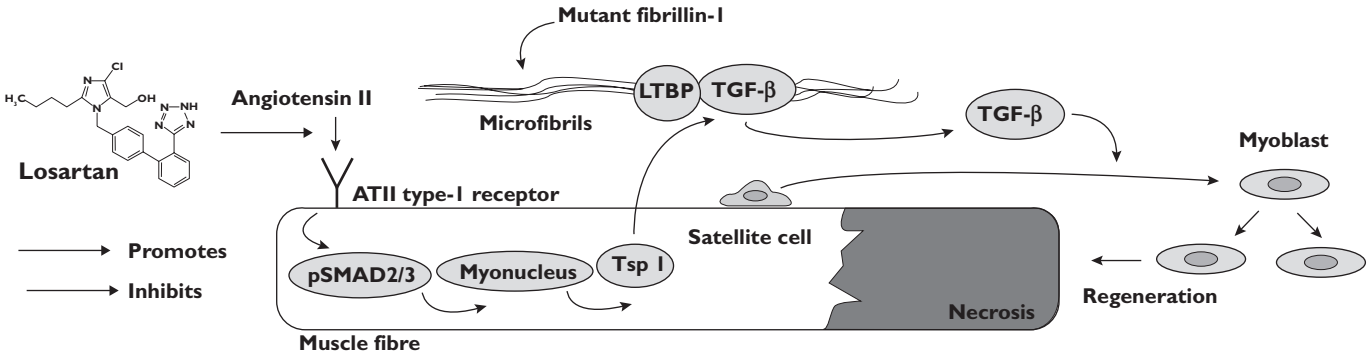

Figure 2

Fibrillin 1 sequesters TGF-β by interacting with the latent TGF-β-binding proteins (LTBP). Mutations in the gene encoding fibrillin-1 (*FBN1*) leads to upregulation of TGF-β. The protein product prevents satellite cells from proliferating and diffusing into damaged myofibres to regenerate and repair the muscle cells. Thrombospondin-1 (Tsp-1), produced in response to activation of angiotensin II type 1 (AT_1) receptor, by angiotensin II, regulates TGF-β activation. Losartan inhibits AT_1 activation and hence inhibits Tsp-1 production (adapted from Chamberlain [95])

Translational research

Clinically, it is generally more practical to deal with the consequential deranged biochemical pathways than targeting the genetic abnormality with techniques such as gene therapy. Treatment is then symptomatic rather than curative although the outcome can nevertheless be dramatic. The management of Marfan syndrome, provides an important illustrative example [20]. The path from molecular insight to gene knock-out animal model and human clinical trial was impressively shortened by recourse to two long-established drugs: losartan first introduced into the clinic in 1995 and doxycycline in 1988.

It was known already, from studies of diabetic nephropathy that angiotensin increased TGF-β concentrations which could be lowered with blockade of its receptor [40]. This led to investigations of whether such blockade, with an angiotensin II type I receptor blocker, could, as previously seen with a neutralizing antibody [38], prevent the complications of Marfan syndrome in a mouse model of the disease [39, 41]. The results using both AT_1R antibody and the small molecule blocker, losartan, were dramatic [39, 41]. The results of a retrospective evaluation of human subjects given an AT_1R blocker because of failure of prior therapy with other agents, were no less impressive [20]. Failure was defined as rapid enlargement in aortic diameter, evidence of aortic diameter approaching the need for surgical intervention (≥4.0 cm), or intolerance to therapy. A sharp reduction in the rate of aortic root dilation was observed in each member of a group of 18 paediatric subjects, aged 1 to 16 years, with severe Marfan syndrome, given losartan (or irbesartan for one subject) with a follow-up of 12 to 47 months. Importantly however, all the subjects continued to receive β-adrenoceptor blocker therapy, and four received ACEi as well, and two switched to the calcium channel blocker, verapamil, because of β-adrenoceptor blocker intolerance.

Doxycycline

Early work on abdominal aortic aneurysms led to the hypothesis that increased production of proteinases in the aneurismal wall was involved [42, 43]. This led to the suggestion that one strategy for limiting progressive aneurysm growth would be direct pharmacological inhibition of the proteinases involved and various studies identified the metalloproteinases (MMP), notably MMP-2 (72-kDA gelatinase) and MMP-9 (92-kDa gelatinase), as being particularly specific targets to aim at [44–47]. Independent evidence that MMP-9 degraded arterial elastin provided further support [48]. With a clear target, the search was therefore directed at identifying inhibitors of the MMPs, including, quite rationally, licensed drugs in clinical use for other conditions. Among these were non-steroidal anti-inflammatory agents (NSAIDs), notably indomethacin, and tetracyclines, notably doxycycline. Both agents provided evidence from rat studies that aneurysmal dilatation could be slowed [49–51]. Studies in humans have since shown that in addition to the known direct inhibitory effect of doxycycline on MMP, pre-operative treatment with doxycycline reduces expression of monocyte/macrophage expression of *MMP-9* and post-translational activation of pro-MMP2 within human aneurysm tissue [52]. In a small randomized controlled trial, Mosorin *et al*. [53] reported that patients given doxycycline, 150 mg daily, showed a significantly lower expansion rate of their abdominal aortic aneurysms than did placebo-control subjects, as early as 6–12 months after treatment initiation. Perhaps lack of financial incentive to support a well powered multicentre study of an out-of- patent drug explains why such a trial has yet to be undertaken. Upregulation of matrix metalloproteinase-2 and -9 (*MMP-2* and *MMP-9*) in thoracic aortic aneurysms, leading to loss of integrity and reduction of vascular smooth muscle contraction, has since been shown in a mouse model (mice heterozygous for a mutant

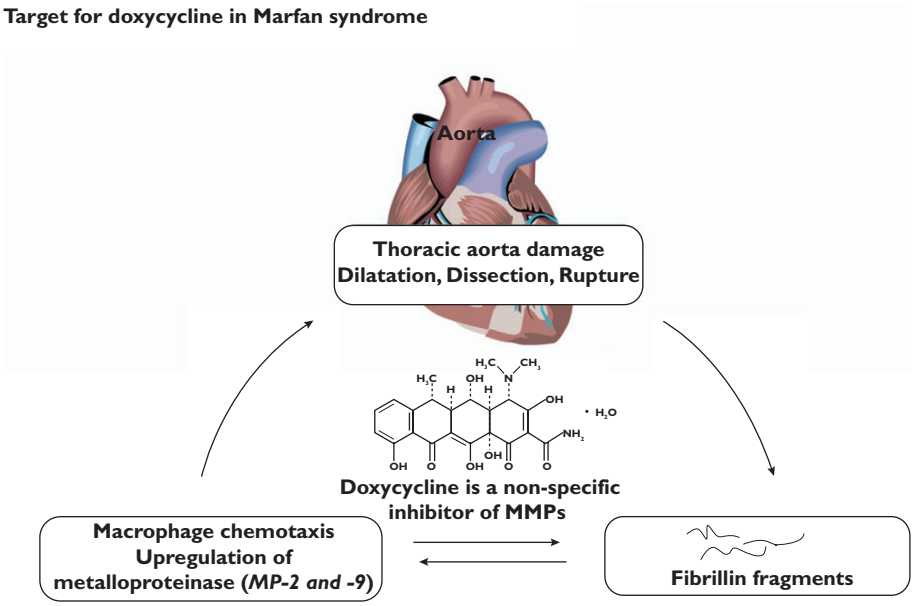

Figure 3

Breakdown of fibrillin leads to fragments which trigger macrophage chemotaxis and upregulate genes encoding several matrix metalloproteinases, notably the zinc endopeptidases, MMP-2 and -9. Both processes amplify the breakdown of the microfibrils through positive feedback. Doxycycline is a non-specific MMP inhibitor

Fbn1 allele encoding a cysteine substitution in fibrillin-1) of Marfan syndrome [54]. Breakdown of fibrillin leads to fragments which trigger macrophage chemotaxis and upregulation of the zinc endopeptidases. Both processes amplify the breakdown of the microfibrils through positive feedback (Figure 3). In the Marfan mouse, long-term doxycycline was more effective than atenolol in preventing thoracic aortic aneurysm through inhibition of MMP-2 and MMP-9 [55].

Although doxycycline also suppressed the upregulation of TGF-β [55], given the evidence that both doxycycine and losartan reduce progression of thoracic aortic aneurysm, and their different spectra of action, combination therapy would appear logical. Investigations in the Marfan mouse, have in fact shown that the combination was considerably more effective at preventing thoracic aortic aneurysm than either losartan or doxycycline alone [56].

Further validation and risk-benefit assessment

Given the known risk of substantial bias inherent in observational studies, poor estimates of efficacy from small human trials, and difficulty in extrapolating from animals to humans, it is proper that confirmatory randomized controlled trial evidence in humans be generated and several trials are underway (Table 1) [57–59]. However, the primary end-point in most of the ongoing trials is a change in aortic root size. Harder outcomes such as change in mortality, incidence of dissection, elective aortic surgery, aortic volume, aortic stiffness and ventricular function, will be secondary end-points. Given the likely difference in molecular mechanisms of action and early promising animal studies [56], a well-designed randomized controlled trial of losartan in combination with doxycycline might also be appropriate. One major problem with further validation of losartan in Marfan syndrome is that the impressive results seen in the recent retrospective evaluation [20] have led many parents to demand off-label use of losartan as first-line prophylaxis for their children, making recruitment to trials more problematic [36].

Widespread use of the AT_1R blockers showed, until recently, a relatively benign adverse effect profile, for this class of drugs. However, an unexpected higher number of cancer deaths with the use of candesartan than with placebo (CHARM programme), in the management of heart failure [60], initially ascribed to the play of chance, caused sufficient concern for several subsequent trials (acronyms LIFE [61], ONTARGET [62], TRANSCEND [63]) of AT_1R blockers to record prospectively the occurrence of cancer. A very recent meta-analysis of those studies [64] suggested that there was cause for concern as it suggested that there was a modestly increased risk of cancer with the AT_1R blockers across a heterogeneous group of trials. In Figure 4 we summarize the observed increased risk of cancer in the randomized controlled trials in which risk estimates are least likely to be subject to bias and to stochastic extremes. We restricted analysis to trials with at least 1000 patients, and which prospectively recorded cancer occurrence. We have refrained from obtaining and

Table 1

Ongoing trials of angiotensin II type I receptor blockers in Marfan syndrome

First author (Country)	Trial ID Estimated completion	Primary outcome measure	Design	Age y	Control	Target *n*	Aortic size at entry
Creager (USA)	NCT00723801 (December 2010)	Aortic and cardiac muscle stiffness	DB PG 6 months	25 years and older	Atenolol	50	ND
De Backer (Belgium)	NCT00782327 (December 2014)	Aortic dilatation	DB PG 3 years	≤10 years	Placebo	174	Z score ≥2
Forteza (Spain)	NCT01145612 (February 2013)	Aortic dilatation	DB PG up to 4 years follow-up	5–60 years	Atenolol	150	<45 mm
Detaint (France) [94]	NCT00763893 (September 2013)	Aortic dilatation	DB PG 3 years	≤10 years	Placebo	300	ND
Gambarin (Italy) [58]	NCT00683124 (July 2012)	Aortic dilatation	DB PG 4 years	1–55 years	Nebivolol alone or combined	291	Z score ≥2.5 with proven FBN1 mutation
Lacro (USA) [59]	NCT00429364 (December 2013)	Aortic dilatation	SB PG 3 years	6 months–25 years	Atenolol	604	Z score >3
Radonic (Netherlands) [57]	2013	Aortic dilatation	Open blinded endpoint 3 years	≤18 years	Placebo	330	≤50 mm
Sandor (Canada)	NCT00593710 (December 2011)	Pulse-wave velocity	DB PG 6 months	12–25 years	Atenolol	30	ND
Wu (Taiwan)	NCT00651235 (June 2011)	Aortic dilatation	Open factorial	≤1 years	Atenolol or propranolol	44	≤55 mm

Notes: The trial reference numbers are those in the trials registry http://www.clinicaltrials.gov. The Z score gives the number of standard deviations of the observed size from the mean of the normal population. DB, double-blind; SB, single-blind; PG, parallel group. All are phase III trials except for the phase study by Wu *et al.* (phase II). The trial by Radonic *et al.* is not phased.

Figure 4

Cancer incidence in randomized controlled trials of angiotensin II type I receptor blockers with cancer prespecified as an endpoint. Note that a risk ratio of 1 indicates no difference between the two study arms. The bars represent 95% confidence intervals

reporting a pooled estimate of relative risk despite statistical data-driven homogeneity, given the obvious non-homogeneity in trial design, most notably in the control treatment used, and in concomitant use of other agents (see labels on the x axis of Figure 4 for details). It is clear that despite some evidence of increased cancer risk with the AT_1R blockers, further exclusion of chance association is still required. This is required [65] to either provide the necessary reassurance or to provide more precise estimates of risk. Any increased risk can be traded-off by all stake-holders against the considerable risk of harm associated with disease progression without treatment. Puzzling questions about the reported associations include (i) why inhibition of a growth factor should stimulate the development of cancer, although dysregulation of a growth signalling pathway may itself be oncogenic and (ii) the relatively short time-frame over which the excess in cancer occurrence was observed, with median or mean follow-ups of no more than 5 years. However, the patients were aged 55 years or older, an age-group known to associated with a dramatic increase in rates of cancer. Therefore, the extent to which the data from this older population might be generalizable to children is unknown. Nevertheless, given that AT_1R blockade is likely to be necessary for life, if a therapeutic benefit is confirmed in Marfan syndrome, urgent further investigations are required so that appropriate trade-offs may be made against the considerable harm associated with disease progression without treat-

ment. Screening requirements can, also then, be formulated for those who decide to opt for prophylactic therapy.

An important question is whether the observed efficacy, and indeed potential adverse consequences are class effects for AT_1R antagonists. Experience with other classes of drugs shows clearly that effectiveness, the balance of efficacy and adverse effect, is generally compound-specific. For example, even within drug classes such as β-adrenoceptor blockers and statins, widely recognized as generally safe for clinical use, specific members have been withdrawn from the market because of their poor harm-benefit balance. Such differences can sometimes only be observed after many years of use in large numbers of patients. In a recent study [66], three AT_1R inhibitors showed marked differences in the extent to which they interacted pharmacokinetically with mycophenolate mofetil. This led the authors to call for therapeutic monitoring with telmisartan. Such intra-class differences in the interaction potential of the AT_1R inhibitors with other drugs are to be expected. Therefore, as AT_1R antagonists are likely to be used for prolonged periods of time in Marfan syndrome, clinical equivalence will be hard to establish. It is important to note that long-term safety in one therapeutic application (e.g. heart failure or hypertension) may not necessarily predict safety in another (e.g. Marfan syndrome). Current evidence suggests that it would be safer to assume clinical non-equivalence since even with respect to blood pressure lowering, intra-class differences are apparent [67]. Moreover, more recent preclinical evidence suggests that the AT_1R antagonists show marked intra-class pharmacological differences [68]. Li *et al*. [68] showed that while candesartan, olmesartan and losartan inhibited pressure-overload-induced cardiac hypertrophy even in the absence of angiotensin II, telmisartan and valsartan could only prevent the hypertrophy in the presence of the pressor peptide. On the available evidence, the first three antagonists are likely to be better candidate AT_1R blockers to take forward into clinical trials.

Increased recognition of the pleiotropic effects of the statins, which extend beyond reduction of low density lipoprotein-cholesterol (LDL-C) to include improvement in enthothelial function, anti-inflammatory effects and immunomodulation, has aroused interest in their possible prophylactic effects in Marfan syndrome [69]. Of particular interest is recent trial evidence which demonstrated that rosuvastatin reduced cardiovascular events in apparently healthy subjects with elevation of high sensitivity C-reactive protein (hsCRP), a marker of inflammation [70]. Despite the positive evidence from this trial (Jupiter) and a number of its extensions [71], drug regulators are still unconvinced about the use of hsCRP as a suitable biomarker for use of rosuvastatin for the primary prevention of cardiovascular events [72]. Interestingly, there was poor correlation between hsCRP and LDL-C concentrations despite both being predictors of efficacy [71], thereby indicating that these act as independent risk factors. Extrapolating from cardiovascular events to aortic dilatation and dissection is however unjustifiable at the present time. Moreover, a recent study indicated that there was no difference in hsCRP concentrations between subjects with large aortic aneurysms and control subjects [73]. However, given the relatively benign adverse reaction profile of statins, validated by an extent of use which has few parallels in the history of drug prophylaxis, clinical trial evaluation of the statins added to a background of optimal prophylaxis, should be undertaken.

The use of statins in combination with peroxisome proliferators-activated γ receptor (PPARγ) agonists has also been proposed as a possible novel strategy as a prophylactic treatment for aortic aneurysm syndromes, including Marfan syndrome [74]. The rationale for evaluating the PPARγ agonists is that PPARγ, a nuclear receptor, when activated exerts potent anti-inflammatory effects, suppresses the expression of MMP-9 and TGF-β-induced profibrotic responses [75–78].

Genetic-test implications

Given the progressive nature of the disease [13, 79, 80], early diagnosis is clearly important. Testing of family members when an *FBN1* mutation has been identified now forms part of routine practice. However, mutation analysis, with commonly used panels of *FBN1* mutations, fails to identify such mutations in up to 10% of cases, suggesting either undetected *FBN1* functional mutations or involvement of other genes [30]. Recent work suggests that deletions in the regulatory and promoter regions may be missed. Beyond molecular diagnosis, there is also work directed towards quantifying clinical disease progression [81].

Biomarker evaluation

Given the importance of TGF-β in the pathogenesis of the aortic complications of Marfan syndrome there is also considerable interest in determining whether the protein encoded by the gene *TGF*-β, can be used as a biomarker [81–85]. In an observational study [82], patients with Marfan syndrome had higher circulating TGF-β concentrations than healthy controls, while patients treated with losartan and/or β-adrenoceptor blockers had lower concentrations than patients not treated with cardiovascular drugs (Figure 5). It is interesting that although the concentrations were lower in the treated patients, they were still elevated relative to healthy controls [82]. Clearly, dose-ranging randomized control trials would help in determining the extent to which the concentrations can be further reduced with such drugs. Importantly, there is also a need to determine the functional consequences of any observed changes in TGF-β concentration with treatment on the aorta.

In some cases there may well be misdiagnosis from an overlap of some features of Marfan syndrome with other syndromes, notably the Loeys-Dietz syndrome [86], with

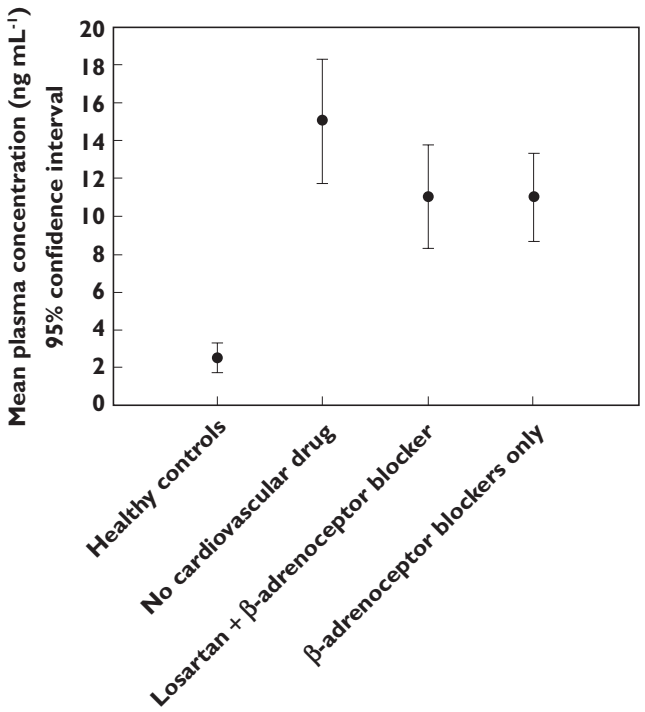

Figure 5

Concentrations of circulating transforming growth factor-β (TGF-β1) in normal controls and Marfan syndrome patients with and without losartan and β-adrenoceptor blocker treatment (data from Matt *et al*. [82])

some common molecular pathways leading to clinical complications similar to those seen in Marfan syndrome. These aneurysm syndromes are caused by heterozygous mutations in *TGFBR1* and *TGFBR2*, the genes encoding the transforming growth factor receptors 1 and 2, respectively [86]. Importantly, despite the apparent loss-of-function effect of these *TGFBR1* or *2* mutations, the overall effect at the tissue level, such as in the aortic wall, is an increase in TGF-β signalling. This suggests that TGF-β blockade may also be beneficial in Loeys-Dietz syndrome. There is also preliminary evidence that TGFBR1 may act as low-penetrance alleles in Marfan syndrome [87]

Recent evidence suggests that circulating concentrations of the MMPs in humans are not useful predictors of the diameter of abdominal aortic aneurysms although post-operative analysis of tissue samples showed that the MMP-9 concentration in the aneurysm wall correlated with aneurysm diameter [88]. Interestingly, a recent study of Marfan patients showed that the matrix metalloproteinase 9 -8202A/G polymorphism was associated with thoracic aortic aneurysms and dissection [89].

Conclusion

The translational path from chance observation to better understanding of the molecular and genetic basis of Marfan syndrome to novel potential treatments has been so far both instructive and fascinating. Whether, the AT_1R inhibitors deliver on all of their promise is yet to be demonstrated [19]. As the recent report of a possible link between use of these agents and cancer highlights, even long established agents in widespread use may not reveal all their adverse effects readily. Over a decade ago, Judah Folkman, who did pioneering work on angiogenesis inhibitors [90–92] calmed excited TIME magazine readers of his latest research in breast cancer by commenting 'I'm flattered, but it's mice, only mice. If you have cancer and you're a mouse, we can take good care of you' [93]. As it turned out, his work did eventually lead to clinically useful advances and several licensed therapies. It is to be hoped that a similar happy outcome will be seen in Marfan syndrome with the AT_1R inhibitors. Pharmacological prophylactic management of Marfan syndrome has moved somewhat beyond the Marfan mouse stage to man, although considerable insights are still being gained by such animal studies. With use of losartan, an AT_1R inhibitor, licensed for other conditions, the translational path has been considerably shortened. The next crucial event is publication of the results of the ongoing randomized controlled trials. An increasing problem in the testing of novel hypotheses generated by new molecular insights on Marfan syndrome is that the small patient population can only sustain a limited number of trials. Therefore parsimonious recruitment of patients and careful choice of investigational therapies to optimize public health gain is essential. In this respect, there is no strong evidence to suggest that any of the AT_1R antagonists is any better than losartan.

Competing Interest

There are no competing interests to declare.

REFERENCES

1 Weve HJM. Ueber Arachnodaktylie (Dystrophia mesodermalis congenita, Typus Marfan). Archiv für Augenheilkunde 1931; 104: 1–46.

2 Marfan AB. Un cas de déformation congénitale des quatre membres, plus prononcée aux extrémités, caracterisée par l'allongement des os avec un certain degré d'aminicissement. Bulletins et memoires de la Société medicale des hôpitaux de Paris 1986; 13: 220–6.

3 McKusick VA. Mendelian disorders. In: The Principles and Practice of Medicine, eds Harvey AM, Johns RJ, McKusick VA, Owens AH, Ross RS. London: Prentice-Hall Inc., 1984; 413–59.

4 Weigang E, Ghanem N, Chang XC, Richter H, Frydrychowicz A, Szabo G, Dudeck O, Knirsch W, von Samson P, Langer M, Beyersdorf F. Evaluation of three

different measurement methods for dural ectasia in Marfan syndrome. Clin Radiol 2006; 61: 971–8.

5 Lundby R, Rand-Hendriksen S, Hald JK, Lilleas FG, Pripp AH, Skaar S, Paus B, Geiran O, Smith HJ. Dural ectasia in Marfan syndrome: a case control study. AJNR Am J Neuroradiol 2009; 30: 1534–40.

6 Voermans N, Timmermans J, van Alfen N, Pillen S, op den Akker J, Lammens M, Zwarts MJ, van Rooij IA, Hamel BC, van Engelen BG. Neuromuscular features in Marfan syndrome. Clin Genet 2009; 76: 25–37.

7 Kohler M, Blair E, Risby P, Nickol AH, Wordsworth P, Forfar C, Stradling JR. The prevalence of obstructive sleep apnoea and its association with aortic dilatation in Marfan's syndrome. Thorax 2009; 64: 162–6.

8 De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet 1996; 62: 417–26.

9 Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, Devereux RB, Hilhorst-Hofstee Y, Jondeau G, Faivre L, Milewicz DM, Pyeritz RE, Sponseller PD, Wordsworth P, De Paepe AM. The revised Ghent nosology for the Marfan syndrome. J Med Genet 2010; 47: 476–85.

10 Yoo EH, Woo H, Ki CS, Lee HJ, Kim DK, Kang IS, Park P, Sung K, Lee CS, Chung TY, Moon JR, Han H, Lee ST, Kim JW. Clinical and genetic analysis of Korean patients with Marfan syndrome: possible ethnic differences in clinical manifestation. Clin Genet 2010; 77: 177–82.

11 Akutsu K, Morisaki H, Takeshita S, Ogino H, Higashi M, Okajima T, Yoshimuta T, Tsutsumi Y, Nonogi H, Morisaki T. Characteristics in phenotypic manifestations of genetically proved Marfan syndrome in a Japanese population. Am J Cardiol 2009; 103: 1146–8.

12 De Backer J. The expanding cardiovascular phenotype of Marfan syndrome. Eur J Echocardiogr 2009; 10: 213–5.

13 Faivre L, Masurel-Paulet A, Collod-Beroud G, Callewaert BL, Child AH, Stheneur C, Binquet C, Gautier E, Chevallier B, Huet F, Loeys BL, Arbustini E, Mayer K, Arslan-Kirchner M, Kiotsekoglou A, Comeglio P, Grasso M, Halliday DJ, Beroud C, Bonithon-Kopp C, Claustres M, Robinson PN, Ades L, De Backer J, Coucke P, Francke U, De Paepe A, Boileau C, Jondeau G. Clinical and molecular study of 320 children with Marfan syndrome and related type I fibrillinopathies in a series of 1009 probands with pathogenic FBN1 mutations. Pediatrics 2009; 123: 391–8.

14 Patel ND, Williams JA, Barreiro CJ, Bethea BT, Fitton TP, Dietz HC, Lima JA, Spevak PJ, Gott VL, Vricella LA, Cameron DE. Valve-sparing aortic root replacement: early experience with the De Paulis Valsalva graft in 51 patients. Ann Thorac Surg 2006; 82: 548–53.

15 Singh MP, Bentall HH. Complete replacement of the ascending aorta and the aortic valve for the treatment of aortic aneurysm. J Thorac Cardiovasc Surg 1972; 63: 218–25.

16 Bentall H, De Bono A. A technique for complete replacement of the ascending aorta. Thorax 1968; 23: 338–9.

17 Pyeritz RE. Marfan syndrome: 30 years of research equals 30 years of additional life expectancy. Heart 2009; 95: 173–5.

18 Shores J, Berger KR, Murphy EA, Pyeritz RE. Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in Marfan's syndrome. N Engl J Med 1994; 330: 1335–41.

19 Pearson GD, Devereux R, Loeys B, Maslen C, Milewicz D, Pyeritz R, Ramirez F, Rifkin D, Sakai L, Svensson L, Wessels A, Van Eyk J, Dietz HC. Report of the National Heart, Lung, and Blood Institute and National Marfan Foundation Working Group on research in Marfan syndrome and related disorders. Circulation 2008; 118: 785–91.

20 Brooke BS, Habashi JP, Judge DP, Patel N, Loeys B, Dietz HC 3rd. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. N Engl J Med 2008; 358: 2787–95.

21 Xiong W, Knispel RA, Dietz HC, Ramirez F, Baxter BT. Doxycycline delays aneurysm rupture in a mouse model of Marfan syndrome. J Vasc Surg 2008; 47: 166–72; discussion 172.

22 Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, Hamosh A, Nanthakumar EJ, Curristin SM, Stetten G, Meyers DA, Fancomano A. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 1991; 352: 337–9.

23 Turner CL, Emery H, Collins AL, Howarth RJ, Yearwood CM, Cross E, Duncan PJ, Bunyan DJ, Harvey JF, Foulds NC. Detection of 53 FBN1 mutations (41 novel and 12 recurrent) and genotype-phenotype correlations in 113 unrelated probands referred with Marfan syndrome, or a related fibrillinopathy. Am J Med Genet A 2009; 149A: 161–70.

24 Pereira L, Andrikopoulos K, Tian J, Lee SY, Keene DR, Ono R, Reinhardt DP, Sakai LY, Biery NJ, Bunton T, Dietz HC, Ramirez F. Targetting of the gene encoding fibrillin-1 recapitulates the vascular aspect of Marfan syndrome. Nat Genet 1997; 17: 218–22.

25 Pereira L, Lee SY, Gayraud B, Andrikopoulos K, Shapiro SD, Bunton T, Biery NJ, Dietz HC, Sakai LY, Ramirez F. Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. Proc Natl Acad Sci USA 1999; 96: 3819–23.

26 Collod-Beroud G, Le Bourdelles S, Ades L, Ala-Kokko L, Booms P, Boxer M, Child A, Comeglio P, De Paepe A, Hyland JC, Holman K, Kaitila I, Loeys B, Matyas G, Nuytinck L, Peltonen L, Rantamaki T, Robinson P, Steinmann B, Junien C, Beroud C, Boileau C. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. Hum Mutat 2003; 22: 199–208.

27 Dietz HC, Pyeritz RE, Puffenberger EG, Kendzior RJ Jr, Corson GM, Maslen CL, Sakai LY, Francomano CA, Cutting GR. Marfan phenotype variability in a family segregating a missense mutation in the epidermal growth factor-like motif of the fibrillin gene. J Clin Invest 1992; 89: 1674–80.

28 Summers KM, Bokil NJ, Baisden JM, West MJ, Sweet MJ, Raggatt LJ, Hume DA. Experimental and bioinformatic characterisation of the promoter region of the Marfan syndrome gene, FBN1. Genomics 2009; 94: 233–40.

29 Judge DP, Biery NJ, Keene DR, Geubtner J, Myers L, Huso DL, Sakai LY, Dietz HC. Evidence for a critical contribution of

haploinsufficiency in the complex pathogenesis of Marfan syndrome. J Clin Invest 2004; 114: 172–81.

30 Matyas G, Alonso S, Patrignani A, Marti M, Arnold E, Magyar I, Henggeler C, Carrel T, Steinmann B, Berger W. Large genomic fibrillin-1 (FBN1) gene deletions provide evidence for true haploinsufficiency in Marfan syndrome. Hum Genet 2007; 122: 23–32.

31 Hutchinson S, Furger A, Halliday D, Judge DP, Jefferson A, Dietz HC, Firth H, Handford PA. Allelic variation in normal human FBN1 expression in a family with Marfan syndrome: a potential modifier of phenotype? Hum Mol Genet 2003; 12: 2269–76.

32 Van Dijk FS, Hamel BC, Hilhorst-Hofstee Y, Mulder BJ, Timmermans J, Pals G, Cobben JM. Compound-heterozygous Marfan syndrome. Eur J Med Genet 2009; 52: 1–5.

33 Patel ND, Williams JA. Valve-sparing aortic root relacement: early experience with the De Paulis. Ann Thorac Surg 2006; 82: 548–53.

34 Matt P, Habashi J, Carrel T, Cameron DE, Van Eyk JE, Dietz HC. Recent advances in understanding Marfan syndrome: should we now treat surgical patients with losartan? J Thorac Cardiovasc Surg 2008; 135: 389–94.

35 Gersony DR, McClaughlin MA, Jin Z, Gersony WM. The effect of beta-blocker therapy on clinical outcome in patients with Marfan's syndrome: a meta-analysis. Int J Cardiol 2007; 114: 303–8.

36 Pyeritz RE. A small molecule for a large disease. N Engl J Med 2008; 358: 2829–31.

37 Ahimastos AA, Aggarwal A, D'Orsa KM, Formosa MF, White AJ, Savarirayan R, Dart AM, Kingwell BA. Effect of perindopril on large artery stiffness and aortic root diameter in patients with Marfan syndrome: a randomized controlled trial. JAMA 2007; 298: 1539–47.

38 Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, Ramirez F, Sakai LY, Dietz HC. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat Genet 2003; 33: 407–11.

39 Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, Gamradt M, ap Rhys CM, Holm TM, Loeys BL, Ramirez F, Judge DP, Ward CW, Dietz HC. Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. Nat Med 2007; 13: 204–10.

40 Ziyadeh FN. Mediators of diabetic renal disease: the case for TGF-beta as the major mediator. J Am Soc Nephrol 2004; 15 (Suppl 1): S55–7.

41 Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, Myers L, Klein EC, Liu G, Calvi C, Podowski M, Neptune ER, Halushka MK, Bedja D, Gabrielson K, Rifkin DB, Carta L, Ramirez F, Huso DL, Dietz HC. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 2006; 312: 117–21.

42 Busuttil RW, Abou-Zamzam AM, Machleder HI. Collagenase activity of the human aorta. A comparison of patients with and without abdominal aortic aneurysms. Arch Surg 1980; 115: 1373–8.

43 Busuttil RW, Rinderbriecht H, Flesher A, Carmack C. Elastase activity: the role of elastase in aortic aneurysm formation. J Surg Res 1982; 32: 214–7.

44 Thompson RW, Holmes DR, Mertens RA, Liao S, Botney MD, Mecham RP, Welgus HG, Parks WC. Production and localization of 92-kilodalton gelatinase in abdominal aortic aneurysms. An elastolytic metalloproteinase expressed by aneurysm-infiltrating macrophages. J Clin Invest 1995; 96: 318–26.

45 Murphy G, Cockett MI, Ward RV, Docherty AJ. Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan. A quantitative comparison of the activities of 95 kDa and 72 kDa gelatinases, stromelysins-1 and -2 and punctuated metalloproteinase (PUMP). Biochem J 1991; 277: 277–9.

46 Thompson RW, Parks WC. Role of matrix metalloproteinases in abdominal aortic aneurysms. Ann N Y Acad Sci 1996; 800: 157–74.

47 Senior RM, Griffin GL, Fliszar CJ, Shapiro SD, Goldberg GI, Welgus HG. Human 92- and 72-kilodalton type IV collagenases are elastases. J Biol Chem 1991; 266: 7870–5.

48 Katsuda S, Okada Y, Okada Y, Imai K, Nakanishi I. Matrix metalloproteinase-9 (92-kd gelatinase/type IV collagenase equals gelatinase B) can degrade arterial elastin. Am J Pathol 1994; 145: 1208–18.

49 Holmes DR, Petrinec D, Wester W, Thompson RW, Reilly JM. Indomethacin prevents elastase-induced abdominal aortic aneurysms in the rat. J Surg Res 1996; 63: 305–9.

50 Petrinec D, Liao S, Holmes DR, Reilly JM, Parks WC, Thompson RW. Doxycycline inhibition of aneurysmal degeneration in an elastase-induced rat model of abdominal aortic aneurysm: preservation of aortic elastin associated with suppressed production of 92 kD gelatinase. J Vasc Surg 1996; 23: 336–46.

51 Curci JA, Petrinec D, Liao S, Golub LM, Thompson RW. Pharmacologic suppression of experimental abdominal aortic aneurysms: acomparison of doxycycline and four chemically modified tetracyclines. J Vasc Surg 1998; 28: 1082–93.

52 Curci JA, Mao D, Bohner DG, Allen BT, Rubin BG, Reilly JM, Sicard GA, Thompson RW. Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. J Vasc Surg 2000; 31: 325–42.

53 Mosorin M, Juvonen J, Biancari F, Satta J, Surcel HM, Leinonen M, Saikku P, Juvonen T. Use of doxycycline to decrease the growth rate of abdominal aortic aneurysms: a randomized, double-blind, placebo-controlled pilot study. J Vasc Surg 2001; 34: 606–10.

54 Chung AW, Au Yeung K, Sandor GG, Judge DP, Dietz HC, van Breemen C. Loss of elastic fiber integrity and reduction of vascular smooth muscle contraction resulting from the upregulated activities of matrix metalloproteinase-2 and -9 in the thoracic aortic aneurysm in Marfan syndrome. Circ Res 2007; 101: 512–22.

55 Chung AW, Yang HH, Radomski MW, van Breemen C. Long-term doxycycline is more effective than atenolol to

prevent thoracic aortic aneurysm in Marfan syndrome through the inhibition of matrix metalloproteinase-2 and -9. Circ Res 2008; 102: e73–85.

56 Yang HH, Kim JM, Chum E, van Breemen C, Chung AW. Effectiveness of combination of losartan potassium and doxycycline versus single-drug treatments in the secondary prevention of thoracic aortic aneurysm in Marfan syndrome. J Thorac Cardiovasc Surg 2010; 140: 305–12 e2.

57 Radonic T, de Witte P, Baars MJ, Zwinderman AH, Mulder BJ, Groenink M. Losartan therapy in adults with Marfan syndrome: study protocol of the multi-center randomized controlled COMPARE trial. Trials 2010; 11: 3.

58 Gambarin FI, Favalli V, Serio A, Regazzi M, Pasotti M, Klersy C, Dore R, Mannarino S, Vigano M, Odero A, Amato S, Tavazzi L, Arbustini E. Rationale and design of a trial evaluating the effects of losartan vs. nebivolol vs. the association of both on the progression of aortic root dilation in Marfan syndrome with FBN1 gene mutations. J Cardiovasc Med (Hagerstown) 2009; 10: 354–62.

59 Lacro RV, Dietz HC, Wruck LM, Bradley TJ, Colan SD, Devereux RB, Klein GL, Li JS, Minich LL, Paridon SM, Pearson GD, Printz BF, Pyeritz RE, Radojewski E, Roman MJ, Saul JP, Stylianou MP, Mahony L. Rationale and design of a randomized clinical trial of beta-blocker therapy (atenolol) versus angiotensin II receptor blocker therapy (losartan) in individuals with Marfan syndrome. Am Heart J 2007; 154: 624–31.

60 Pfeffer MA, Swedberg K, Granger CB, Held P, McMurray JJ, Michelson EL, Olofsson B, Ostergren J, Yusuf S, Pocock S. Effects of candesartan on mortality and morbidity in patients with chronic heart failure: the CHARM-Overall programme. Lancet 2003; 362: 759–66.

61 Dahlof B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, Fyhrquist F, Ibsen H, Kristiansson K, Lederballe-Pedersen O, Lindholm LH, Nieminen MS, Omvik P, Oparil S, Wedel H. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 2002; 359: 995–1003.

62 Yusuf S, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P, Anderson C. Telmisartan, ramipril, or both in patients at high risk for vascular events. N Engl J Med 2008; 358: 1547–59.

63 Yusuf S, Teo K, Anderson C, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P. Effects of the angiotensin-receptor blocker telmisartan on cardiovascular events in high-risk patients intolerant to angiotensin-converting enzyme inhibitors: a randomised controlled trial. Lancet 2008; 372: 1174–83.

64 Sipahi I, Debanne SM, Rowland DY, Simon DI, Fang JC. Angiotensin-receptor blockade and risk of cancer: meta-analysis of randomised controlled trials. Lancet Oncol 2010; 11: 627–36.

65 Nissen SE. Angiotensin-receptor blockers and cancer: urgent regulatory review needed. Lancet Oncol 2010; 11: 605–6.

66 Miura M, Satoh S, Kagaya H, Saito M, Inoue T, Ohkubo T, Habuchi T, Suzuki T. Effect of telmisartan, valsartan and candesartan on mycophenolate mofetil pharmacokinetics in Japanese renal transplant recipients. J Clin Pharm Ther 2009; 34: 683–92.

67 Oparil S. Comparative antihypertensive efficacy of olmesartan: comparison with other angiotensin II receptor antagonists. J Hum Hypertens 2002; 16 (Suppl 2): S17–23.

68 Li L, Zhou N, Gong H, Wu J, Lin L, Komuro I, Ge J, Zou Y. Comparison of angiotensin II type 1-receptor blockers to regress pressure overload-induced cardiac hypertrophy in mice. Hypertens Res 2010; 33: 1289–97.

69 Blum A, Shamburek R. The pleiotropic effects of statins on endothelial function, vascular inflammation, immunomodulation and thrombogenesis. Atherosclerosis 2009; 203: 325–30.

70 Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008; 359: 2195–207.

71 Ridker PM, Genest J, Boekholdt SM, Libby P, Gotto AM, Nordestgaard BG, Mora S, MacFadyen JG, Glynn RJ, Kastelein JJ. HDL cholesterol and residual risk of first cardiovascular events after treatment with potent statin therapy: an analysis from the JUPITER trial. Lancet 2010; 376: 333–9.

72 Ridker PM, Glynn RJ. JUPITER, rosuvastatin, and the European Medicines Agency. Lancet 2010; 375: 2071.

73 Touat Z, Lepage L, Ollivier V, Nataf P, Hvass U, Labreuche J, Jandrot-Perrus M, Michel JB, Jondeau G. Dilation-dependent activation of platelets and prothrombin in human thoracic ascending aortic aneurysm. Arterioscler Thromb Vasc Biol 2008; 28: 940–6.

74 Sorice GP, Folli F. A combination of PPAR-gamma agonists and HMG CoA reductase inhibitors (statins) as a new therapy for the conservative treatment of AAS (aortic aneurysm syndromes). Med Hypotheses 2009; 73: 614–8.

75 Ishibashi M, Egashira K, Hiasa K, Inoue S, Ni W, Zhao Q, Usui M, Kitamoto S, Ichiki T, Takeshita A. Antiinflammatory and antiarteriosclerotic effects of pioglitazone. Hypertension 2002; 40: 687–93.

76 Shiomi T, Tsutsui H, Hayashidani S, Suematsu N, Ikeuchi M, Wen J, Ishibashi M, Kubota T, Egashira K, Takeshita A. Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates left ventricular remodeling and failure after experimental myocardial infarction. Circulation 2002; 106: 3126–32.

77 Ghosh AK, Bhattacharyya S, Lakos G, Chen SJ, Mori Y, Varga J. Disruption of transforming growth factor beta signaling and profibrotic responses in normal skin fibroblasts by peroxisome proliferator-activated receptor gamma. Arthritis Rheum 2004; 50: 1305–18.

78 Soumian S, Gibbs R, Davies A, Albrecht C. mRNA expression of genes involved in lipid efflux and matrix degradation in occlusive and ectatic atherosclerotic disease. J Clin Pathol 2005; 58: 1255–60.

79 Willis L, Roosevelt GE, Yetman AT. Comparison of clinical characteristics and frequency of adverse outcomes in patients with Marfan syndrome diagnosed in adulthood versus childhood. Pediatr Cardiol 2009; 30: 289–92.

80 Klintschar M, Bilkenroth U, Arslan-Kirchner M, Schmidtke J, Stiller D. Marfan syndrome: clinical consequences resulting from a medicolegal autopsy of a case of sudden death due to aortic rupture. Int J Legal Med 2009; 123: 55–8.

81 Mortensen K, Aydin MA, Rybczynski M, Baulmann J, Schahidi NA, Kean G, Kuhne K, Bernhardt AM, Franzen O, Mir T, Habermann C, Koschyk D, Ventura R, Willems S, Robinson PN, Berger J, Reichenspurner H, Meinertz T, von Kodolitsch Y. Augmentation index relates to progression of aortic disease in adults with Marfan syndrome. Am J Hypertens 2009; 22: 971–9.

82 Matt P, Schoenhoff F, Habashi J, Holm T, Van Erp C, Loch D, Carlson OD, Griswold BF, Fu Q, De Backer J, Loeys B, Huso DL, McDonnell NB, Van Eyk JE, Dietz HC. Circulating transforming growth factor-beta in Marfan syndrome. Circulation 2009; 120: 526–32.

83 Braverman AC. Transforming growth factor-{beta}: a biomarker in Marfan syndrome? Circulation 2009; 120: 464–6.

84 Ahimastos AA, Aggarwal A, Savarirayan R, Dart AM, Kingwell BA. A role for plasma transforming growth factor-beta and matrix metalloproteinases in aortic aneurysm surveillance in Marfan syndrome? Atherosclerosis 2010; 209: 211–4.

85 Attias D, Stheneur C, Roy C, Collod-Beroud G, Detaint D, Faivre L, Delrue MA, Cohen L, Francannet C, Beroud C, Claustres M, Iserin F, Kien PK, Lacombe D, Le Merrer M, Lyonnet S, Odent S, Plauchu H, Rio M, Rossi A, Sidi D, Steg PG, Ravaud P, Boileau C, Jondeau G. Comparison of clinical presentations and outcomes between patients with TGFBR2 and FBN1 mutations in Marfan syndrome and related disorders. Circulation 2009; 120: 2541–9.

86 Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H, De Backer JF, Oswald GL, Symoens S, Manouvrier S, Roberts AE, Faravelli F, Greco MA, Pyeritz RE, Milewicz DM, Coucke PJ, Cameron DE, Braverman AC, Byers PH, De Paepe AM, Dietz HC. Aneurysm syndromes caused by mutations in the TGF-beta receptor. N Engl J Med 2006; 355: 788–98.

87 Lucarini L, Evangelisti L, Attanasio M, Lapini I, Chiarini F, Porciani MC, Abbate R, Gensini G, Pepe G. May TGFBR1 act also as low penetrance allele in Marfan syndrome? Int J Cardiol 2009; 131: 281–4.

88 Wilson WR, Choke EC, Dawson J, Loftus IM, Thompson MM. Plasma matrix metalloproteinase levels do not predict tissue levels in abdominal aortic aneurysms suitable for elective repair. Vascular 2008; 16: 248–52.

89 Chen L, Wang X, Carter SA, Shen YH, Bartsch HR, Thompson RW, Coselli JS, Wilcken DL, Wang XL, LeMaire SA. A single nucleotide polymorphism in the matrix metalloproteinase 9 gene (-8202A/G) is associated with thoracic aortic aneurysms and thoracic aortic dissection. J Thorac Cardiovasc Surg 2006; 131: 1045–52.

90 Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971; 285: 1182–6.

91 Wu Z, O'Reilly MS, Folkman J, Shing Y. Suppression of tumor growth with recombinant murine angiostatin. Biochem Biophys Res Commun 1997; 236: 651–4.

92 Boehm T, Folkman J, Browder T, O'Reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature 1997; 390: 404–7.

93 Folkman J. I'm flattered, but it's mice, only mice. If you have cancer and you're a mouse, we can take good care of you. *Time Magazine* May 18th 1998.

94 Detaint D, Aegerter P, Tubach F, Hoffman I, Plauchu H, Dulac Y, Faivre LO, Delrue MA, Collignon P, Odent S, Tchitchinadze M, Bouffard C, Arnoult F, Gautier M, Boileau C, Jondeau G. Rationale and design of a randomized clinical trial (Marfan Sartan) of angiotensin II receptor blocker therapy versus placebo in individuals with Marfan syndrome. Arch Cardiovasc Dis 2010; 103: 317–25.

95 Chamberlain JS. ACE inhibitor bulks up muscle. Nat Med 2007; 13: 125–6.